Understanding *Klebsiella pneumoniae* Clonality, Virulence and Antimicrobial Resistance at the Human-Animal-Environment Interface from Bangladesh

Saifur Rahman

Linacre College, University of Oxford

Department of Biology

A thesis submitted for the degree of Doctor of Philosophy

Trinity 2025

A smooth sea never made a skilled sailor.

— English proverb —

Table of Contents

Abstract

The One Health approach is increasingly recognised as crucial to addressing the global threat of antimicrobial resistance (AMR). However, data on the epidemiology of AMR at the human-animal-environmental interface remain limited from low- and middle-income countries. This thesis presents the first systematic One Health survey of *Klebsiella pneumoniae* and related species in Bangladesh, integrating phenotypic and genomic analyses with clinical and demographic metadata.

A total of 3759 samples were collected between December 2021 and March 2023 from humans (urinary tract infections, surgical site infections, and healthy rectal carriage), livestock (chicken, domestic animals, free-flying birds) and environmental sources (water and flies) in Mymensingh, Bangladesh. *K. pneumoniae* was isolated from 14.4% (541/3759) of samples, with marked variation across sources and higher isolation rates during warmer and wetter seasons. Notably, flies showed the highest prevalence (36.4%, 80/220), with significant carriage during summer and monsoon, highlighting their potential as mechanical vectors.

Hospital settings were hotspots for AMR, particularly for extended-spectrum β-lactamase (predominantly *bla*$_{\text{CTX–M–15}}$) and carbapenemase (predominantly *bla*$_{\text{NDM–5}}$) producers, especially in hospital-associated surgical site infections (SSIs). Convergent multidrug-resistant (MDR) hypervirulent strains, including carbapenem-resistant hypervirulent *K. pneumoniae,* were restricted to SSIs, representing an acute clinical threat. Clonal dissemination of MDR *K. pneumoniae* ST48 and ST490 indicated potential outbreak dynamics within surgical wards.

Although overall prevalence was low, plasmid-mediated mobile colistin resistance (mainly *mcr*-8) was detected in poultry-associated settings, including isolates from flies, water, and healthy humans, but not in clinical isolates. Conversely, carbapenemase genes were rarely identified outside hospitals. Non-pneumoniae *Klebsiella* species comprised 56.6% (705/1246) of isolates. Despite species diversity, they generally exhibited low levels of AMR and virulence.

While AMR-associated genes were sporadically identified in community and environmental sources, ESBLs, carbapenemases, and hypervirulence determinants were predominantly detected in hospital settings. Collectively, these findings highlight hospitals as critical intervention points to interrupt the dissemination of AMR in *Klebsiella* and support periodic surveillance across farms, wastewater, and vectors to detect emerging resistance and track high-risk genes within a One Health framework.

Acknowledgements

Foremost among my acknowledgements is Almighty God, to whom I owe the strength, patience, and perseverance that made this work possible.

I am profoundly grateful to my supervisor, Professor Timothy R. Walsh, for his scholarly guidance, steadfast encouragement, constructive criticism, and kindness throughout this study. Despite his demanding schedule, he invariably offered me his thoughtful supervision and placed his trust in me, the support without which neither this research nor this writing would have been possible.

I am equally grateful to my co-supervisors, Dr Refath Farzana and Professor Julian Parkhill. From the preparation of my scholarship application through to the completion of this study, Dr Refath Farzana provided unwavering academic support and guidance that proved invaluable. She encouraged me to seize every opportunity, sharpened my academic and research skills, and guided me with care and dedication at every step. The thoughtful advice and incisive suggestions of Professor Julian Parkhill strengthened the quality and depth of this research.

I extend my heartfelt thanks to Professor Sukumar Saha for his guidance and support throughout the study, especially during the sampling work in Bangladesh.

My sincere thanks go to my friend, Amrita Pondit, for her kind support and cooperation during the sampling for this study. Although this thesis is written in the first person, Amrita and I worked together on the sampling. I deeply appreciate her contribution to this vital part of the research.

I gratefully acknowledge funding and support for this study from the Prime Minister’s Fellowship Authority (Government of Bangladesh), the Ineos Oxford Institute for Antimicrobial Research (IOI), and Linacre College, University of Oxford. I also thank collaborators at Bangladesh Agricultural University and Mymensingh Medical College Hospital. Special thanks are due to Professor Md. Abul Kalam Azad (Department of Surgery, Mymensingh Medical College Hospital), S M Rafiqul Islam (Department of Surgery, Mymensingh Medical College Hospital) and Professor Syeda Anjuman

Nasreen (Department of Microbiology, Mymensingh Medical College) for their cooperation in setting up the study and sampling at the hospital.

I have been fortunate to work with excellent colleagues in the lab, who created a friendly, supportive, and inspiring environment. I am grateful for their collaboration and camaraderie.

My greatest debt is to my family. To my beloved wife, Zannatul Firdous, and our little boy, Zohaib Rahman, your love, sacrifice, and resilience have steadied me at every turn. To my parents and cousin Babul Mia, whose constant support sustained my family during my absence, I offer my heartfelt thanks for your encouragement and selfless devotion. Whatever success this thesis represents is as much yours as mine, and I dedicate it to you with all my heart.

Finally, to my late father, who planted this dream but did not live to see it fulfilled, I hope he watches from heaven.

Declaration

I declare that this thesis is my own work, undertaken with appropriate support and assistance from my supervisors and colleagues. My supervisory team provided scientific guidance throughout all the stages of this work.

Specific assistance I have received from others in relation to this thesis is outlined below.

I performed field sampling and data collection with the assistance of a team from Bangladesh Agricultural University and Mymensingh Medical College Hospital, who provided support during the sample and data collection process. I acknowledge the assistance from the team members, especially Amrita Pondit. I am also grateful to Professor Md. Abul Kalam Azad (Department of Surgery, Mymensingh Medical College Hospital), S M Rafiqul Islam (Department of Surgery, Mymensingh Medical College Hospital) and Professor Syeda Anjuman Nasreen (Department of Microbiology, Mymensingh Medical College) for their cooperation during hospital-based sampling.

I am especially thankful to Dr Refath Farzana for her guidance on the bioinformatics analysis of whole genome sequencing data.

Presentations at International Conferences

1. **Rahman S,** *et al*. Emergence of mobile colistin resistance in *Klebsiella* at the human-animal-environment interface in Bangladesh. Oral presentation at the 35th ESCMID Global Congress, April 2025, Vienna, Austria.

2. **Rahman S,** *et al*. Surveillance of surgical site infections: a prospective study in a tertiary care hospital in Bangladesh. Poster presentation at the 34th ECCMID Congress, April 2024, Barcelona, Spain.

List of Abbreviations

3GCs	Third-generation cephalosporins
AAHC	Antibiotic-associated haemorrhagic colitis
AMC	Amoxicillin-clavulanic acid
AMK	Amikacin
AMR	Antimicrobial resistance
AMRSurME	Antimicrobial Resistance Surveillance Monitoring and Evaluation
AMS	Antimicrobial stewardship
AMU	Antimicrobial usage
ARC	Antimicrobial resistance containment
ARGs	Antimicrobial resistance genes
AST	Antimicrobial susceptibility testing
ATM	Aztreonam
AWEEC	Animal Welfare and Experimentation Ethics Committee
BARA	Bangladesh AMR Response Alliance
BAU	Bangladesh Agricultural University
BBS	Bangladesh Bureau of Statistics
BF	Bird faeces
bla	β-lactamase gene
BLAST	Basic Local Alignment Search Tool
BLASTn	Basic Local Alignment Search Tool for nucleotides
BNAP	Bangladesh National Action Plan
BPPL	Bacterial Priority Pathogen List
BRIG	BLAST Ring Image Generator
CAIs	Community-acquired infections
CAPTURA	Collecting Antibiotic Prescription data for Universal Access and Use
CAZ	Ceftazidime
CCS	Chicken cloacal swabs
CDC	Communicable Disease Control
CF	Conversion Factor
CFU	Colony-forming unit
CGE	Centre for Genomic Epidemiology
CI	Confidence interval
CIP	Ciprofloxacin
cKP	Classical *Klebsiella pneumoniae*

CLSI	Clinical Laboratory Standard Institute
CMS	Chicken meat swabs
CRF	Case record form
CR-hvKP	Carbapenem-resistant hypervirulent *Klebsiella pneumoniae*
CRK	Colistin-resistant *Klebsiella* spp.
CR-KP	Carbapenem-resistant *Klebsiella pneumoniae*
CSK	Colistin-sensitive *Klebsiella* spp.
CST	Colistin
CTX	Cefotaxime
CTX-M	Cefotaximase-M
CZA	Ceftazidime-avibactam
DARS	Domestic animal rectal swabs
df	Degrees of freedom
DGDA	Directorate General of Drug Administration
DGHS	Directorate General of Health Services
DMSO	Dimethyl Sulfoxide
ECDC	European Centre for Disease Prevention and Control
ESBLs	Extended-spectrum β-lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organisation
FEP	Cefepime
FOS	Fosfomycin
gDNA	Genomic DNA
GDP	Gross Domestic Product
GEN	Gentamicin
GLASS	Global Antimicrobial Resistance and Use Surveillance System
HAIs	Hospital-acquired infections
HCCA	α-Cyano-4-hydroxycinnamic acid
HGT	Horizontal gene transfer
HRS	Human rectal swabs
hvKP	Hypervirulent *Klebsiella pneumoniae*
IBM SPSS	International Business Machines Statistical Package for the Social Sciences
ICEs	Integrative and conjugative elements
ICU	Intensive care unit
IEDCR	Institute of Epidemiology, Disease Control and Research

IMP	Imipenemase
Indels	Insertions and deletions
IOI	Ineos Oxford Institute for Antimicrobial Research
IPC	Infection Prevention and Control
IPM	Imipenem
IRB	Institutional Review Board
IS	Insertion sequence
iTOL	Interactive Tree of Life
Km	Kilometre
KoSC	*Klebsiella oxytoca* species complex
KPC	*Klebsiella pneumoniae* carbapenemase
KpSC	*Klebsiella pneumoniae* species complex
LMICs	Low- and middle-income countries
LPS	Lipopolysaccharide
LVX	Levofloxacin
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionisation-Time of Flight Mass Spectrometry
mcr	Mobile colistin resistance
MCRNK	*mcr*-negative *Klebsiella* pp.
MCRPK	*mcr*-positive *Klebsiella* pp.
MDR	Multidrug-resistant
MDR-hvKP	Multidrug-resistant hypervirulent *K. pneumoniae*
MEM	Meropenem
MGEs	Mobile genetic elements
MIC	Minimum inhibitory concentration
ML	Maximum Likelihood
MLST	Multilocus Sequence Typing
MMC	Mymensingh Medical College
MMCH	Mymensingh Medical College Hospital
NAP	National Action Plan
NCBI	National Centre for Biotechnology Information
NDM	New Delhi Metallo-β-lactamase
OD	Optical density
ONT	Oxford Nanopore Technologies
OR	Odds ratio
OTC	Over-the-counter

OXA	Oxacillinase
OxTREC	Oxford Tropical Research Ethics Committee
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PMQR	Plasmid-mediated quinolone resistance
PR	Prevalence ratio
QC	Quality control
rcf	Relative centrifugal force
rpm	Rotations per minute
SDGs	Sustainable Development Goals
SHV	Sulfhydryl variable
SNP	Single-nucleotide polymorphism
SOPs	Standard operating procedures
SSI	Surgical site infection
ST	Sequence type
TGC	Tigecycline
TZP	Piperacillin-tazobactam
UK	United Kingdom
UN	United Nations
UNEP	United Nations Environment Programme
UNICEF	United Nations Children's Fund
UOX	University of Oxford
UTI	Urinary tract infection
VFDB	Virulence Factors of Pathogenic Bacteria Database
VIM	Verona Integron-encoded Metallo-β-lactamase
WASH	Water, Sanitation, and Hygiene
WGS	Whole genome sequencing
WHO	World Health Organisation
WOAH	World Organisation for Animal Health
XDR	Extensively drug-resistant

Chapter 1

General Introduction

1.1 Antimicrobial resistance (AMR): The impending global pandemic

1.1.1 A brief history of antibiotics and the evolution of AMR

Antibiotics mark a long arc from ancient antimicrobial remedies, such as applying mouldy bread on wounds, to modern chemotherapeutics (Gould, 2016). The term ‘antibiotic’ was first used by Selman Waksman in 1942, who defined an antibiotic as a substance produced by microorganisms that inhibits other microbes (Waksman, 1947; Waksman & Flynn, 1973). Early breakthroughs included arsphenamine (salvarsan, 1909) as the first modern antimicrobial, as well as prontosil and other sulfonamides in the 1930s, and penicillin, discovered by A. Fleming in 1928 and used clinically in the 1940s (Fleming, 1929; Christensen, 2021). A golden age, spanning from the 1940s to the 1970s, yielded many new classes of antibiotics. After this golden period, the drug discovery pipeline began to dry up, and very few new drugs have been introduced in the last few decades. Unfortunately, resistance emerged rapidly after each introduction of new classes of antibiotics, highlighting a continual evolutionary arms race between bacterial adaptation and drug development (Figure 1.1) (Coates *et al*., 2011; Hutchings *et al*., 2019). For instance, the first sign of antibiotic resistance to penicillin was reported by Abraham and Chain at Oxford in 1940 (Abraham & Chain, 1940). Moreover, rapid discovery and availability of these drugs within a relatively short period led to excessive, irresponsible, and misuse in medical and veterinary practices as well as in farming systems. Therefore, the process was accelerated, and bacteria became immune to most antibiotic classes. The effectiveness of most antibiotics has been eroded with the rise of AMR, resulting in some infections effectively being untreatable (Hutchings *et al*., 2019).

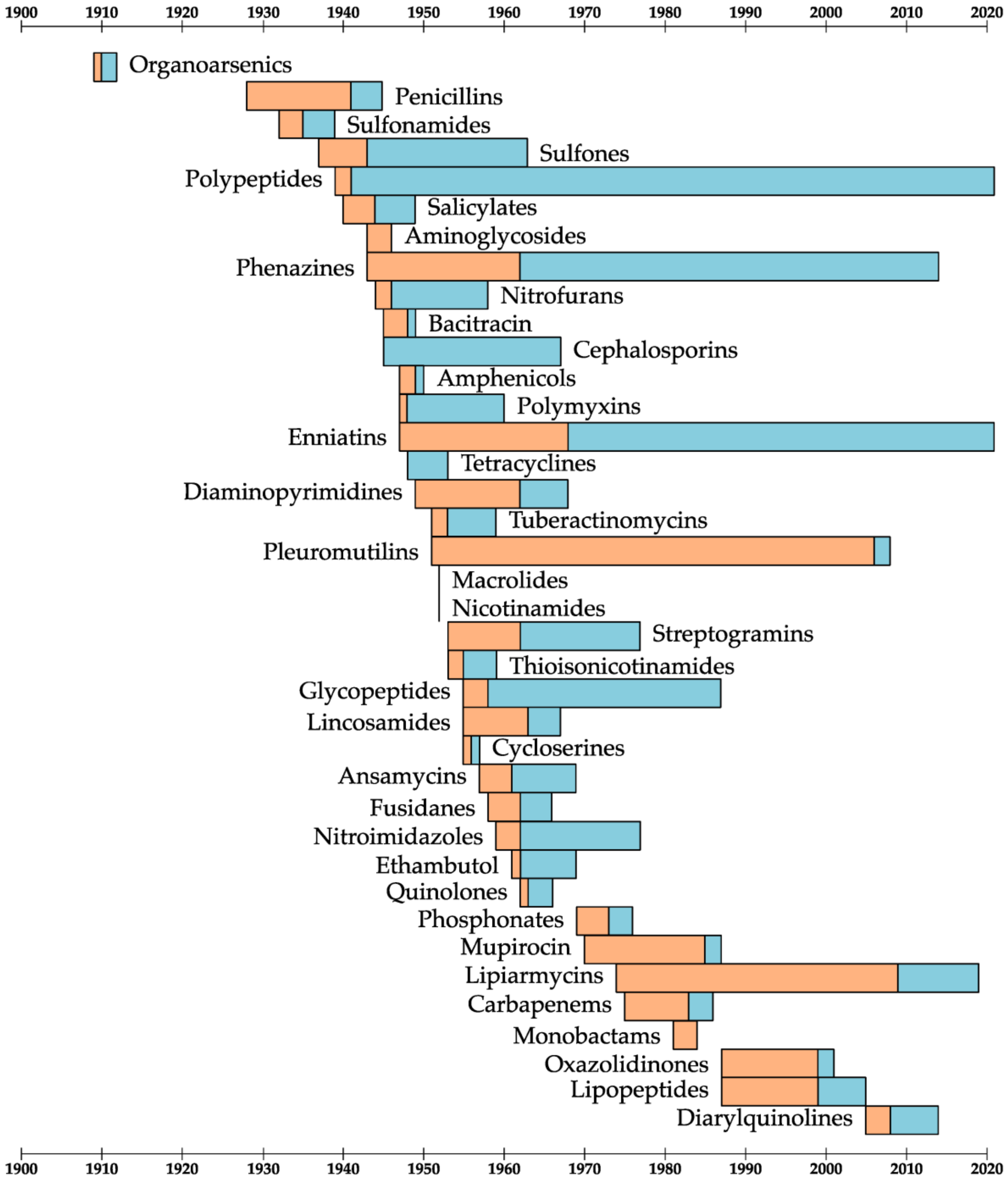


Figure 1.1 Timeline showing the discovery, first clinical use, and first reported clinical resistance across 38 selected antibiotic classes (Stennett *et al*., 2022). For each antibiotic class, the orange bars represent the ‘development windows’ (the period after its discovery when the antibiotic was first used in the clinic) and the blue bars represent the ‘resistance windows’ (the period after its first use, when clinical resistance was reported).

1.1.2 Mechanism of AMR

AMR is the phenomenon in which microorganisms (such as bacteria, viruses, fungi and parasites) develop or acquire mechanisms that render antimicrobial agents ineffective, making infections difficult to treat and increasing the risk of disease spread, severe illness and death. In public health terms, AMR occurs when germs defeat the drugs designed to kill them (WHO, 2023).

Bacterial AMR occurs as a natural process resulting from the selection pressure that antibiotics exert on bacterial populations. It can be either natural or acquired. Natural resistance may be intrinsic (always expressed in the species) or induced (the genes are naturally present in the bacteria but are only expressed at resistance levels after exposure to an antibiotic) and is not related to horizontal gene transfer (HGT, the process by which genetic material is transferred between bacterial cells independently of cell division, allowing bacteria to rapidly acquire new traits such as antibiotic resistance or virulence). Acquired resistance confers resistance through acquisition of genetic material via HGT mechanisms: transformation (incorporation of naked DNA), transduction (phage-mediated), and conjugation (by "sex" pili); and the bacteria may experience mutations to their own chromosomal DNA. The acquisition may be temporary or permanent. Plasmid-mediated transmission of resistance genes is the most common route for acquiring genetic material from outside sources (Reygaert, 2018).

Antibiotics generally act on five main targets (inhibit cell wall synthesis, depolarize the cell membrane, inhibit protein synthesis, inhibit nucleic acid synthesis, and inhibit metabolic pathways in bacteria), and antibiotic resistance can essentially be acquired through four different pathways (transformation, transduction, conjugation, and mutation) and expressed by four different mechanisms (limiting uptake of a drug, modifying a drug target, inactivating a drug, active drug efflux) (Figure 1.2) (Kapoor *et al.*, 2017; Reygaert, 2018).

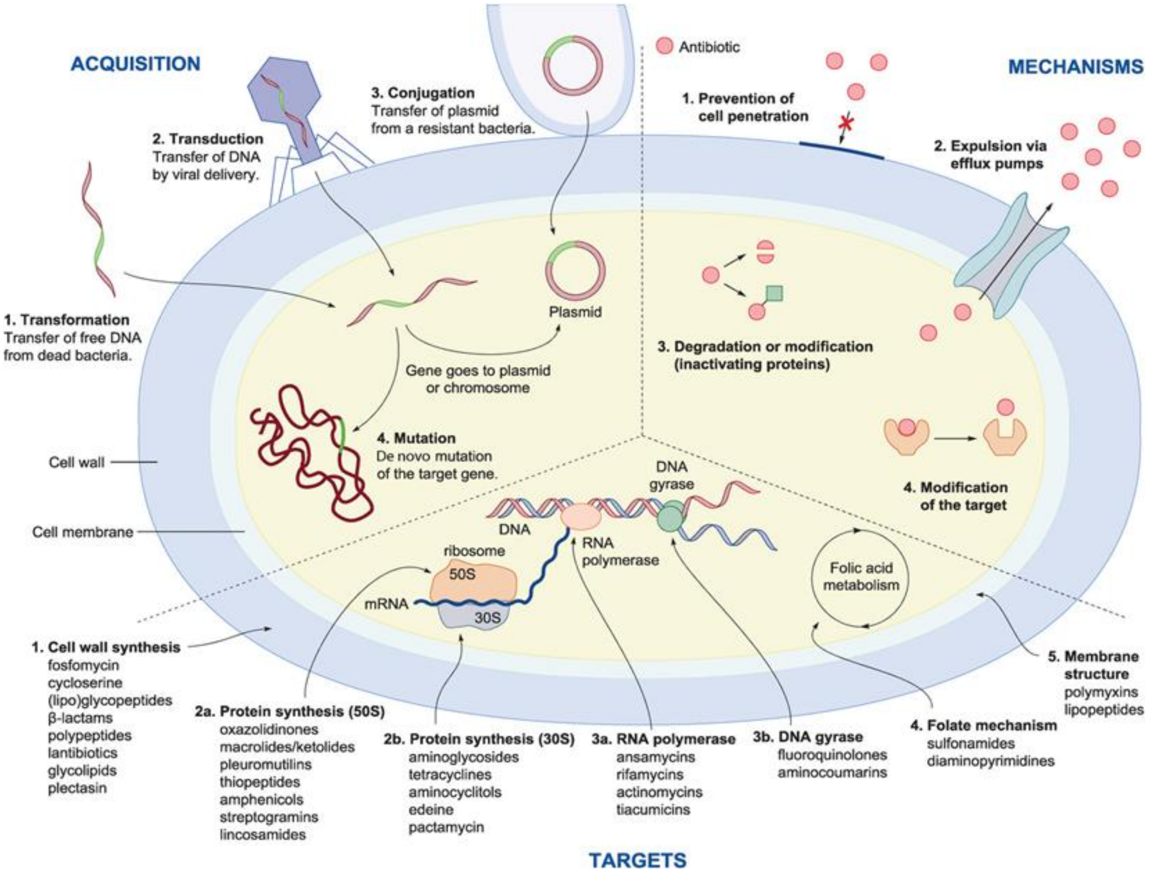


Figure 1.2 The four resistance acquisition pathways, the four main mechanisms of resistance, and the five main targets for antibiotics (Chellat *et al*., 2016).

1.1.3 Why AMR commands global attention?

AMR is recognised as an emerging health security threat in the 21st century, menacing a century of medical progress. No country or region is exempt from the consequences of AMR (Murray *et al*., 2022). The estimated global health burden due to AMR will be at least 10 million annual deaths and a reduction of 1.1–3.8% of GDP (Gross Domestic Product) annually by 2050, which will disproportionately affect the low- and middle-income countries (LMICs) (O’Neill, 2016). An estimated 24 million people worldwide would face extreme poverty by 2030, particularly in LMICs, if no immediate action is taken to combat AMR (The World Bank, 2016).

Reanalyses of the global burden of bacterial AMR estimated that in 2021, 4.71 million deaths were associated with AMR, including 1.27 million deaths directly attributable to resistant bacteria. In this analysis, deaths associated with AMR included all deaths in which a resistant bacterial infection was present, irrespective of whether resistance was the primary cause of death, as patients might have died even if the infecting bacteria were susceptible, due to underlying disease (e.g., cancer), severity of illness, or other factors. In contrast, deaths directly attributable to resistant bacteria represent mortalities that would not have occurred if the infecting bacteria had been susceptible to antibiotics, thereby indicating the direct effect of AMR. By 2050, this is expected to rise to 8.22 million deaths associated with AMR, including 1.91 million directly attributable to resistant bacteria (Figure 1.3). The highest AMR mortality rates are expected in the South Asia, Latin America, and the Caribbean regions (Naghavi *et al*., 2024). Moreover, during the COVID-19 pandemic, most patients in intensive care units (ICUs) have been prescribed antibiotics to rule out secondary bacterial infections, which perhaps exacerbate the burden of pre-existing pandemic AMR (Langford *et al*., 2020).

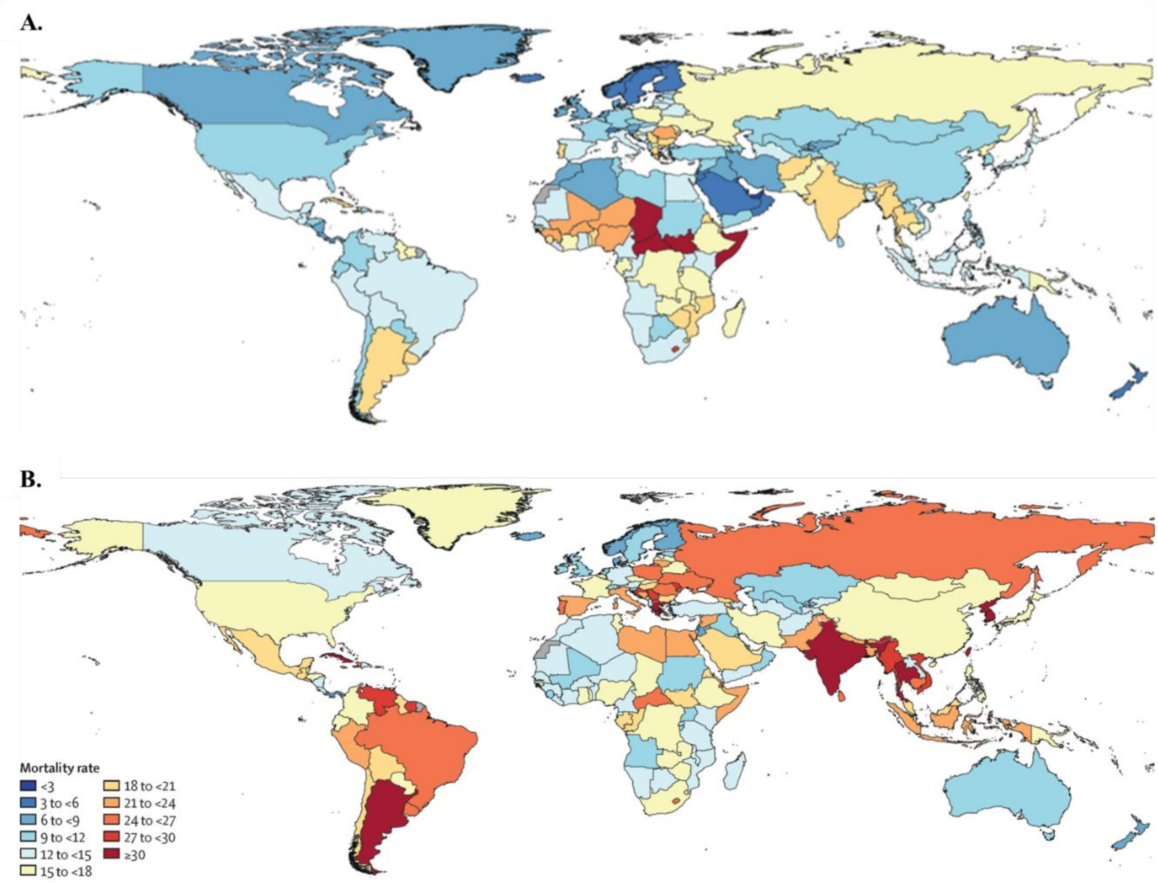


Figure 1.3 Global distribution of AMR-attributable death rates per 100,000 population (Naghavi *et al*., 2024). **A.** Death rate attributable to AMR, 2021. **B.** Projected death rate attributable to AMR, 2050*,* highlighting disproportionate burdens in South Asia and Latin America.

1.1.4 AMR and sustainable development goals (SDGs): How drug resistance threatens progress?

The SDG initiative by the United Nations (UN) includes 17 well-defined goals to serve a better, more equitable and sustainable life on this planet. AMR, often referred to as a silent pandemic, not only poses a threat to global health but also to the achievement of the SDGs. AMR impacts more than 10 out of 17 SDGs either directly or indirectly (Figure 1.4). It directly affects SDG 3 (Good health and well-being), with estimates of up to 10 million deaths annually due to AMR by 2050 (O'Neill, 2016). A World Bank assessment warns that inadequate action could push more than 24 million people into poverty by 2030, endangering SDG 1 (poverty reduction), SDG 8 (decent work and economic growth) and SDG 10 (reduced inequalities), through its negative effects on livelihoods and increased healthcare costs (The World Bank, 2016). The increased burden of AMR, especially on women and children in LMICs like Bangladesh, is hindering progress towards SDG 4 (education) and SDG 5 (gender equality) (Strunk *et al.*, 2024; WHO, 2024).

AMR directly affects SDG 6 (clean water and sanitation) since poor hygiene, untreated wastewater, and limited access to safe water facilitate the spread of resistant pathogens (UNEP, 2023). Meeting anticipated food demand with an estimated 50–70% production increase from 2010 to 2030 risks a parallel rise in antimicrobial use in livestock and aquaculture, threatening sustainable food systems and farmer livelihoods, and thereby SDG 2 (zero hunger) and SDG 12 (responsible consumption and production) (Aslam *et al.*, 2024). The association between global climate change (Goal 13) and AMR is also significant, while a 1°C rise in ambient temperature has been associated with a 5–10% increase in foodborne infections, incurring substantial healthcare and economic costs (Scallan *et al.*, 2011). AMR also threatens the sustainability of aquatic and terrestrial ecosystems (SDG 14 and 15) through environmental contamination and antimicrobial overuse (WHO, 2024). Moreover, addressing AMR requires sustained international cooperation under a One Health approach, which aligns with SDG 17 (partnerships for the goals) (Aslam *et al.*, 2024).

- 1 – No poverty
- 2 – Zero hunger
- 3 – Good health and well-being
- 4 – Quality education
- 5 – Gender equality
- 6 – Clean water and sanitation
- 7 – Affordable and clean energy
- 8 – Decent work and economic growth
- 9 – Industry, innovation and infrastructure
- 10 – Reduced inequalities
- 11- Sustainable cities and communities
- 12 – Responsible consumption and production
- 13 – Climate action
- 14 – Life below water
- 15 – Life on land
- 16 – Peace, justice and strong institutions
- 17 – Partnership of the goals

Figure 1.4 SDGs relevant to AMR (adapted from United Nations, 2015). Arrows indicate the SDGs intersect with AMR. Goals addressed about AMR are highlighted in red text.

1.1.5 A One Health approach to combat AMR

One Health is an integrated approach that aims to sustainably improve the health of humans, animals, plants, and ecosystems by recognising their deep interdependence and coordinating actions across sectors such as human and veterinary medicine, agrifood systems, and the environment. Although initially introduced to address zoonotic diseases, this concept has since been proposed to combat more complex problems like AMR (Walsh, 2018).

To address AMR globally, the quadripartite partners (World Health Organisation, WHO; Food and Agriculture Organisation, FAO; World Organisation for Animal Health, WOAH/formerly OIE; and United Nations Environment Programme, UNEP) have all advocated for incorporating One Health principles into national and global AMR action plans. This concept is formalised in the One Health

Joint Plan of Action (2022–2026)**,** emphasising coordinated multisectoral actions to tackle AMR. This approach builds on the WHO Global Action Plan on AMR (2015) by incorporating antimicrobial stewardship (AMS, the coordinated optimisation of antimicrobial use to improve patient outcomes and limit antimicrobial resistance) and infection prevention and control (IPC, measures implemented to prevent and reduce the transmission of infectious agents, particularly in healthcare settings) in both humans and animals. It also includes WASH (water, sanitation, and hygiene) and waste management, integrated surveillance of resistance and antimicrobial usage (AMU, the prescribing, consumption, and patterns of use of antimicrobial agents in clinical, community, and agricultural settings), and aligned governance, regulation and incentives across sectors (OECD, 2023).

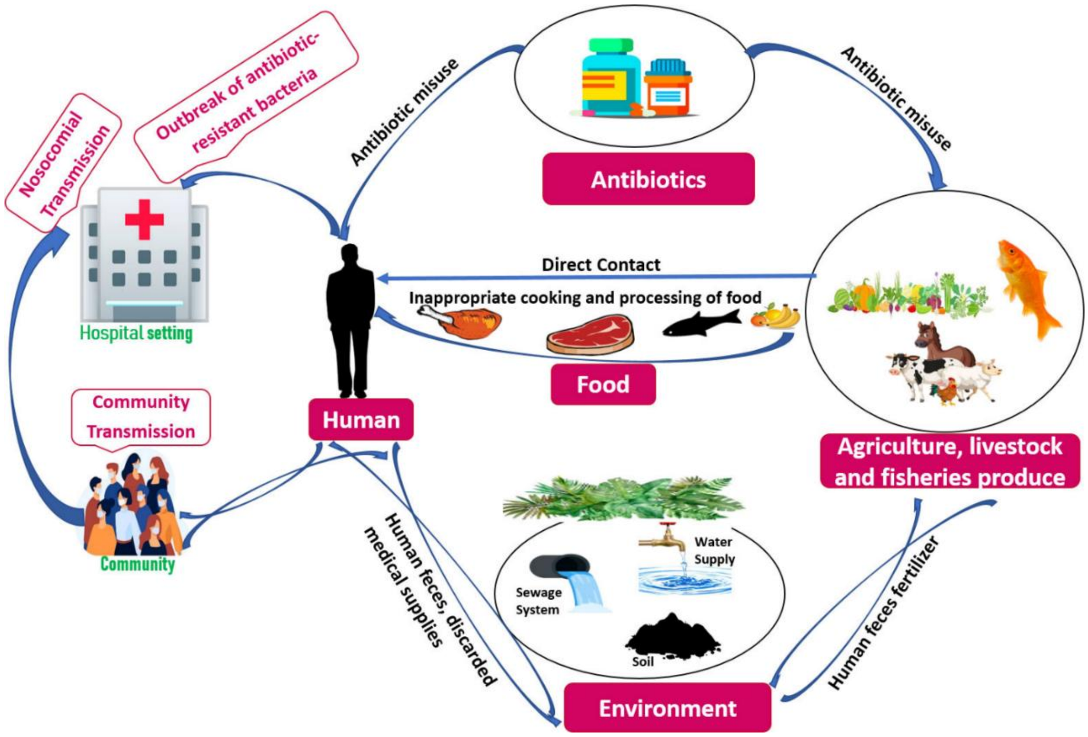


Figure 1.5 A potential schematic for the complex AMR interactions across the One Health sectors (Ahmad *et al*., 2023).

AMR presents a global health challenge that affects not only humans but also animals and the environment. Resistant pathogens and their genes can transfer between humans, animals, and the environment through various pathways, including food chains, direct contact, and contaminated water sources (Figure 1.5) (Ahmad *et al*., 2023). The shift in the global AMR landscape has also been marked by the rise of multidrug-resistant (MDR) bacteria (resistant to ≥3 antimicrobial classes), especially *Enterobacteriaceae* (Naghavi *et al*., 2024). Within this landscape, *Klebsiella* has emerged as a globally important pathogen due to its capacity to disseminate AMR through mobile genetic elements (MGEs, genetic elements capable of mobilising within or between genomes, thereby facilitating HGT), driven largely by extended-spectrum β-lactamases (ESBLs, enzymes produced by certain Gram-negative bacteria that hydrolyse and confer resistance to a broad range of β-lactam antibiotics), notably cefotaximase-M (CTX-M), carbapenemases (KPC, NDM, OXA-48-like), and last-line colistin resistance (Wyres & Holt, 2018; Gürbüz *et al*., 2024).

1.2 An order, a family, a genus: Locating *Klebsiella* in Enterobacterales

Enterobacterales is a large order of Gram-negative, facultatively anaerobic bacteria within the class Gammaproteobacteria, under the phylum Pseudomonadota. In 2016, a genome-based phylogenetic study replaced the order 'Enterobacteriales' with 'Enterobacterales' and clarified family-level evolutionary relationships using conserved signature insertions and deletions (signature indels), which are specific, shared insertions and deletions in conserved protein sequences that serve as molecular markers of common ancestry. Based on these markers, seven families (*Enterobacteriaceae*, *Morganellaceae*, *Yersiniaceae*, *Erwiniaceae*, *Pectobacteriaceae*, *Hafniaceae* and *Budviciaceae*) were recognised (Adeolu *et al*., 2016; Oren & Garrity, 2021).

Among the seven families, *Enterobacteriaceae* is regarded as one of the most widely studied bacterial families. Although there are many exceptions, the common characteristics of the members of this family include being rod-shaped, non-spore forming, often motile, catalase-positive and oxidase-negative, reducing nitrate to nitrite, and glucose fermenters. Ecologically, members of this family are ubiquitous, and many occur as part of the normal intestinal microbiota in humans and animals; hence, they are referred to as "enterobacteria" or "enteric bacteria". They also occur widely in soil, water, plants, and other environmental niches. Most of them are mesophiles with optimal growth at 20–45°C and rarely thrive in extreme temperature or pH conditions (Octavia & Lan, 2014; Jenkins *et al*., 2017; Yin *et al*., 2025).

The *Enterobacteriaceae* family is one of the most taxonomically diverse bacterial families, which contains more than 60 genera and 250 species (Parte, 2014; Adeolu *et al*., 2016). Although many species reside as commensals to humans and animals, *Enterobacteriaceae* are frequently associated with both intestinal and extraintestinal diseases and can develop fatal infections. Within this family, above 95% of clinically significant species belong to ten well-known human and animal-associated genera (*Escherichia, Klebsiella, Salmonella, Shigella, Enterobacter, Citrobacter, Proteus*, *Serratia, Morganella, Providencia*). Within *Enterobacteriaceae*, *Klebsiella* is considered one of the most important genera, which stands out for its dual identity as a frequent coloniser of the human and animal guts that can also cause severe diseases and a genetically diverse lineage that readily acquires virulence and AMR determinants (Donnenberg, 2014; Kang *et al*., 2018; Cabrera *et al*., 2025).

The genus *Klebsiella* was named by V. Trevisan in 1885 in honour of German Swiss microbiologist Edwin Klebs (1834–1913) (Brisse *et al*., 2006). *Klebsiella* consists of Gram-negative, non-motile, typically encapsulated, facultatively anaerobic, rod-shaped bacteria (0.3–1.5 µm wide × 0.5-5.0 µm long), found singly, in pairs, in chains, or linked end to end. They can grow in standard laboratory media with special requirements, optimally between 35-37°C and at pH 7.2 (Ristuccia & Cunha, 1984; Dong *et al*., 2022). *Klebsiella* are widely distributed in nature and recovered from humans, animals, soil, water, plants, and insects. Although the members of this genus are usually present as normal flora in the nose, mouth, and intestines of humans and other warm-blooded animals, they are considered significantly opportunistic pathogens connected with several severe nosocomial bacterial infections, including pneumonia, urinary tract infections, sepsis, meningitis, diarrhoea, peritonitis, and soft tissue infections. Their ability to colonise human and animal intestines, persist in diverse environmental reservoirs, and cause severe infections in both community and healthcare settings makes the genus suitable for AMR studies through the One Health approach (Brisse *et al*., 2006; Wyres *et al*., 2020; Calland *et al*., 2023).

1.3 Species composition of the genus *Klebsiella*

The genus *Klebsiella* is genetically diverse and currently comprises more than 15 recognised species, including species belonging to the *K. pneumoniae* species complex (KpSC), *K. oxytoca* species complex (KoSC) and other *Klebsiella* species (*K. aerogenes*, *K. indica*, *K. terrigena*, *K. ornithinolytica*, *K. planticola*, *K. electrica*) that share an average of around 90% nucleotide identity (Figure 1.6) (Wyres *et al*., 2020; Yang *et al*., 2021). *K. terrigena*, *K. ornithinolytica*, *K. planticola* were moved from *Klebsiella* to *Raoultella* in 2001 (*R. terrigena*, *R. ornithinolytica*, *R. planticola*). However, a later genome-wide analysis proposed reunifying *Raoultella* with *Klebsiella* and specifically classified *R. electrica* as *K. electrica* (Wyres *et al*., 2020; Ma *et al*., 2021).

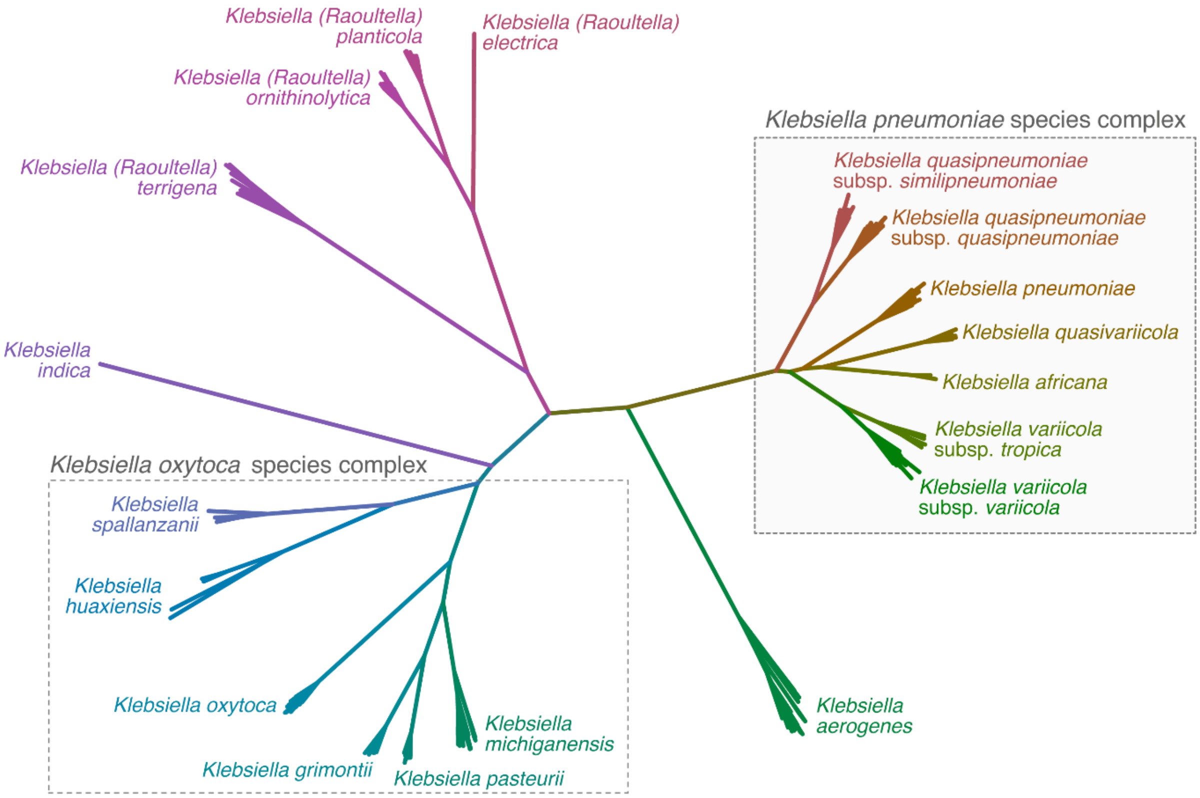


Figure 1.6 Core-genome-based tree showing the phylogenetic relationships between *K. pneumoniae* and related species. This figure was taken from the Kleborate module for KpSC (Kleborate Modular v.3.0.0 documentation), which was updated from previously published literature (Wyres *et al*., 2020; Wick *et al*., 2024).

KpSC and KoSC have no formal taxonomic designation. KpSC commonly refers to closely related species with an average nucleotide identity of 95-96% to *K. pneumoniae*. Three distinct phylogroups, namely *K. pneumoniae* (KpI), *K. quasipneumoniae* (KpII) and *K. variicola* (KpIII) were initially identified (Holt *et al*., 2015). To date, seven phylogroups belonging to KpSC have been recognised, including *K. pneumoniae* (Kp1) and *K. quasipneumoniae* subsp. *quasipneumoniae* (Kp2), *K. variicola* subsp. *variicola* (Kp3), *K. quasipneumoniae* subsp. *similipneumoniae* (Kp4), *K. variicola* subsp. *tropica* (Kp5), *K. quasivariicola* (Kp6) and *K. africana* (Kp7) (Lam *et al*., 2021). KoSC is likewise an informal complex which includes at least six closely related species, including *K. oxytoca*, *K. michiganensis*, *K. pasteurii*, *K. grimontii*, *K. huaxiensis*, and *K. spallanzanii* (Yang *et al*., 2021). Due to close genetic relatedness, members of KpSC or KoSC are often misidentified by conventional biochemical tests or earlier PCR (polymerase chain reaction) schemes (Fontana *et al*., 2019). *K. variicola* or *K. quasipneumoniae* are frequently reported as *K. pneumoniae* by routine tests. Accurate species assignment matters as ecological niches, virulence and AMR determinants can differ among KpSC members. For instance, *K. variicola* is often associated with plants and animals while also causing human diseases. Recent identification methods therefore rely on genome-based approaches (e.g., whole genome sequencing, WGS) and a mass spectrometry platform (MALDI-TOF, matrix-assisted laser desorption/ionisation time-of-flight) with updated libraries for accurate taxonomy (Rodrigues *et al*., 2018; Lam *et al*., 2021; Ohama *et al*., 2022).

Advances in WGS and associated bioinformatic tools have become central to the species-level identification and genotypic characterisation of *K. pneumoniae* and related species. Short-read sequencing platforms (e.g., Illumina) are widely used for routine surveillance due to their high accuracy and throughput, while long-read technologies (e.g., Oxford Nanopore Technologies) facilitate complete genome and plasmid reconstruction. Genomic data are typically analysed using standard bioinformatic pipelines that include quality control, genome assembly, and annotation, followed by in silico genotyping approaches (e.g., multilocus sequence typing, MLST and core-genome phylogenetics), and the identification of resistance, virulence determinants, plasmid replicons using curated databases and tools (e.g. ResFinder, VirulenceFinder, PlasmidFinder, Kleborate, Kaptive). These combined approaches provide the high resolution required to identify specific species, high-risk clones, characterise resistance and virulence profiles, and to support source-attribution and outbreak investigations (Wyres & Holt, 2018; Wyres *et al*., 2020; Wick *et al*., 2024).

Within *Klebsiella*, *K. pneumoniae* is considered the most clinically important species, and the WHO has recognised it as a critical priority healthcare-associated pathogen (Tacconelli *et al*., 2018; Thorpe *et al*., 2022). *K. pneumoniae* is accountable for more than 70% of the infections due to the *Klebsiella* genus and is a vital menace to public health because of its resistance and virulence profile (Holt *et al*., 2015; Quansah *et al*., 2019). Beyond hospitals, *K. pneumoniae* can colonise humans, livestock, and companion animals, and is recovered from the food chain, surface water, hospital wastewater, soil and insect vectors such as flies, underscoring its ecological plasticity and the likelihood of cross-sector transmission (Thorpe *et al*., 2022). These features make *K. pneumoniae* a good candidate for understanding how clonality, virulence and AMR interact at the human-animal-environment interface.

1.4 *K. pneumoniae*: a One Health AMR threat

1.4.1 Ecology, disease spectrum and transmission of *K. pneumoniae*

K. pneumoniae (previously called Friedlander's bacillus) was first described by Carl Friedlander in 1882 as an encapsulated, non-motile, rod-shaped organism and the causative agent of pneumonia. This bacterium can either be a commensal organism or a potential pathogen. It is usually an opportunistic, hospital-associated pathogen, responsible for about one-third of all Gram-negative infections (Navon-Venezia *et al*., 2017). Classical and hypervirulent, these two principal pathotypes are commonly associated with *K. pneumoniae* infections. Classical *K. pneumoniae* (cKP) strains are often multidrug-resistant and primarily associated with hospital-acquired infections (HAIs), where the common manifestations are pneumonia, urinary tract infections (UTIs), and wound infections, which can progress to bloodstream infections. Neonates, older adults, patients with indwelling medical devices, and the immunocompromised are at the highest risk of HAIs. Unlike cKP, hypervirulent *K. pneumoniae* (hvKP) shows increased pathogenicity and is capable of causing severe, rapidly progressing, community-acquired infections (CAIs), with the common manifestations including necrotising pneumonia, endophthalmitis, soft tissue infections, meningitis, and pyogenic liver abscess with metastatic spread. The ability to cause metastatic infections is characteristic of hvKP infections. People with alcoholism and diabetes are at risk of developing CAIs (Russo & Marr, 2019; Wyres *et al*., 2020; Kochan *et al*., 2023; Russo *et al*., 2024).

Apart from humans, *K. pneumoniae* can cause clinically important diseases in multiple livestock species and companion animals (Figure 1.7). In dairy cattle, it is a well-recognised cause of acute mastitis, where contaminated bedding and milking equipment are common sources (Zheng *et al.,* 2022). In horses, it can cause infectious endometritis, leading to infertility (Song *et al.,* 2023). In poultry, *K. pneumoniae* is associated with upper respiratory tract infections and septicaemia (Alchalaby *et al.,* 2024; Scholtz *et al.,* 2024). In pigs, it can cause pneumonia, meningitis, enteritis and septicaemia (Bowring *et al*., 2017; Collins & Mizzi, 2025). In companion animals (dogs and cats), *K. pneumoniae* can cause a broad spectrum of disease, including UTIs, pneumonia, pyometra, soft tissue infections and septicaemia (Marques *et al*., 2019; Soliman *et al*., 2024).

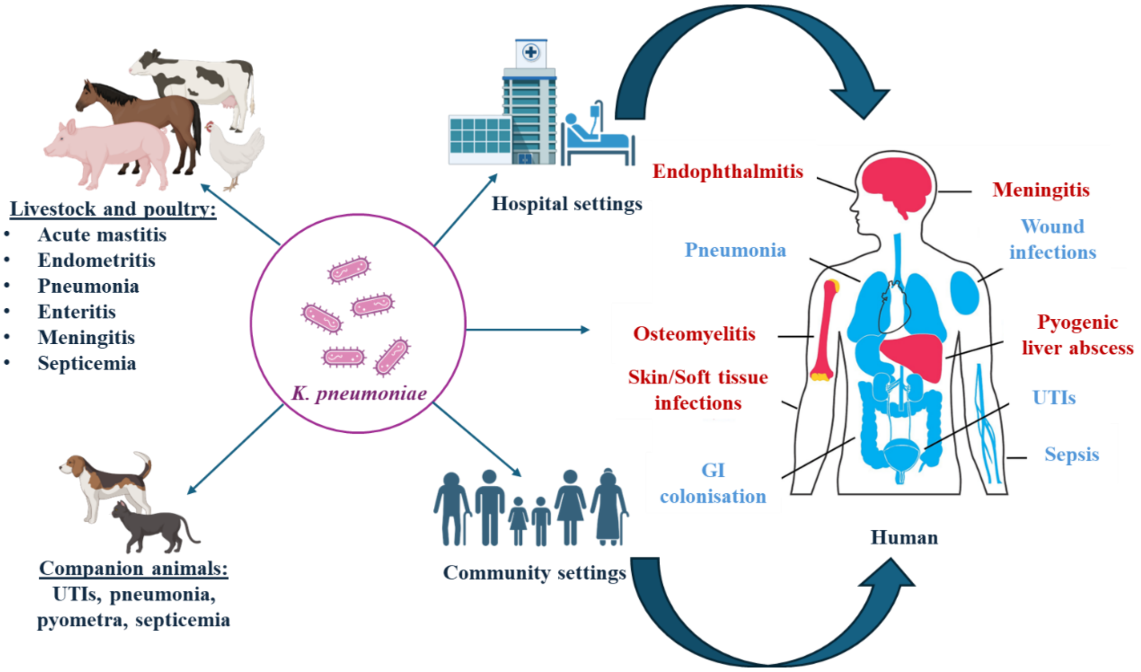


Figure 1.7 Clinical and veterinary disease spectrum of *K. pneumoniae* (updated from Assoni *et al*., 2024). In case of human infection sites, the blue colour indicates the infections caused by cKp strains,

while diseases commonly associated with hvKP are marked in red. The figure was partly created with BioRender.com.

K. pneumoniae can survive in various ecological niches and its transmission reflects this ecological diversity (Figure 1.8). Within healthcare settings, *K. pneumoniae* can spread quickly between hosts through direct contact (person-to-person) via contaminated hands of healthcare personnel or patients, as well as via contaminated devices (e.g., ventilators or intravenous catheters) and surfaces. Patients with medical conditions such as wounds (caused by injury or surgery) can be exposed to *K. pneumoniae*, leading to serious nosocomial infections and outbreaks (Gorrie *et al*., 2017). *K. pneumoniae* typically exhibits a higher transmission rate than *Escherichia coli*, especially in healthcare settings, because of its ability to persist in the environment and form biofilms (Wyres & Holt, 2018). Outside the hospital, environmental contamination can play an essential role in community transmission. Foodborne transmission routes, such as contaminated food and food products, can lead to human colonisation and infections. Occupational exposure can affect farmers, veterinarians, and animal handlers due to close contact with colonised animals (Zong *et al*., 2021). *K. pneumoniae* is frequently recovered from water, soil, sewage, and hospital wastewater, likely owing to contamination by human or animal wastes. Contaminated water sources, agricultural lands, insects and flies can serve as a medium for the persistence and spread of this bacterium (Hassan *et al.,* 2021; Zhang *et al.,* 2023; Cook *et al.,* 2025).

In recent years, the rise in difficult-to-treat MDR strains has become a global concern, particularly third-generation cephalosporins (3GCs, a class of broad-spectrum β-lactam antibiotics with enhanced activity against Gram-negative bacteria relative to earlier generations) and carbapenem-resistant *K. pneumoniae* (CR-KP, strains resistant to carbapenem antibiotics, which are last-line β-lactams) associated with hospital outbreaks and hypervirulent strains associated with severe CAIs (Holt *et al*., 2015). Highlighting the urgency of this issue, the WHO prioritised *K. pneumoniae* listing in the ESKAPE pathogen group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which are responsible for the majority of HAIs worldwide and emphasised the need for new antimicrobial development (Pendleton *et al*., 2013; WHO, 2017; Wyres & Holt, 2018). Most recently, the WHO BPPL (bacterial priority pathogen list) 2024 ranked CR-KP as the top priority bacterial threat globally (Sati *et al*., 2025).

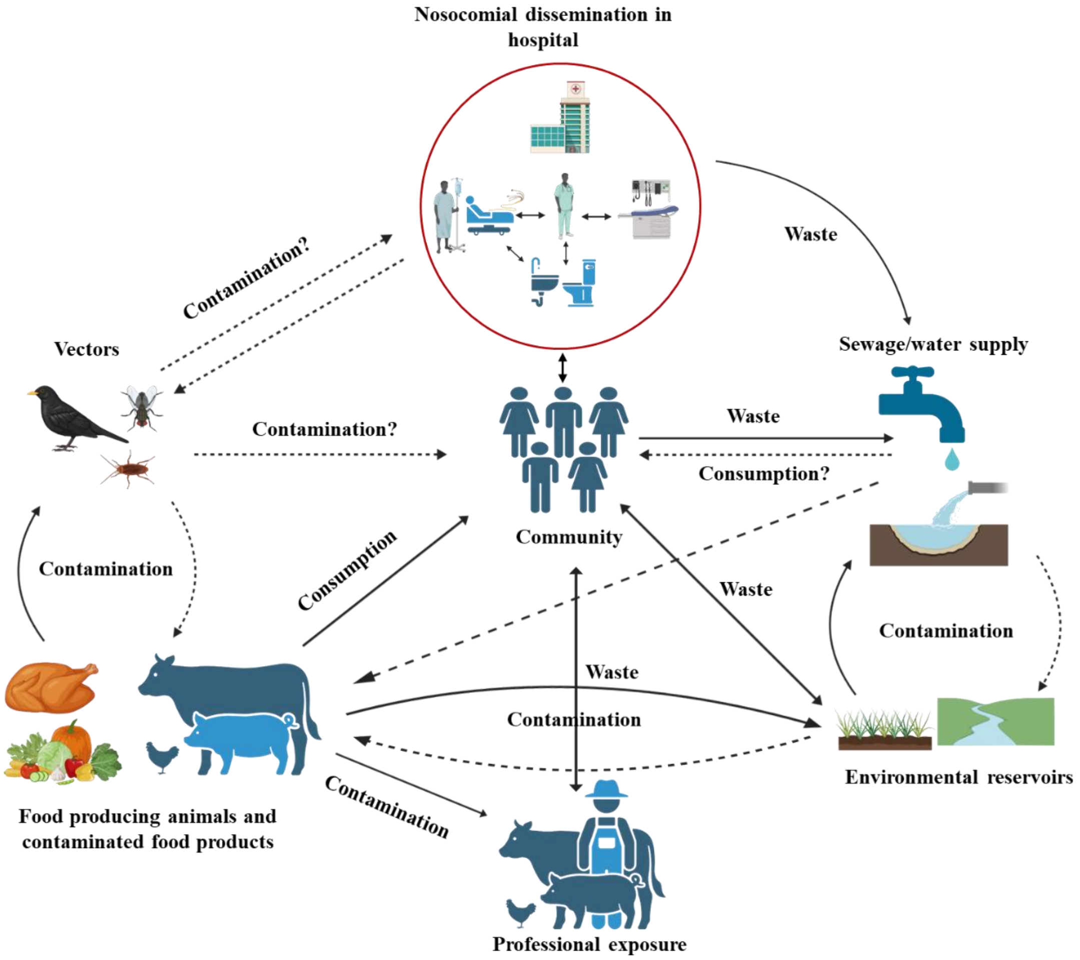


Figure 1.8 A potential schematic for transmission pathways of *K. pneumoniae* between human, environmental, agricultural and clinical reservoirs (updated from Walsh, 2018; Chawla *et al*., 2025). Dashed lines indicate putative transmission paths. The figure was created with BioRender.com.

1.4.2 The virulence repertoire of *K. pneumoniae*

K. pneumoniae deploys various strategies to evade the host immune defences and establish infection (Figure 1.9). The pathogenic potential of *K. pneumoniae* in human and animal hosts is shaped by multiple virulence determinants. At present, four main classes of well-characterised virulence factors are recognised in *K. pneumoniae*. These factors are the capsule, lipopolysaccharide (LPS), fimbriae (also known as pili), and siderophores. Capsular polysaccharides (K antigen), along with the O antigen

(LPS), confer resistance to host immune cells. Fimbriae (predominantly type 1 and 3) are the adhesive organelles that drive biofilm formation and attachment to abiotic surfaces (e.g., catheters or other medical devices), thereby enabling colonisation of host cells and persistence in patients.

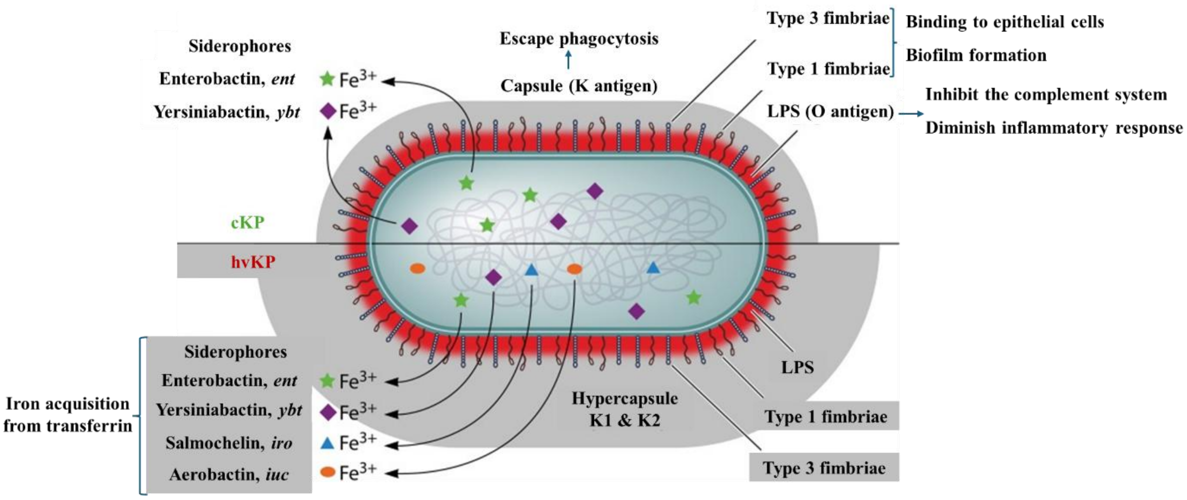


Figure 1.9 Schematic presentation of well-characterised virulence factors in *K. pneumoniae* strains (cKP and hvKP) (updated from Paczosa & Mecsas, 2016).

In *K. pneumoniae*, four siderophores have been described: enterobactin (*ent*), yersiniabactin (*ybt*), salmochelin (*iro*), and aerobactin (*iuc*), which are utilised for iron acquisition from host cells, a vital process for its pathogenesis (Figure 1.9). Apart from these canonical factors, several other factors (not yet thoroughly characterised) have been identified as important for *K. pneumoniae* virulence. These include outer membrane proteins (e.g., OmpA), the major porins (OmpK35 and OmpK36), the *acrAB* efflux system, the *kfu* iron transport systems, genes enabling allantoin catabolism for carbon and nitrogen acquisition, and biofilm formation (Paczosa & Mecsas, 2016; Piperaki *et al*., 2017; G. Wang *et al*., 2020; Wyres *et al*., 2020; Xu *et al*., 2024).

All *K. pneumoniae* (both cKP and hvKP) possess a group of chromosomally encoded virulence factors that underpin their basic ability to establish opportunistic infection in hosts. These include the core *ent* locus for enterobactin biosynthesis, *fim* and *mrk* loci for type 1 and type 3 fimbriae, together with variable K antigen and O antigen biosynthesis loci, required for growth in most niches (Wyres *et al*., 2020). A chromosomal operon called the *cps* gene cluster harbours several genes (*wzi, wza, wzb, wzc,* *wzx, wzy, gnd, wca, cpsACP,* and *galF*) involved in capsule production (Pan *et al*., 2015; Wyres *et al*., 2020). So far, at least 186 genetically distinct capsule types (K locus) have been identified, while classical serology recognises 77 K serotypes (K1–K77). Different serotypes exhibit significant variations in virulence traits. For instance, K1 and K2 serotypes are found to be significantly associated with invasive hvKP infections worldwide (Xu *et al*., 2024). In *K. pneumoniae*, O antigen biosynthesis is mainly encoded at O loci (also known as *rfb* loci) and proceeds via an ABC transporter pathway where *wzm* and *wzt* genes mediate export of the assembled unit. Genes involved in LPS production also include *uge*, which is present in the majority of *K. pneumoniae* isolates (both clinical and commensal strains), while *wabG* is likely to be mainly present in clinical isolates. To date, 13 distinct O loci have been identified, where O1 and O2 serotypes are most commonly found in *K. pneumoniae* isolates (Paczosa & Mecsas, 2016; Wyres *et al*., 2020; Lam *et al*., 2022).

In addition to core chromosomal pathogenicity factors, *K. pneumoniae* harbours various virulence factors encoded by accessory genes and mobile genetic elements, which can exacerbate disease severity. Unlike the *ent* locus, *ybt*, *iuc* and *iro* are typically acquired siderophore loci associated with enhanced virulence. The *ybt* gene is associated with both cKP and hvKP populations. In *K. pneumoniae*, the *ybt* locus is usually mobilised by the integrative conjugative elements (ICEs), but occasionally also by plasmids (Lam *et al*., 2018). The *iuc* and *iro* loci occur at a low prevalence (in <10% of *K. pneumoniae*) and are mainly disseminated through large virulence plasmids. For example, pK2044 (*K. pneumoniae* virulence plasmid, KpVP-1) carries *iuc1* and *iro1*, while Kp52.145pII (KpVP-2) carries *iuc2* and *iro2*. These plasmids typically cannot transfer on their own but can be mobilised between bacterial cells via other conjugative elements or by fusing with other conjugative plasmids. Although *iro* is most often plasmid-borne, certain lineages (*iro3*) can be carried on ICEs (Lam *et al*., 2018, 2019).

The *clb* (*pks*) locus encoding colibactin (a genotoxin), first described in *E. coli*, is found in approximately 10% of *K. pneumoniae* and is typically embedded in ICEs. Colibactin promotes gastrointestinal colonisation and metastatic spread, and its presence is an indicator of liver abscess with hvKP (Wyres *et al*., 2020; Tan *et al*., 2024). Another important virulence trait in *K. pneumoniae* is hypermucoviscosity, often driven by excess capsule production due to the presence of accessory genes *rmpA* or *rmpA2* (regulator of mucoid phenotype). The *rmpA* and *rmpA2* loci are frequently found adjacent to *iro* or *iuc* on the virulence plasmids but can also occur on ICEs. Importantly, hypermucoviscosity is not always strictly synonymous with capsule overproduction and can be genotypically and phenotypically uncoupled in some strains. Phenotypically, hypermucoviscosity is characterised by sticky growth and identified by a positive string test (production of a ≥5 mm viscous filament when a colony is stretched with an inoculation loop) (Walker *et al.*, 2019; Walker & Miller, 2020; Wyres *et al.*, 2020; Dey *et al.*, 2022).

The cKP strain generally exhibits lower virulence potential with the presence of basic virulence factors such as fimbriae, diverse K antigens, O antigens, the primary iron uptake system *ent* and sometimes *ybt*. On the contrary, hvKP is best characterised as a highly virulent pathogen and can be distinguished from cKP in many aspects (Table 1.1). The hvKP has emerged as a distinct pathotype since it was first reported in Taiwan in the mid-1980s and is now reported globally (Dong *et al.*, 2022). The hvKP infections are clinically characterised by severe invasive, metastatic CAIs. Although early differentiation leaned on the hypermucoviscous phenotype (identified by the string test) and K1/K2 capsule types, recent definitions prioritise the genomic criteria. Besides the core virulence determinants and *ybt*, few hypervirulence biomarkers (*iucA, iroB*, *rmpA*, *rmpA2* and *peg-344*), virulence plasmids, and *clb*, have been identified for the differentiation between hvKp and cKP. Within these, *iucA* and *rmpA/rmpA2* carried on plasmids are recognised as the most predictive hypervirulence biomarkers (Choby *et al.*, 2019; Russo & Marr, 2019; Wyres *et al.*, 2020; Spadar *et al.*, 2022; Lei *et al.*, 2024; Stanton & Wyres, 2024). Additionally, animal models can be used to validate suspected hvKP strains with a preference for the murine model over the *Galleria mellonella* model (Russo & MacDonald, 2020; Dong *et al.*, 2022).

Table 1.1 General differential features between hvKP and cKP strains. (updated from Paczosa & Mecsas, 2016; Choby *et al*., 2019; Russo & Marr, 2019; Dong *et al*., 2022; Wyres *et al*., 2020; Kochan *et al*., 2023; Lei *et al*., 2024)

Features	**hvKP**	**cKP**
Location for the development of infection	Primarily in the community	Primarily in the healthcare settings
Hosts	All age groups, often in a healthy population	Neonates, older adults and immunocompromised
Geographical concentration	Primarily in Taiwan and Southeast Asia, now reported globally	Worldwide
Typical clinical syndromes	Pyogenic liver abscess, endophthalmitis, meningitis and metastatic infections	Pneumonia, UTIs, wound infections, device-associated infections and septicaemia
Sites of infection	Often multiple	Mostly single
Co-pathogens at the site of infection	Usually, monomicrobial	Can be polymicrobial
Key hypervirulence markers	Harbours hypervirulence genes such as *iucA, iroB*, *rmpA*, *rmpA2*, *peg-344*, and virulence plasmids	Lacks the canonical hvKP marker and carries core virulence genes only (e.g., *ent*, sometimes *ybt* via ICEs)
Capsule (K) types	Strongly associated with K1 and K2	Diverse K loci
Virulence potential	High	Low
Antimicrobial resistance	Historically, more susceptible	High propensity for MDR

1.4.3 Evolution of AMR in *K. pneumoniae*

In the present era of antibiotic resistance, MDR *K. pneumoniae* represents one of the most concerning pathogens worldwide. *K. pneumoniae* readily acquires AMR determinants via plasmids, integrons and transposons (Figure 1.10). It is also recognised as a key trafficker of AMR genes between *Klebsiella* species and other Enterobacterales due to the abundance of AMR genes carried on plasmids and mobile genetic elements, signifying its importance in the spread and development of AMR (Wyres & Holt, 2018). The European Antimicrobial Resistance Surveillance Network reported that the resistance rate of *K. pneumoniae* against the four major antibiotic classes (the 3GCs, aminoglycosides, fluoroquinolones and carbapenems) was higher than that of *E. coli* (Navon-Venezia *et al*., 2017).

Moreover, resistance to all important drug classes (β-lactams, aminoglycosides, quinolones, tigecycline and polymyxins) used to treat *K. pneumoniae* infections has been observed clinically (Wyres *et al*., 2020). In *K. pneumoniae*, resistance to antibiotics occasionally arises through chromosomal mutation, but mostly results from acquisition of AMR genes via horizontal gene transfer (HGT). More than 100 distinct acquired AMR genes have been identified in *K. pneumoniae*, mainly carried by plasmids (Wyres & Holt, 2016).

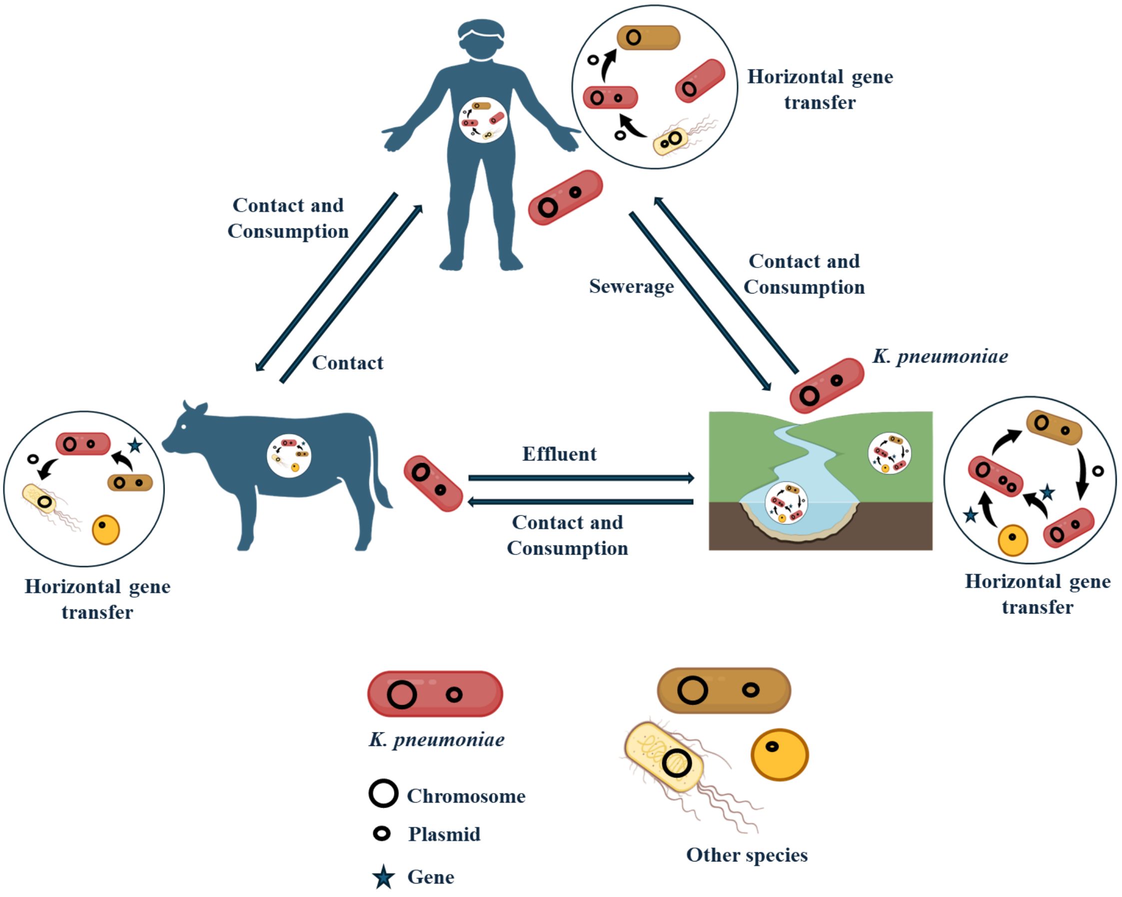


Figure 1.10 Schematic presentation of AMR gene and plasmid trafficking by *K. pneumoniae* (updated from Wyres & Holt, 2018). *K. pneumoniae* strains can move between environmental, human and animal niches, carrying AMR genes and/or plasmids. Movement can occur from the environment to hosts via contact or consumption of contaminated water sources or plant matter, between human and animal hosts via contact or consumption, and back to the environment via effluent and sewerage (including human and animal waste streams (e.g., wastewater and effluent from households, healthcare facilities, and livestock operations). Across all these settings, *K. pneumoniae* can both receive or donate plasmids via

HGT with a wide range of donor species, providing routes for transfer of AMR genes from environmental microbes to human pathogens. The figure was created with BioRender.com.

K. pneumoniae is naturally resistant to ampicillin (β-lactam) due to production of the chromosomal β-lactamase enzyme, sulfhydryl variable (SHV) by the core gene $\mathit{bla}_{\text{SHV-1}}$. Resistance to β-lactam in *K. pneumoniae* was reported in the early 1960s, with the discovery of the first β-lactamase genes, $\mathit{bla}_{\text{SHV-1}}$ and $\mathit{bla}_{\text{TEM-1}}$. Subsequently, the first extended-spectrum β-lactamase (ESBL) gene, $\mathit{bla}_{\text{SHV-2}}$, was identified in *K. pneumoniae* from Germany in the 1980s, with an extended-spectrum activity against third-generation cephalosporins and monobactams (Kliebe *et al*., 1985). Shortly after that, another plasmid-mediated ESBL variant, $\mathit{bla}_{\text{TEM-3,}}$ was reported in France (Sirot *et al*., 1987). During the 1980s-1990s, *K. pneumoniae* was the principal hospital host for plasmid-mediated ESBLs and harboured TEM (Temoniera) and SHV-type ESBLs. In the 1990s, a new ESBL family known as the cefotaximase-M (CTX-M) emerged, conferring resistance to expanded-spectrum cephalosporins. About 200 ESBL variants have been identified since their first identification in 1982, and many of them subsequently transferred to *E. coli* (Navon-Venezia *et al*., 2017; Piperaki *et al*., 2017; Wyres & Holt, 2018). In parallel, the plasmid-mediated AmpC appeared in *K. pneumoniae* (e.g., CMY-1 in 1989; later ACC-1, ACT, DHA variants) (Jacoby, 2009). As ESBL producers expanded, carbapenems became the first-line treatment against *K. pneumoniae* infections. The extensive use of carbapenems led to the emergence and rapid dissemination of plasmid-mediated carbapenemases in *K. pneumoniae*, which hydrolyse all β-lactams, including the last-line carbapenems, posing a significant public health threat. CR-KP have now been reported in all WHO regions. The prevalent carbapenemase types in *K. pneumoniae* are the *K. pneumoniae* carbapenemases (KPCs), the metallo-β-lactamases (Verona integron-encoded metallo-β-lactamase, VIM; imipenemase, IMP; and New Delhi metallo-β-lactamase, NDM types such as NDM-1) and the oxacillinase (OXA)-type enzymes (mainly OXA-48-like) (Tzouvelekis *et al*., 2012). The earliest detected carbapenemase in *K. pneumoniae* was IMP-1 in 1991 in Japan, followed by $\mathit{bla}_{\text{KPC}}$ in 1996 in the US, $\mathit{bla}_{\text{OXA-48}}$ in 2003 in Turkey, and $\mathit{bla}_{\text{NDM-1}}$ in 2008 from a patient who travelled to India (Figure 1.1) (Haruta *et al*., 2000; Yigit *et al*., 2001; Poirel *et al*., 2004; Yong *et al*., 2009). Subsequently, other variants were also reported. Besides enzyme production, carbapenem resistance also occurs via permeability or efflux changes, such as permeability alterations due to porin loss (e.g., loss of OmpK35/OmpK36) and overexpression of efflux pumps (e.g., *acrAB* efflux pump). Furthermore, carbapenemase genes can become chromosomally integrated, stabilising resistance (e.g., mobilisation of determinants like *bla*$_{\text{NDM-1}}$, *bla*$_{\text{VIM-1}}$ from plasmids onto the chromosome via mobile genetic elements). The global spread of the ESBL gene *bla*$_{\text{CTX-M-15}}$, along with carbapenemase genes *bla*$_{\text{KPC}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{NDM-1}}$, and *bla*$_{\text{NDM-5,}}$ poses a major threat due to their clinical significance (Navon-Venezia *et al*., 2017; Sakamoto *et al*., 2018; Wyres & Holt, 2018).

Aminoglycosides dominated therapeutic use from the mid-1940s to the 1980s before being replaced with third-generation cephalosporins, carbapenems and fluoroquinolones (Krause *et al*., 2016). During that period, *K. pneumoniae* developed resistance through aminoglycoside modification enzymes, inactivating the drugs by acetylation, adenylation or phosphorylation. Subsequently, plasmid-mediated resistance genes from *aac*, *ant*, *aad*, *aph* families, as well as 16S rRNA methyltransferases such as *arm* and *rmtB* were identified in *K. pneumoniae* (Figure 1.11). Among gentamicin- or amikacin-resistant *K. pneumoniae*, *aac (6')-Ib* is the most frequently found, followed by *aac (3')-II, aph (3')-IV*, and *ant (3')-I* (El-Badawy *et al*., 2017). Chromosomal resistance mechanisms also play a role, including reduced cell permeability and active efflux (alterations in *acrAB-tolC* and *kpnEF* efflux pump systems and loss of putative porin, kpnO), as well as aminoglycoside-modifying enzyme genes (Padilla *et al*., 2010; Wang *et al*., 2020).

Quinolones were introduced into clinical use in the 1960s, but their use expanded markedly with the advent of fluoroquinolones in the 1980s, leading to the development of quinolone resistance. *K. pneumoniae* encompasses all known resistance mechanisms for quinolone resistance in Gram-negative bacteria (Naeem *et al*., 2016). The mechanisms include target-site gene mutations, enhanced activity of MDR efflux systems and drug-modifying enzymes (Redgrave *et al*., 2014). The dominant resistance mechanism in *K. pneumoniae* is chromosomal mutations in the quinolone binding targets, DNA gyrase (*gyr*A-*gyr*B) and topoisomerase IV (*par*C-*par*E), with mutations in *parC* and *gyrA* found earlier and more commonly than those in *gyrB* and *parE* (Nam *et al*., 2013). Efflux pump upregulation (notably *oqxAB* and *acrAB-tolC*) also promotes quinolone resistance. The *oqxAB*, although chromosomal in origin, can also occur in plasmids and contributes to plasmid-mediated quinolone resistance (PMQR) (Wong *et al*., 2015). PMQR determinants in *K. pneumoniae* include the *qnr* genes (such as *qnrA*, *qnrB, qnrS*, *qnrD*), first described on a plasmid from *K. pneumoniae* isolated in the USA in 1994 (Surleac *et al*., 2020). Another PMQR gene, the aminoglycoside acetyltransferase variant *aac(6')-Ib-cr*, can modify certain quinolones and decrease susceptibility (Figure 1.11) (Ruiz *et al*., 2012).

With the rise of carbapenemase-producing MDR *K. pneumoniae* in the early 2000s, polymyxin became the last therapeutic option. The first polymyxin non-sensitive *K. pneumoniae* was reported from a nosocomial infection in Greece in 2004, and since then, reports have increased (Antoniadou *et al*., 2007). Polymyxin resistance in *K. pneumoniae* is dominated by chromosomal mechanisms, where certain regulatory genes (*phoPQ*, *pmrA/pmrB*, *pmrD, mgrB*) activate lipid A modifications (known as the LPS modification system), often through activating mutations in *phoPQ, pmrA/pmrB* or inactivation of *mgrB* (Jayol *et al*., 2014; Poirel *et al*., 2015). Other mechanisms include enhanced capsule production (which can shield outer membrane charge), and overexpression of *acrAB-tolC* and *kpnEF* efflux pumps (De-Majumdar *et al*., 2013; Srinivasan & Rajamohan, 2013). Plasmid-mediated polymyxin resistance has also emerged in *K. pneumoniae* recently, and the *mcr-1* (mobile colistin resistance) gene was identified in China in 2015 (Liu *et al*., 2016). From then, several *mcr* variants (*mcr-1*, *mcr-3*, *mcr-7*, *mcr-8*, *mcr-9* and *mcr-10*) have been identified in *K. pneumoniae* (Figure 1.11) (Zhang *et al*., 2025).

Tigecycline has been used against *K. pneumoniae* infections since 2005 due to its ability to evade classical tetracycline resistance, but resistance emerged soon thereafter (Schedlbauer *et al*., 2015). Known resistance mechanisms are largely chromosomal, including modifications in 30S/16S ribosomal unit targets and reduced cell permeability. Upregulation of *acrAB-tolC* and *oqxAB* via changes in their regulators (*rarA*, *ramA*, *ramR* and *acrR*) and decreased *ompK35* expression are also shown to contribute to resistance (Osei *et al*., 2016; Xu *et al*., 2025). Furthermore, the *tetA* genes encoding an efflux pump that resists tetracyclines were identified in tigecycline-resistant *K. pneumoniae* isolates (Ahn *et al*., 2016). Recently, plasmid-mediated tigecycline resistance has been reported in *K. pneumoniae* in 2020 with a transferable RND-type efflux pump cluster, *tmexCD1–toprJ1* on large IncHI1B/IncFIB plasmids (Wang *et al*., 2021; Qu *et al*., 2023). Concurrently, plasmid-encoded *tet(X4)* has been reported in clinical

K. pneumoniae, conferring resistance via the drug inactivation beyond efflux systems (Figure 1.11) (Zhai *et al*., 2022).

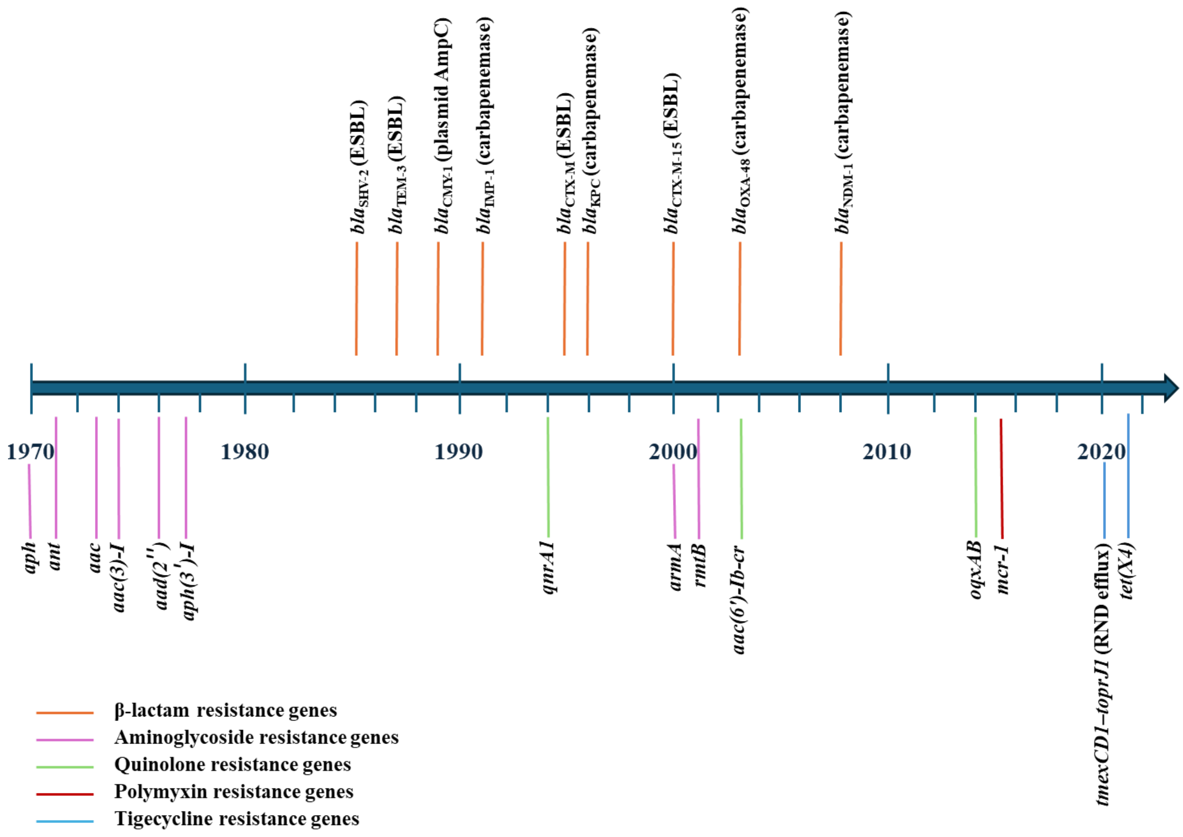


Figure 1.11 Timeline of mobile antimicrobial resistance genes (ARGs) first detected in *K. pneumoniae*. Timelines present identified resistance genes in *K. pneumoniae* against five important antibacterial classes. All ARGs are grouped by resistance mechanism, with line colours corresponding to the legend. The first year of isolation of *K. pneumoniae* carrying the indicated ARG is marked based on a PubMed search. Preference was given to the first members of a new resistance gene family, or for the first gene allele within a gene family conferring a new activity, or the first evidence of a family member in a new genomic context. Rare gene families were excluded. (Updated from Navon-Venezia *et al*., 2017; Wyres & Holt, 2018).

1.4.4 Convergence of AMR and hypervirulence in *K. pneumoniae*: an emerging threat

The MDR cKP and hvKP have traditionally been associated with two distinct lineage groups (hospital-associated and community-associated lineages) of *K. pneumoniae*, distinguished by the presence of acquired AMR determinants and several hypervirulence loci, respectively. For around three decades after hvKP was first recognised, these groups were considered essentially non-overlapping, with only occasional and sporadic reports of convergence (Wyres *et al*., 2020). In the mid to late 2010s, a fatal outbreak of *K. pneumoniae* in China with combined carbapenem resistance and hypervirulence firmly established the convergence phenomenon (Yao *et al*., 2018). Recently, an increasing number of reports have described convergent strains that combine MDR with hypervirulence (MDR-hvKP) (Wyres *et al*., 2020). The emergence of convergence strains poses a significant threat to global health, as it combines severe invasive disease potential with limited treatment options. Considering the public health importance of MDR-hvKP, particularly carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP), the WHO issued a global risk communication, highlighting the increasing detections (WHO, 2024). Geographically, Asia appears to be the focal point for the convergence of CR-hvKP (Lei *et al*., 2024). This pattern likely reflects a combination of true epidemiological drivers and enhanced detection and reporting, rather than being solely due to surveillance or reporting bias. High carbapenem consumption, an increased burden of CR-KP, and the widespread circulation of hvKP lineages in Asia create favourable conditions for convergence through HGT. In addition, extensive genomic surveillance and sequencing-based studies of *K. pneumoniae* in countries like China increase the likelihood of identifying convergent strains compared with regions with limited surveillance. Consistent with this interpretation, global genome-wide analyses demonstrate that while CR-hvKP strains are now present across all six WHO regions, China represents a major endemic setting for their emergence and diversification (Figure 1.12) (Heng *et al*., 2024; Lei *et al*., 2024).

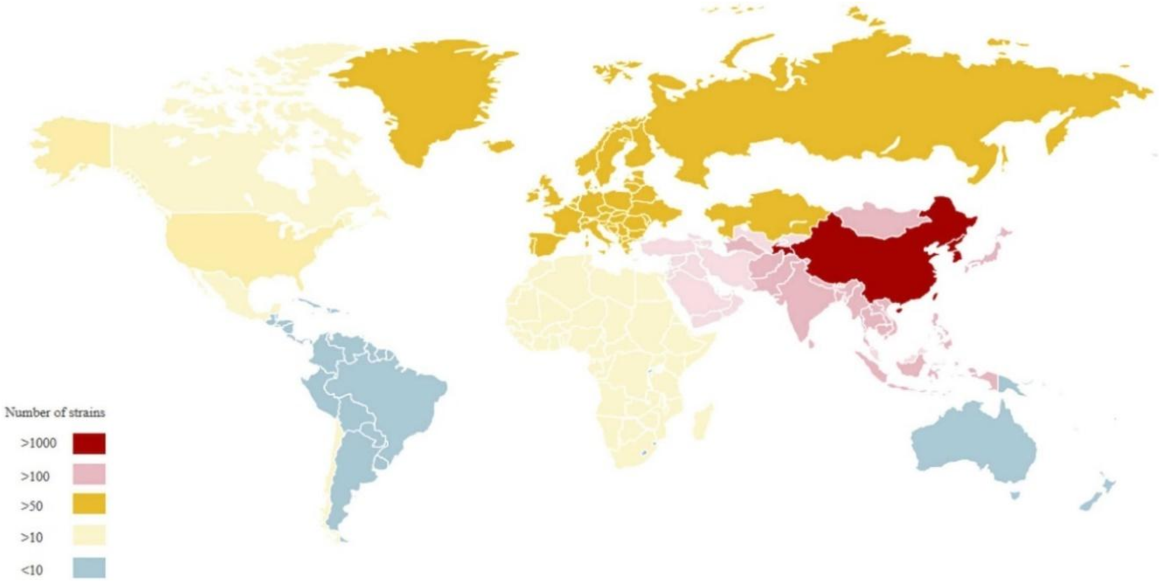


Figure 1.12 Map showing the global distribution of CR-hvKP strains (adapted from Lei *et al*., 2024).

Convergence of resistance and virulence can occur through three evolutionary mechanisms: the acquisition of resistance determinants or plasmids by hvKP strains, the acquisition of virulence determinants or plasmids by MDR cKP lineages, or through the acquisition of hybrid or mosaic plasmids carrying both resistance and virulence determinants (Wyres *et al*., 2020; Lan *et al*., 2021; Pu *et al*., 2023; Chen *et al*., 2024; Lei *et al*., 2024). Hybridisation can occur through several mechanisms, including the insertion of resistance loci into common virulence plasmids or the insertion of virulence loci into AMR plasmids, or the recombination of AMR and virulence plasmid backbones (e.g., IncHI1B/IncFIB backbones bearing *iuc/iro/rmpA2* plus *bla*KPC/NDM). So far, the most common scenario involves MDR clones harbouring virulence plasmids or hybrid plasmids encoding both AMR and hypervirulence determinants (Lam *et al*., 2019; Spadar *et al*., 2023; Wyres *et al*., 2020).

1.4.5 Clonality and epidemiology of MDR, hypervirulent and convergent *K. pneumoniae*

In bacteria, clonality means populations that originate asexually (e.g., binary fission) from a single ancestral cell, resulting in genetically similar lineages with shared evolutionary traits and playing a significant role in the global spread of AMR. In *K. pneumoniae,* clones are defined either by the classical 7-locus multilocus sequence typing (MLST) scheme (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) or core-genome MLST (cgMLST), which aggregates closely related STs into clonal groups (CGs)

(Diancourt *et al*., 2005; Hennart *et al*., 2022). *K. pneumoniae* genomes are typically about 5–6 Mbp in size, encoding around 5,500 genes. Approximately 2000 genes are core (conserved) and present in almost all members of the species, whereas the rest are accessory and vary widely between clones. This pangenome architecture facilitates both AMR and virulence plasticity (Holt *et al*., 2015; Wyres *et al*., 2020). For *K. pneumoniae*, a very large number of STs have been defined by 7-locus MLST. According to the Institut Pasteur MLST database, thousands of distinct ST profiles have been described to date (currently over 9500 unique allelic profile combinations used to assign STs) (Institut Pasteur MLST database, 2025).

Although *K. pneumoniae* infections worldwide are caused by diverse STs or clones, these can be categorised into MDR or high-risk clones responsible for HAIs and hypervirulent clones responsible for CAIs (Wyres *et al*., 2020). In *K. pneumoniae*, MDR clones are defined as resistant to ≥3 antimicrobial classes, in addition to ampicillin (which is intrinsically resistant), and are genetically diverse. Across regions, genomics studies consistently highlight ST258/512, ST11, ST15, ST37, ST101, ST147 and ST307 as prevalent MDR or high-risk clones (Figure 1.13). These MDR hospital-adapted clones frequently carry ESBLs (especially CTX-M group 1/9) and carbapenemases (KPC, NDM, OXA-48-like), often on conjugative plasmids with broad host range, enabling both local outbreaks and cross-border dissemination (Wyres *et al*., 2020; Spadar *et al*., 2022; Heng *et al*., 2024). These highly resistant clones are unrelated and important geographical differences exist in their contributions to the MDR infection burden (Wyres & Holt, 2016; Navon-Venezia *et al*., 2017; Wyres *et al*., 2019; Elias *et al*., 2023). Although ST258 and its derivative ST512 are dominant CR-KP in the Americas and southern Europe, they are comparatively rare in other regions of the world, whereas ST11 is the dominant cause of CR-KP infections in China (Liao *et al*., 2020; Karampatakis *et al*., 2023). Long-term genomic surveillance shows that dominant clones can replace one another over time, highlighting the importance of monitoring for local shifts (e.g., ST307 increases at the expense of older ST258 in a hospital in Sub-Saharan Africa) (Heinz *et al*., 2024).

In contrast to MDR clones, hvKP clones are globally dominated by the same subset of lineages. So far, ST23 has been the most common, followed by ST65 and ST86, whereas ST25, ST66, and ST380 occurred less often (Wyres *et al*., 2020; García-Cobos *et al*., 2025). These hypervirulent clones frequently carry the plasmid-borne loci *iuc*, *iro* and *rmpA/rmpA2* (hypermucoviscosity), while the ICEs mobilise *ybt* and often *clb*. The capsular antigen K1 is highly conserved in hypervirulent clone ST23, whereas K2 is found in other hypervirulent clones (Russo & Marr, 2019; Stanton & Wyres, 2024). Geographically, hvKP clones (ST23) are more prevalent in the Asia-Pacific region compared to other regions, but their prevalence is increasing globally (Zhang *et al*., 2016; McElheny *et al*., 2024; Nguyen *et al*., 2024).

Convergence has been reported among both MDR and hvKP clones. For example, carbapenem-resistant or MDR ST11 strains carrying the virulence plasmid with *iuc* (and often *rmpA2*) are reported in China (Gu *et al*., 2018). Carbapenem-resistant genes (OXA-48, NDM) have been identified in the hypervirulent *K. pneumoniae* ST23 lineage (Roulston *et al*., 2018; Zhao *et al*., 2019).

MDR or hvKP clones have also been isolated from different non-human sources, including a range of animals, for example, ST11 from poultry, ST15 from companion animals, ST23 from non-human primates and horses (Ewers *et al*., 2014; Anzai *et al*., 2017; Gravey *et al*., 2023; Mourão *et al*., 2024). MDR (ST11, ST15, and ST147) and occasionally hvKP clones (ST23) are recovered from environmental reservoirs such as hospital wastewater, surface water and soil, often carrying ESBLs or carbapenemases and virulence loci (Araújo *et al*., 2025; Liao *et al*., 2025). Despite the widespread presence of *K. pneumoniae* MDR and hypervirulent clones in non-human environments, whole genome sequencing (WGS) surveys outside humans remain limited, restricting robust source attribution (Wyres *et al*., 2020).

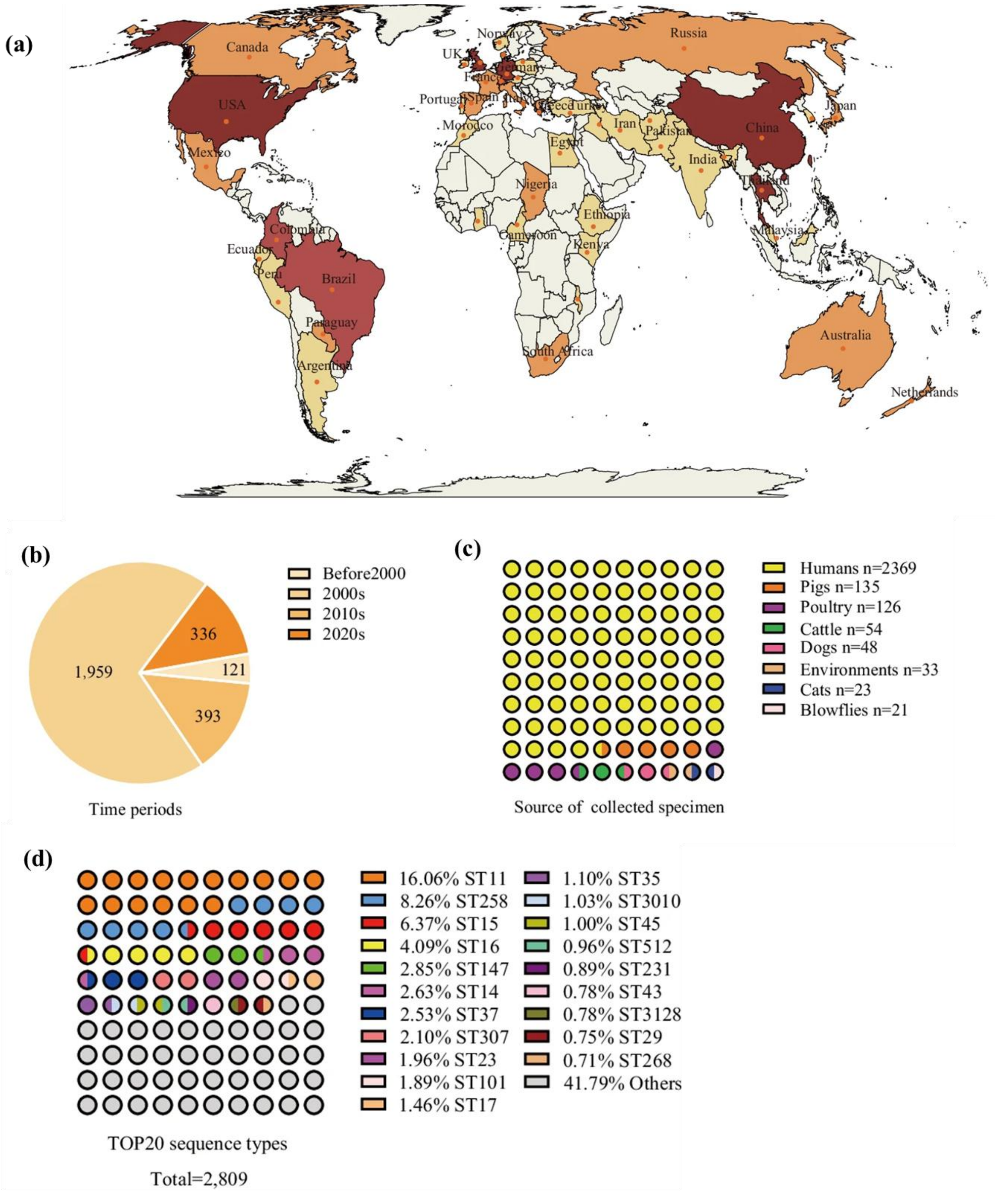


Figure 1.13 Global distribution, sources and dominant sequence types of *K. pneumoniae* isolates (n=2809) from 57 countries (adapted from Huang *et al*., 2025). **a.** World map showing the distribution of samples across 57 countries. **b.** Temporal distribution of the samples. **c.** Host distribution of the samples. **d.** Relative abundances of the 20 most prevalent sequence types (STs).

1.4.6 Plasmid diversity in *K. pneumoniae*

In *K. pneumoniae*, plasmids serve as a major vehicle for transferring AMR, virulence, and other accessory genes among species and related genera, facilitating the emergence of MDR and convergent strains in both clinical and non-clinical environments. This self-replicating extrachromosomal DNA material can spread rapidly via HGT, thereby accelerating the evolution of AMR. Plasmid-borne ESBLs, carbapenemases, and mobile colistin resistance (*mcr*) genes are among the most pressing AMR threats (Wyres *et al*., 2020; Pan & Li, 2025).

K. pneumoniae carries highly diverse plasmids, including various replicon types such as narrow-host-range IncF backbones (IncFII(K)/IncFIB(K)), IncR that are prevalent in clinical lineages, broad-host-range vehicles (IncR/IncN/IncX3), small ColKP3 plasmids, and virulence-associated IncHI1B/IncFIB(K) (KpVP-1/2) (Ramirez *et al*., 2019; Wyres *et al*., 2020; Sahoo *et al*., 2024; Hinthong *et al*., 2025). Analyses of fully closed *K. pneumoniae* genomes available in the NCBI GenBank database indicate a median of three plasmids per complete *K. pneumoniae* genome (interquartile range, 2–5; range, 0–10), which is significantly higher than that of *E. coli*, where complete genome analyses typically report a median of one plasmid per genome (interquartile range, 0–3; range, 0–9). This high plasmid load suggests *K. pneumoniae* is particularly permissive for plasmids, meaning it readily acquires plasmid-borne materials from diverse donors and maintains them long enough to transmit among human and animal-associated niches (Figure 1.10) (Wyres & Holt, 2018).

Most horizontally acquired AMR genes in *K. pneumoniae* are carried on large conjugative plasmids, which belong to a small number of incompatibility groups (IncFII, IncN, IncR, and IncX3). However, small plasmids that are mobilisable but not self-transmissible can also harbour AMR genes (Navon-Venezia *et al*., 2017; Ramirez *et al*., 2019). Notably, MDR clones tend to harbour a higher plasmid load and diversity than hypervirulent clones (Wyres *et al*., 2019). Large-scale genomic studies report that the IncFIBK is one of the most commonly found plasmids in *K. pneumoniae* (associated with both MDR and virulence plasmids), along with IncFIIK, R replicons, small (Col) plasmids, other F plasmid variants (FII, FIA, and FIB) and incompatibility types X3, N, HI1B (Wyres *et al*., 2020; Spadar *et al*., 2023).

Key resistance loci often show characteristic plasmid backbones such as *bla*$_{\text{KPC}}$ on pKpQIL-like IncFII(K)/IncFIB(pQil), *bla*$_{\text{OXA-48-like}}$ largely on IncL pOXA-48–like plasmids, *bla*$_{\text{NDM}}$ frequently on IncX3, *bla*$_{\text{CTX-M-15}}$ often on IncF platforms including IncFIB(K), *mcr-1* most often on IncX4, IncI2, and IncHI2/HI2A plasmids and *mcr-8* frequently on IncFII(K) (Wang *et al*., 2018; Zhu *et al*., 2020; Wu *et al*., 2021; Hammad *et al*., 2023; Liu *et al*., 2023; Ikhimiukor *et al*., 2024; Calvo-Villamañán *et al*., 2025). Although classical hvKp virulence plasmids (KpVP-1/2) are typically non-conjugative, conjugation-competent derivatives and resistance–virulence cointegrates are now documented, allowing transfer across sequence types and even species (Yang *et al*., 2022).

In *K. pneumoniae*, various plasmids, especially IncC (A/C2), IncHI2, and IncI1, are frequently identified across human, livestock, companion animals, and environmental (wastewater and surface water) isolates, indicating a clear One Health connection in AMR transmission (Sekizuka *et al*., 2018; Matlock *et al*., 2023; Verburg *et al*., 2024). Since similar plasmids are disseminating across sectors and hosts, understanding their role in AMR ecology is crucial for risk assessment, outbreak tracing, and developing effective interventions, especially in LMICs, where surveillance systems are still in development (Spadar *et al*., 2023; Pan & Li, 2025).

1.5 Extending beyond *K. pneumoniae*: why do other *Klebsiella* spp. matter?

K. quasipneumoniae, a member of the KpSC, comprises two subspecies (subsp. *quasipneumoniae* and *similipneumoniae*). In addition to human clinical infections, *K. quasipneumoniae* can be found in various environmental sources, including animals, plants, water and soil. The ecological diversity indicates that it is environmentally established rather than an incidental contaminant, although it is less abundant than *K. pneumoniae* in both clinical and environmental settings (Sękowska *et al*., 2025). *K. quasipneumoniae* has often been under-recognised or misidentified as *K. pneumoniae* in routine diagnostics using traditional microbiological assays. However, advances in genome-based approaches, including WGS, have revealed its widespread distribution and pathogenic potential (Rodríguez-Medina *et al*., 2024; Tian *et al*., 2025). It can cause both HAIs (e.g., pneumonia, septicaemia) and often CAIs (invasive infections). The genomic studies further suggest that, although not very common, *K. quasipneumoniae* is increasingly detected in mixed-species or mixed-lineage infections, particularly alongside *K. pneumoniae*, in both clinical and environmental samples. Such co-occurrence may facilitate horizontal gene transfer within the KpSC. Importantly, this species can harbour and spread both AMR and virulence determinants as seen in *K. pneumoniae*. Recent reports have identified MDR clinical strains harbouring carbapenemases $bla_{\mathrm{NDM\text{-}1}}$ and $bla_{\mathrm{KPC\text{-}2}}$ (Arena *et al*., 2015; Tian *et al*., 2025). Another report shows *K. quasipneumoniae* isolated from pigs harboured *mcr-8* (Phetburom *et al*., 2021). Additionally, an MDR environmental strain initially misidentified as *K. pneumoniae* has been reclassified as *K. quasipneumoniae* by WGS, underscoring extra-clinical reservoirs (Altayb *et al*., 2023). These findings together highlight the emergence and spread of MDR in this species, as well as the need for genome-based, accurate identification in surveillance (Sękowska *et al*., 2025; Tian *et al*., 2025).

K. variicola, another member of the KpSC, historically has been plant-associated but is now recognised as an opportunistic human pathogen causing respiratory, urinary tract and bloodstream infections. It can also cause disease in animals (such as mastitis in cows). Its clinical importance was obscured due to misidentification as *K. pneumoniae*. Recent genomic studies from clinical and environmental sources (including wastewater) highlight a broad host range and carriage of ESBLs/carbapenemases/plasmid-mediated colistin resistance in some lineages, underscoring relevance among human-animal-environment interfaces (Podder *et al*., 2014; Rodríguez-Medina *et al*., 2019; Cherak *et al*., 2021; Phetburom *et al*., 2021; Lekota *et al*., 2024; Yang *et al*., 2024; Ohno *et al*., 2025).

K. oxytoca, a member of the KoSC, inhabits diverse environmental niches and the gut microbiota. It causes opportunistic human infections, and its AMR profile includes ESBLs and emerging carbapenem resistance, with reports from both human and hospital environments (Lowe *et al*., 2012; Chapman *et al*., 2020; Yang *et al*., 2021; Jiang *et al*., 2024). Its key virulence feature includes the kleboxymycin biosynthetic gene cluster, which encodes genotoxin (tilimycin/tilivalline) responsible for antibiotic-associated haemorrhagic colitis (AAHC) and is widely distributed across KoSC members (Shibu *et al*., 2020, 2021). Environmental and wildlife studies further indicate that non-clinical reservoirs can also harbour MDR strains (Yang *et al*., 2021; Quintelas *et al*., 2024).

K. michiganensis, another member of KoSC, is increasingly found in nosocomial infections and has developed significant AMR, including ESBLs and carbapenemases (e.g., KPC, NDM). This has been observed in hospital wastewater-derived and neonatal clinical isolates, underscoring the environmental spread beyond clinical settings (Prah *et al*., 2022; Long *et al*., 2024). *K. pasteurii* (formerly Ko4) and *K. grimontii*, part of KoSC, have also been reported from humans and animals, indicating potential for cross-sector circulation (Merla *et al*., 2019; Campos-Madueno *et al*., 2021). Notably, carbapenem-resistant *K. grimontii* has also been reported from clinical settings, indicating that KoSC members can contribute to the broader *Klebsiella* AMR pool (Liu *et al*., 2018).

K. aerogenes, reclassified from *Enterobacter aerogenes* based on comparative genomic analysis. This species differs from other *Klebsiella* spp. in two notable ways. First, it carries an intrinsic, inducible chromosomal *ampC* gene (a class C β-lactamase), which is different from other *Klebsiella* spp. that characteristically encode class A β-lactamase genes (e.g., SHV in *K. pneumoniae*, OKP in *K. quasipneumoniae*, LEN in *K. variicola* and OXY in KoSC) (Long *et al*., 2017; Yang *et al*., 2021; Feng *et al*., 2024). Second, it is motile while the *Klebsiella* genus is typically non-motile (Podschun & Ullmann, 1998). This species is recognised as an emerging healthcare-associated pathogen with inducible *ampC* and rising reports of ESBLs and carbapenemases (Rodrigues *et al*., 2024). Population genomic analyses reveal an open pangenome with substantial accessory resistome, and outbreaks of carbapenem-resistant *K. aerogenes* emphasise the need for species-specific genomic surveillance and antimicrobial stewardship (Feng *et al*., 2024; Morgado *et al*., 2024).

1.6 Rationale for the study design

K. pneumoniae ranks among the top causes of HAIs in both national and global reports. International surveillance systems such as GLASS (Global Antimicrobial Resistance and Use Surveillance System) consistently flag *K. pneumoniae* due to rising reports of resistance to 3GCs and carbapenems in many regions. In response, the WHO has recently placed 3GC-resistant *K. pneumoniae* and CR-KP on the top bacterial priority pathogen list (BPPL 2024), for which urgent R&D (research and development) and public health actions are emphasised. Additionally, the emergence of the CR-hvKP (often considered a superbug) has added further complications, leading to therapeutic failures and increased mortality (Ajulo & Awosile, 2024; Chong *et al*., 2024; Sati *et al*., 2025).

Global AMR research on *K. pneumoniae* is primarily patient-focused and hospital-based. However, several studies aimed to determine how different sectors contribute to the public health burden of AMR in *K. pneumoniae* and other *Klebsiella* species. These cross-sectoral genomic surveys collected samples from various clinical and non-clinical settings to identify the strain frequency and potential cross-sector transmission dynamics. Studies report that the transmission of AMR strains and genes between clinical and non-clinical settings may be less common. But they also document spillover of MDR strains and plasmids at human-animal-environment interfaces, so the overall picture remains mixed (Ludden *et al.,* 2020; Butaye *et al.,* 2021; Dereeper *et al.,* 2022; Thorpe *et al.,* 2022; Calland *et al.,* 2023; Jacob *et al*., 2024). Although the evidence shows substantial diversity beyond hospitals and potential for resistance spread, the uneven sampling and limited cross-sector genomics leave major gaps in our understanding of the abundance, distribution and transmission of AMR and virulent strains and genes within and between humans, animal farms, and environmental settings.

According to the WHO report on global surveillance of AMR, Southeast Asia has been identified as a region with significant gaps in surveillance, along with a lack of standardised methodologies, data sharing, and coordination. Bangladesh, a developing country within this region, is particularly vulnerable due to its high AMR burden, posing both regional and global threats (WHO, 2022).

MDR, ESBL, and CR-KP are frequently reported in the Indian subcontinent (e.g., Bangladesh, India, Pakistan), recovered from humans, food animals, and environmental reservoirs such as wastewater (Kumarasamy *et al.,* 2010; Farzana *et al.,* 2013; Islam *et al.,* 2013; Mohanty *et al.,* 2017; Nahid *et al*., 2017; Wyres & Holt, 2018). However, information regarding the prevalence and molecular epidemiological features of MDR *K. pneumoniae* is limited in Bangladesh (Khan *et al.,* 2018). As part of the scoping exercise for this study, I reviewed the published literature relevant to *Klebsiella* spp. in Bangladesh from 2015 onwards (Appendix A). Database search included PubMed with the relevant keywords '*Klebsiella*', '*Klebsiella* spp.', '*Klebsiella pneumoniae*', '*Klebsiella quasipneumoniae*', '*Klebsiella variicola*', '*Klebsiella oxytoca*', '*Klebsiella aerogenes*' and 'Bangladesh'. Reviews and meta-analyses were excluded. Briefly, scoping searches recognise ESBL (*bla*$_{\text{CTX-M-15}}$) and carbapenemase-producing *K. pneumoniae* and other *Klebsiella* spp. in hospitals, with *bla*$_{\text{NDM}}$ and *bla*$_{\text{OXA-48-like}}$ enzymes common. Global high-risk or MDR clones (ST11, ST15, ST14, ST307) have also been reported in clinical isolates. Notably, plasmid-mediated *mcr-8* positive, colistin-resistant *K. pneumoniae* has been detected in clinical isolates and isolates recovered from water (Farzana *et al*., 2020; Mondol *et al*., 2025). Environmental, wastewater and food chain investigations further show that *Klebsiella* spp. and other Enterobacterales carrying multiple AMR genes, showing potential spillover to people via food and water (Appendix A). Additionally, national surveillance reports also flag high resistance in *Klebsiella* from clinical specimens (IEDCR, 2024). Despite increased evidence, my scoping review identifies the following gaps in *Klebsiella* AMR surveillance in Bangladesh:

- First, sampling is heavily biased towards hospitals, while sources like animals, food, and wastewater are not consistently monitored, making it harder to pinpoint where and how resistance is developing and their transmission dynamics.
- Second, limited use of genomics. Although recent studies are shifting towards WGS, many studies still rely on phenotypic antimicrobial susceptibility testing (AST) and PCR, which cannot identify high-risk clones, mobile elements, or convergence of hypervirulence with resistance.
- Third, inadequate metadata, which weakens outbreak detection and risk assessment.

- Fourth, insufficient attention to the monitoring of high-end resistance mechanisms, despite documented *mcr-8* in *K. pneumoniae* clinical isolates and nearby reservoirs.
- Finally, capacity constraints. Although sequencing and bioinformatics capacity are growing, routine genomic linkage between hospital, veterinary, and environmental data is limited (Sujan *et al*., 2023).

While several studies from Bangladesh have investigated AMR in *Klebsiella* spp. from individual domains, such as human, animal, or environmental sources, an integrated One Health approach remains limited. In particular, no studies have comprehensively applied a One Health framework that simultaneously incorporates human (hospital and community), animal, and environmental sampling, while integrating epidemiological, clinical, and genomic data within a single analytical framework to assess the burden of AMR in *K. pneumoniae* or other *Klebsiella* spp. in Bangladesh. Consequently, there remains a critical gap in understanding the prevalence, resistance profiles, genomic diversity, and virulence potential of this WHO critical priority pathogen across the human–animal–environment interface in the country. Addressing this gap is essential for informing effective surveillance, risk assessment, and control strategies for *K. pneumoniae* and related species in Bangladesh.

1.7 Objectives and scope of this thesis

To the best of my knowledge, this is the first prospective, systematic One Health survey of AMR in *Klebsiella* spp. in Bangladesh. This thesis implements a comprehensive One Health sampling framework, covering site selection, sample size determination, and coordinated sampling and data collection across human, animal and environmental sources, to generate representative data. The phenotypic approach (such as AST) is combined with WGS, and the genomic data were merged with relevant demographic and clinical metadata to explore associations, potential outbreaks, and putative drivers of AMR. Overall, this thesis aims to investigate the genomic epidemiology of AMR, virulence, clonality and plasmid diversity in *Klebsiella* spp., with particular focus on *K. pneumoniae*, within a One Health framework in Bangladesh. The specific objectives of this thesis are as follows:

1. To determine the prevalence of *K. pneumoniae* at the human-animal-environment interface in the study area and to examine whether prevalence varies significantly by sample source, sampling location and time period.
2. To characterise dominant resistance mechanisms in circulating *K. pneumoniae* at the human–animal–environmental settings, identify their clonality and determine the role of plasmids in the AMR dissemination across various sectors.
3. To determine the virulence profile of *K. pneumoniae*, including hvKP and convergent strains from various sources, assess their clonal relatedness and plasmid-mediated spread in community, farm, and healthcare settings.
4. To determine the prevalence, AMR and virulence profiles of non-pneumoniae *Klebsiella* spp. across human, animal, and environmental compartments in Bangladesh.
5. To investigate the emergence and dissemination of mobile colistin resistance in *Klebsiella* spp. among human, animal, and environmental sectors in Bangladesh.

Taken together, this thesis describes the most comprehensive assessment to date of AMR and virulence in *Klebsiella* in Bangladesh from both epidemiological and molecular perspectives. My findings represent a baseline dataset to inform the Bangladeshi national AMR strategy, strengthen GLASS-aligned reporting, guide One Health policy and support targeted interventions across human health, animal health, and environmental management to contain this high-risk pathogen regionally and globally.

Chapter 2

Materials and Methods

2.1 Study design

Effective One Health AMR surveillance depends on designing and implementing context-specific, optimised sampling strategies that can guide targeted interventions and policy (WHO, 2017). Such optimisation is essential for selecting an appropriate sampling method and determining a suitable sample size based on study duration and available resources, to ensure sufficient precision of the findings. A sample size that is too small can produce misleading estimates, while an oversized sample can be resource intensive. Therefore, developing an operational, resource-efficient sampling protocol is important, particularly in LMICs like Bangladesh (ECDC, 2023).

In this study, I have adopted the One Health sampling framework outlined by Walsh (2018) and Zhou *et al*. (2022) and have optimised it to align with the local context of Bangladesh. I have systematically selected sampling sites, sample sources, and designed a sampling protocol that ensures objectivity and representative findings for both urban and rural settings. A schematic diagram of the study design and sampling strategy for this study is presented in Figure 2.1, illustrating the conceptual, preparatory, and operational stages of this One Health AMR surveillance study.

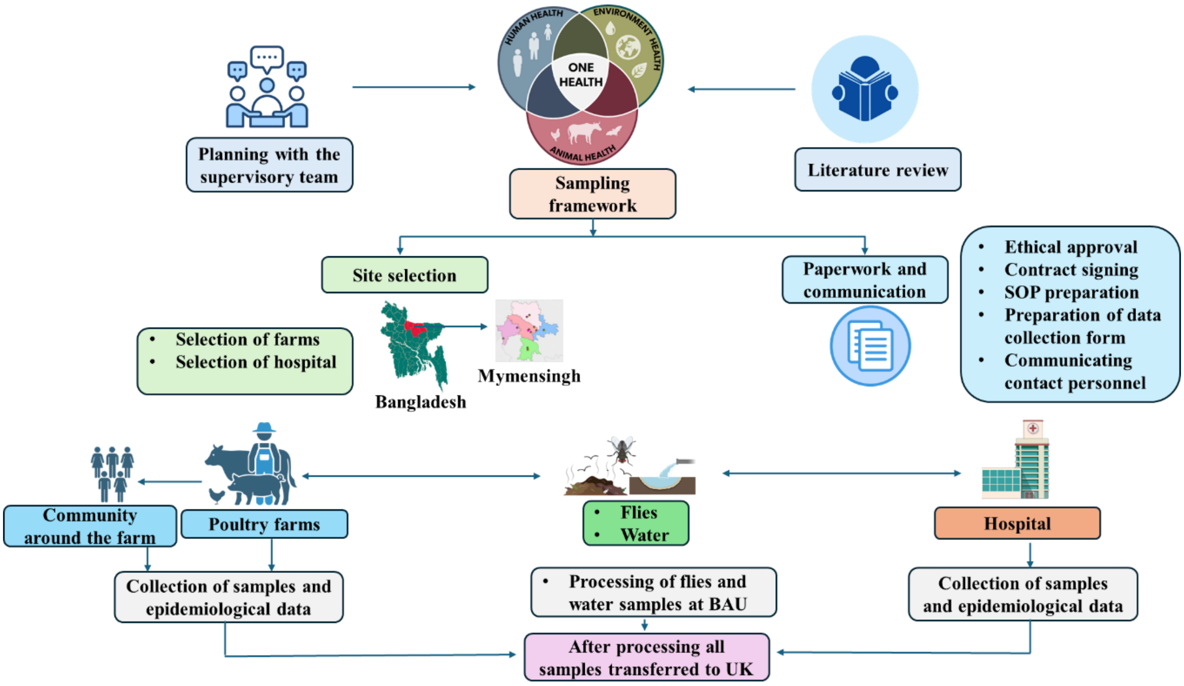


Figure 2.1 A schematic diagram of the study design and sampling strategy for One Health AMR

surveillance in Mymensingh, Bangladesh. BAU, Bangladesh Agricultural University; SOP, Standard Operating Procedure.

From December 2021 to March 2023, a prospective cross-sectional survey was performed to investigate the epidemiology of MDR *K. pneumoniae* in clinical infections from outpatient departments and hospitalised patients and in the faecal carriage of healthy human volunteers from the community. Simultaneously, a cross-sectional carriage study was conducted during this period to understand the prevalence of MDR *K. pneumoniae* in livestock and the environment.

A comprehensive 'One Health' concept was employed for sampling humans, including normal flora, urinary tract infections (UTIs), surgical site infections (SSIs), livestock (chickens, chicken meat, domestic animals and free-flying birds), and the environment (flies and water). After collection and processing, all samples were transferred to the University of Oxford, UK, for further analysis, including microbiological characterisation, WGS and downstream data analysis.

Hospital-based samples (UTIs and SSIs from patients) and community-based samples (normal flora of healthy humans from the community) were collected as part of a cross-sectional, source-comparative design and were not linked at the individual, household, or community level. No longitudinal follow-up of patients, families, or surrounding communities was undertaken. The sampling framework aimed to capture contemporaneous *K. pneumoniae* populations from distinct human, animal, and environmental sources within a shared geographic setting to enable comparative analysis of clonality, virulence, and antimicrobial resistance patterns.

The study was set up following a formal collaboration agreed upon between the Chancellor, Masters, and Scholars of the University of Oxford (UOX), UK, and Bangladesh Agricultural University (BAU), Bangladesh [Lead Ref: R78974/CN001] (Enclosed in Appendix B). The agreement outlines the management of Ineos Oxford Institute for Antimicrobial Research (IOI), Oxford funding for sampling-related costs, and sets terms for data security and protection, copyright, and authorship for publications resulting from my research. The study was conducted in Mymensingh, Bangladesh and Oxford, UK, where BAU and Mymensingh Medical College Hospital (MMCH) from Bangladesh and UOX from the UK were involved.

2.2 Ethical approvals

Ethical approval was taken for both human and farm samples. This study was ethically approved by the following ethical review committees: (i) Institutional Review Board (IRB), Mymensingh Medical College [Memo no: MMC/IRB/2021/420], Mymensingh, Bangladesh; (ii) Animal Welfare and Experimentation Ethics Committee (AWEEC), Bangladesh Agricultural University [Memo no: AWEEC/BAU/2021 (23)], Mymensingh, Bangladesh; (iii) Oxford Tropical Research Ethics Committee (OxTREC), University of Oxford, UK [OxTREC Reference: 30-21] following the Helsinki Declaration. Written consent was taken from all the participants in the study. Patients' data was anonymised using spreadsheets and protected by encryption and passwords, in accordance with the Helsinki Declaration (General Assembly of the World Medical Association, 2014). Ethical permissions are attached in Appendix C, and the participant consent form is in Appendix D. I contacted all the farm owners enrolled in the study, explained the objective of my study to them and obtained their consent for farm sampling. Designated contact persons from MMCH were also approached to secure consent and coordinate the collection and processing of clinical samples in the microbiology lab at MMC. The collaboration agreement between the UOX and BAU enabled sample processing at the microbiology laboratory of BAU.

2.3 Site selection

Bangladesh is a densely populated country with an average population density of approximately 1,333 people per square kilometre (United Nations, 2024). Therefore, human and animal cohabitation is very common (Figure 2.2). The waste management system is also poorly developed, resulting in household, hospital, and animal wastes often being found near roads, hospitals, residences, and water bodies. Periodic floods and cyclones further disrupt these systems, thereby heightening public health risks from this untreated waste. Together, these factors can facilitate the spread of pathogenic bacteria and AMR and make Bangladesh a compelling setting for a One Health study of AMR dissemination.

Figure 2.2 Context for One Health AMR surveillance in Bangladesh: population density, inadequate sanitation and waste management, flood impacts and human-animal cohabitation. A. shows high urban population density; (B–C) show unmanaged waste and poor sanitation are adjacent to dwellings and waterways; D. shows residential areas are submerged during flooding. (E-F) show proximity of human-animal households.

Administratively, Bangladesh is divided into eight divisions and 64 districts. Within the Mymensingh Division, Mymensingh is the oldest district, with a high population density (approximately 1,400 people per square kilometre) (Bangladesh Bureau of Statistics, 2022). The district hosts a major tertiary hospital (MMCH) and an agricultural university (BAU). Mymensingh is also recognised as a hub for poultry farming, making it a suitable setting for this study (Alam *et al.,* 2014). Within the Mymensingh district, there are 13 subdistricts. Sampling was performed in 5 subdistricts. Mymensingh Sadar was selected for its central location, population concentration and presence of the tertiary hospital MMCH. Subsequently, four adjacent subdistricts (Tarakanda, Muktagachha, Trishal, and Gauripur) surrounding Mymensingh Sadar from four directions were included as sampling sites (Figure 2.3)

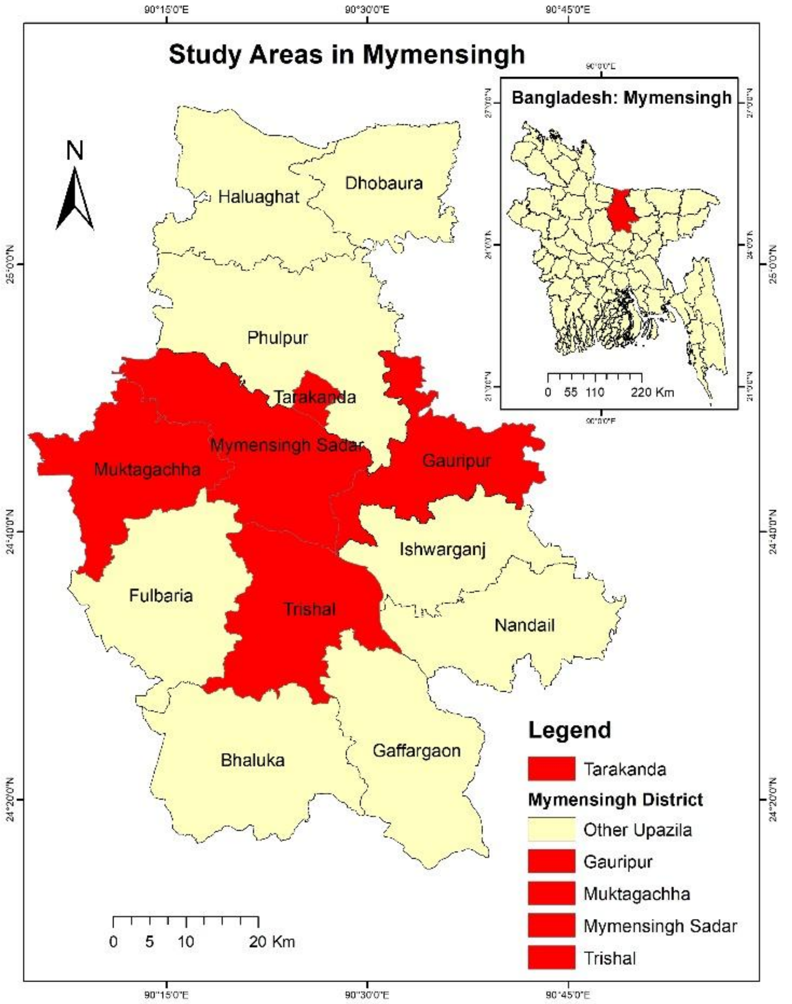


Figure 2.3 Sampling locations (● red marked) in Mymensingh district, Bangladesh.

2.4 Sample size calculation

The statistical analyses assessing the prevalence and trends of antimicrobial resistance mechanisms rely on the assumptions of Chi-square testing. In this study, to ensure adequate sampling, a Chi-square test of homogeneity was performed to determine the sample size (Table 2.1). For Chi-square tests involving categorical outcomes, Cohen's w is the appropriate standardised effect size measure. Cohen's w quantifies the magnitude of deviation between observed and expected proportions across multiple categories. This approach is particularly suitable for One Health surveillance studies in which resistance patterns are compared across heterogeneous sources without a single predefined reference group. The following assumptions were made for the sample size determination:

- Level of statistical significance (alpha, α) was set at 0.05, and statistical power (beta, β) was set at 80% (β=0.20).
- A buffer of 15% was included to account for dropouts and other unanticipated missingness.
- Three scenarios were calculated for different Cohen's w values, representing different levels of effect size (small: w=0.10, medium: w=0.30, and large: w=0.50).
- Four sampling time points were included per source group; therefore, sample size requirements were calculated per time point per sample category, with and without buffer, and then aggregated (total) across time points.

The sample size for this study was determined through a thorough review of existing literature. The primary goal was not to investigate any specific resistance mechanism but rather to estimate the prevalence of resistance to various clinically significant antibiotics at the human-animal-environment interface. Therefore, to calculate the sample size, this study relied on prior studies from Bangladesh that focused on commonly identified resistance mechanisms, particularly ESBL and the emerging colistin resistance (*mcr*). Given the nature (surveillance-oriented) of the study and recognising that even modest differences in resistance prevalence may be epidemiologically and clinically meaningful in endemic settings, the final sample size was conservatively based on the small effect size scenario (w =0.10). This resulted in a total required sample size of 1,656 observations (184 for each of the nine source categories and 46 for each sampling time after accounting for the 15% buffer) (Table 2.1).

Although the odds ratio (OR) was used in the analytical phase of the study to quantify associations between groups and resistance outcomes, it was not used for sample size determination. Odds ratios are model-dependent, pairwise measures requiring explicit reference categories and careful interpretation when outcomes are common, making them more suitable for inferential analyses following data collection. In contrast, Cohen's w provides a model-agnostic measure of overall heterogeneity in proportions, making it suitable for sample size planning based on global Chi-square tests.

Table 2.1 Sample size calculation scenarios for Chi-square test of homogeneity**.**

Source group	**Effect size Cohen's w**	**df (n-1)**	**N (each category, each time point)**	**Total + 15% buffer (each time point)**	**N (each category)**	**Total + 15% buffer**	**Total per group**	**Total per group + 15% buffer**
Farm (CCS, CMS, DARS, BF)	0.10	8	40	46	160	184	640	736
	0.30	8	5	15	20	60	200	240
	0.50	8	2	3	8	12	32	64
Environment (Water, flies)	0.10	8	40	46	160	184	320	368
	0.30	8	5	15	20	60	100	120
	0.50	8	2	3	8	12	16	32
Human (Isolates from UTI, isolates from SSI, HRS)	0.10	8	40	46	160	184	480	552
	0.30	8	5	15	20	60	150	180
	0.50	8	2	3	8	12	24	36

N, number; df, degrees of freedom; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs.

2.5 Collection of samples

For livestock sampling, a total of 10 poultry farms (one broiler farm and one layer farm from each selected subdistrict) were included based on the following inclusion criteria: (i) farms having more than 3000 birds, (ii) farms raising the birds of a single age group, and all birds in a batch fed or watered from common sources, (iii) computer-generated randomisation to select the farms. Retail meat shops within 10 km of the selected poultry farms were chosen for chicken meat sampling. Human (normal flora) and environmental sampling were performed from different points within a 10 km radius surrounding the selected poultry farms. Clinical sampling was performed at MMCH, a public tertiary care hospital, with 1000 allocated beds (DGHS, 2021). Human rectal swabs were also collected from community people living around the MMCH. For farm (CCS, CMS, DARS, BF) and environmental samples (water and flies), five samples were collected from each sampling site during each sampling time. From the community, fifteen human rectal swabs were collected from each site during each sampling time.

Outpatients with UTIs and patients with SSIs admitted at MMCH from January 2022 to March 2023, whose urine specimens and wound swabs were sent to the MMC microbiology lab for diagnostic purposes, were included in this study based on inclusion and exclusion criteria. Samples were collected from farms, human volunteers, and the environment every four months during the 16-month sampling period from December 2021 to March 2023 to identify any seasonal variations. In contrast, clinical samples from UTIs and SSIs were collected continuously throughout this period from the enrolled patients according to the inclusion criteria who visited or were admitted to MMCH.

For farm (CCS, CMS, DARS, BF) and environmental samples (water and flies), five samples were collected from each sampling site during each sampling time. From the community, fifteen human rectal swabs were collected from each site during each sampling time. It is worth noting that the same farms (enrolled during the first sampling) were sampled for each subsequent period of sampling. However, the sampling from the poultry and human subjects was non-repetitive. All samples from each category were collected following preparation and approval of the standard operating procedures (SOPs). The distribution of samples and total number of samples collected during the sampling period has been presented in tabular form (Table 2.2).

Table 2.2 Total samples collected from December 2021 to March 2023.

Sample types	**Sector**	**Quarter-1 (Dec'21-Mar'22)**	**Quarter-2 (Apr'22-Jul'22)**	**Quarter-3 (Aug'22-Nov'22)**	**Quarter-4 (Dec'22-Mar'22)**	**Total number of samples**
Water	Environment	55	55	55	55	**220**
Flies		55	55	55	55	**220**
CCS	Farm	50	50	50	50	**200**
CMS		50	50	50	50	**200**
DARS		50	50	50	50	**200**
BF		6	22	18	29	**75**
UTIs	Human	216	391	301	262	**1170**
SSIs		94	196	336	188	**814**
HRS		165	165	165	165	**660**
Total		**741**	**1034**	**1080**	**904**	**3759**

CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs; Quarter-1 (December 2021 – March 2022); Quarter-2 (April 2022 – July 2022); Quarter-3 (August 22 – November 2022); Quarter-4 (December 2022 – March 2023).

2.5.1 Collection of rectal swabs from healthy human volunteers

An equal number (n = 15) of participants from each sampling site were selected based on inclusion and exclusion criteria. The inclusion criteria were: (i) inhabiting within 10 km of the selected poultry farms and the hospital, (ii) participants who are willing and able to give informed consent, (iii) male or female, aged 18 years or above, whereas the exclusion criteria were: (i) pregnant women, (ii) diabetic patient, (iii) participants with any gastrointestinal or chronic diseases, (iv) any individual who is not able to give consent themselves.

A prior schedule was arranged for sampling and interviewing healthy volunteers. They were informed about the purpose and details of the research, and their written consent was obtained before providing rectal swabs. Rectal swabs were collected using transport swabs with Amies and charcoal (COPAN Diagnostics, VWR, UK). The volunteers received detailed instructions on how to collect samples using Amies charcoal swabs, ensuring that they understood the procedure clearly to perform the sampling correctly (Figure 2.4). Aseptic measures such as hand washing and gloves during sampling were appropriately maintained. Each participant was given a unique identifier number, which was anonymised. The swab package was opened, and the swab was removed carefully from the tube. The cotton bud end of the swab was inserted about 3-4 cm into the anus and gently rotated for about 10 seconds. The swab stick was then placed in the tube and labelled properly. After providing samples, an interview was conducted in accordance with the case report form (CRF) (Appendix E).

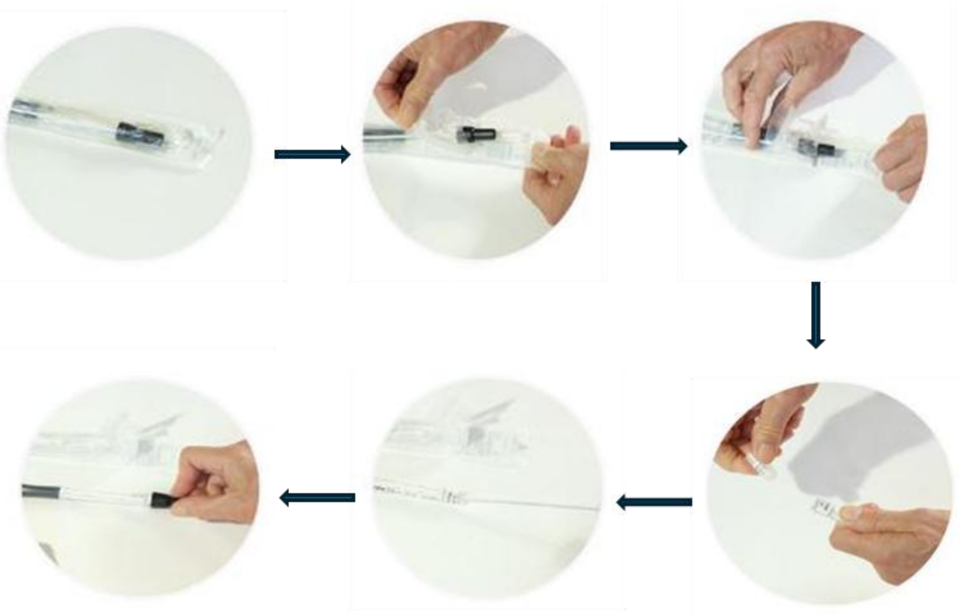


Figure 2.4 Procedure of using Amies charcoal transport swab.

2.5.2 Collection and processing of SSI samples

Hospitalised patients who were suspected of infections by the physicians of MMCH, and whose specimens (wound swabs from SSIs) were referred for microbiology to the microbiology laboratory of MMC, were the target group. For patients with SSIs, the inclusion criteria were: (i) patients whose wound swabs were sent to the microbiology lab of MMCH for diagnostic purposes, (ii) patients had the following symptoms: fever, redness, pain around the area of surgery, and drainage of cloudy fluid from the surgical wound, (iii) male or female, aged 18 years or above, (iv) participants willing and able to give informed consent. The exclusion criteria were: (i) pregnant women, (ii) diabetic patients, and (iii) participants with any malignancy or chronic diseases.

Once potential participants were identified, they were contacted by phone to explain the study protocol. Participants were given as much time as they needed to think about the information and were encouraged to ask questions to the investigator, their healthcare provider, or other independent parties to help them decide whether to take part in the study. After this period of reflection, a visit was scheduled to conduct interviews with the participants according to the CRF (Appendix E).

The SSI sampling procedure involved collecting swabs from infection sites when the wound was opened for dressing (Figure 2.5). The swabs were taken before dressing, and the surrounding skin and mucosal surfaces were cleaned to prevent contamination prior to swabbing. Swabs were then sent to the MMC microbiology laboratory for culture and sensitivity testing. Primary cultures were performed in accordance with routine clinical microbiology protocols using MacConkey agar and blood agar, with incubation overnight at 37°C. In addition, chromogenic UTI agar (supplied specifically for this study) was used in parallel to enhance selective isolation and presumptive identification of Enterobacterales, with particular emphasis on *K. pneumoniae*. Colonies exhibiting chromogenic characteristics on chromogenic UTI agar with vancomycin (10 mg/L) (Liofilchem, Roseto, Italy), consistent with *K. pneumoniae* (green colonies), were subcultured and then transferred to Amies charcoal transport swabs and stored until they could be sent to the UK.

Figure 2.5 Collection of wound swabs from a patient with suspected SSIs at MMCH.

2.5.3 Collection and processing of urine samples

For UTI samples, patients visiting the outpatient department of MMCH with symptoms of UTI were enrolled in this study, based on the inclusion and exclusion criteria. The inclusion criteria were: (i) patients whose urine specimens were sent to the microbiology lab of MMCH for diagnostic purposes, (ii) patients had at least one of the following symptoms: fever (>38.0°C), suprapubic tenderness, costovertebral angle pain or tenderness, urinary urgency, dysuria, (iii) if patient had a urine culture with no more than two species of organisms identified, at least one of which is a bacterium of $\geq 10^5$ CFU/ml (iv) male or female, aged 18 years or above, (v) participants willing and able to give informed consent. The exclusion criteria were: (i) pregnant women, (ii) diabetic patients, and (iii) participants with any malignancy or chronic diseases.

Eligible participants were contacted by phone, informed about the study procedures, and consent was obtained. Interviews were then scheduled for those who agreed and were interviewed according to the CRF (Appendix E). Patients were instructed on the proper method for urine collection. Specimens were collected in sterile, screw-cap containers that were appropriately labelled according to standard procedures. The containers were then transferred to the MMC laboratory. Initial culture of urine specimens was performed following routine clinical diagnostic procedures using MacConkey agar and blood agar. To enhance selective recovery and presumptive identification of the uropathogen of interest, particularly *K. pneumoniae,* chromogenic UTI agar (supplied specifically for this study) was used concurrently for all urine samples. For processing, 30 ml of urine from each sample tube was transferred to 50 ml sterile conical centrifuge tubes and centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded, and 5 µl of the pellet was placed onto MacConkey agar, blood agar and chromogenic UTI agar media with vancomycin (10 mg/L) (Liofilchem, Roseto, Italy) using a micropipette. The plates were incubated at 37°C overnight and then inspected for growth. Green colonies, consistent with the chromogenic characteristics of *K. pneumoniae* on chromogenic UTI agar, were subcultured, then transferred to Amies charcoal transport swabs, and stored for shipment to the UK.

2.5.4 Collection of chicken cloacal swabs (CCS)

Samples were collected using Amies transport swabs with charcoal. Hygienic measures were properly followed during sample collection. Firstly, the bird was appropriately restrained by a person. Then the swab was removed from the package, and the cotton end (entire tip) was inserted into the cloaca through the vent carefully. The swab was moved in two to four circular motions while applying gentle pressure against the mucosal surface and then placed into the tube (Figure 2.6).

Figure 2.6 Collection of cloacal swabs from a chicken.

2.5.5 Collection of chicken meat swabs (CMS)

Chicken meat swabs were collected from retail meat shops within a 10 km radius of the selected poultry farms. In Bangladesh, live bird markets generally involve on-site slaughter and processing of birds (Figure 2.7). For the collection of CMS, sterile gloves were used and changed between two collections. The swab was removed from the package carefully and moistened in sterile phosphate-buffered saline (PBS). Then the swab was wiped over the preferred sites of the carcass. It was moved vertically and horizontally while applying gentle pressure against the surface. Then it was placed into the tube, tightening the cap and labelled properly.

Figure 2.7 Live bird market condition and sampling. A. slaughter area with poor hygiene, B. collection of meat swabs from a chicken.

2.5.6 Collection of domestic animal rectal swabs (DARS)

The animals (cattle and goats) were adequately restrained to avoid any injury during sample collection, and care was taken not to frighten the animals. Sampling was performed aseptically (Figure 2.8). The samples were collected using Amies charcoal transport swabs. The swab was removed from the package, and the cotton bud end of the swab was inserted about 5 cm into the anus and gently rotated for about 10 seconds. The swab was then removed, placed in the tube and labelled.

Figure 2.8 Collection of rectal swabs from a goat (left) and a calf (right).

2.5.7 Collection of bird faeces

Faeces from backyard poultry, pigeons, turkeys, and wild birds on the premises of the selected poultry farms and near the hospital area were collected. The bird droppings I collected were mostly from pigeons, sparrows, and common mynas. Sterile paper sheets were placed in the chosen areas (around the sheds on the farms, over the roof of the shed, and under the trees within the farm premises, on the wall of the hospital boundary and near the dustbin), and then I waited and checked periodically. At least 1 gram of freshly voided bird faeces was collected from the sterile surface and transferred to Amies transport media using a transport swab. Due to sampling difficulties and time constraints, I could not collect the required number of samples from free-flying birds from all the locations.

2.5.8 Collection and processing of water samples

Five points at each sampling site were selected, and water samples were collected from various sources around the hospital and farms, including drains, ponds, canals and the river (Figure 2.9). The water sample was collected aseptically using 50 ml sterile falcon tubes. The cap was removed (care was taken not to touch the underside of the lid), and the tube was filled up to 30 ml by inserting it horizontally to a depth of approximately 30 cm below the water surface. Then, the cap was closed immediately. The outside of the falcon tube was sterilised and placed in the tube rack after labelling. The tube rack was placed in the icebox to avoid sunlight exposure and then transported to the microbiology lab at BAU.

In the lab, the sample in a falcon tube was centrifuged at 3600 rpm for 10 minutes, and the supernatant was removed. The pellet was washed twice with 10 ml of Luria Broth. Then, 1.4 ml of sterile Luria broth was poured into the cryo-tube, and 1 ml of the pellet was added. 600 µl sterile glycerol was added to the tube and mixed properly to store the bacteria. The tube lid was sealed with parafilm tape and labelled. Then, they were stored at -80°C in cryoboxes until they were transferred to the UK.

Pond near the waste disposal area

Drain outside the hospital

Residential wastewater discharge

Drain inside the hospital

Farm wastewater discharge

Drain near the poultry farm

Figure 2.9 Collection of water samples from various sources.

2.5.9 Collection and processing of fly samples

Fly samples were collected using commercially available sticky fly traps placed in carefully chosen high-risk locations around the poultry farms and the hospital. Fly traps were positioned near waste pits, dustbins, and open drains as these sites are known aggregation points for synanthropic flies and represent potential interfaces for the dissemination of antimicrobial-resistant bacteria between animal, environmental, and human settings (Figure 2.10). At each sampling site, five trap locations were selected. The selection of trap locations was guided by ecological relevance and accessibility rather than random spatial sampling. To maintain consistency across sampling rounds, the same types of locations were targeted at each time point, although exact trap placement varied slightly according to local site conditions and operational feasibility.

After a 12-hour exposure period, the fly traps were collected carefully, and each trap was kept in a separate sterile plastic zipper bag. The bags were transported to the microbiology laboratory at BAU. The number of flies trapped in the fly trap varied depending on the location; however, five flies (one from each trap) were randomly selected for culture from each sampling site at each time point to maintain a consistent sample size. Flies from the trap were separated using sterile forceps and placed into sterile screw cap test tubes (5 ml) containing 3 ml of Luria broth (one fly in one tube). The screw cap test tubes were incubated at 37°C in a shaking incubator at 200 rpm overnight. To stock, 2.4 ml Luria broth from the screw cap test tubes was transferred into a cryo-tube, and 600 µl sterile glycerol was added and mixed properly. The tube lid was sealed with parafilm tape, labelled, and stored at -80°C in cryo-boxes until it was transferred to the UK.

Figure 2.10 Flies are trapped in the sticky fly trap.

2.5.10 Methodological considerations in the sampling approach

Although chromogenic UTI agar is primarily designed for urinary pathogens, it was used in this study as a selective and differential medium to facilitate targeted screening for *K. pneumoniae* across multiple sample types. The medium was employed as a supplementary research tool rather than a replacement for routine diagnostic culture, and all isolates underwent subsequent confirmatory analyses.

Environmental sampling was designed to capture *K. pneumoniae* populations and resistance signals from flies and water in epidemiologically high-relevance settings rather than to generate spatially representative prevalence estimates; findings should therefore be interpreted as indicators of presence and diversity in high-risk interfaces under a purposive, risk-based sampling strategy.

2.6 Collection of demographic and clinical data

During the collection of rectal swabs from healthy volunteers and samples from patients with UTIs and SSIs, demographic data and clinical histories were collected. In the case of data collection, the nature of the study was explained to each participant, and both verbal and written consent were obtained from them (Figure 2.11). The participants were interviewed based on a standardised case report form (CRF) developed for this study (Appendix E). In case of patients, the data collected in this study included participants' names, age, sex, locality, family member, clinical symptoms, reason for hospitalisation, ward name, type of specimen, outcome, date of admission, date of sample collection, ongoing antibiotics, and antibiotics used during hospitalisation. The data collected from healthy human volunteers included name, age, sex, locality, family member, animal rearing, sanitary status, previous hospitalisation history, and previous or current antibiotic use. The metadata collected in this study are described in Table 2.3.

Table 2.3 Definitions for metadata collected in this study.

Metadata	**Description**
Gender	Male or female
Village	Based on locality
Occupation	Whether the participants were either crop farmers, livestock farmers or unemployed
Education	Whether the participants were educated or uneducated
Methods of waste disposal	Either the household waste was disposed of near the house or away from the house in the waste pit
Raising domestic animals	Whether the participants raise any domestic animal or not
Own farm	Whether the participants were either farm owners or not
Drinking water availability	Source of daily drinking water (filtered water/tube well water/tap water)
Consumption of protein	How many days of the week the participants could have the main protein source, fish or chicken
Toilet facilities	Either a communal or a private toilet
Types of toilets	Water-based flush toilet/ connected to septic tank/ simple pit toilet
Access to soap and water	Whether the participants had access to soap and water after using the toilet
Previous antibiotic usage history within three months	Whether the participants had a history of taking antibiotics within 3 months of sampling or not
Antibiotics taken with or without a prescription from a physician	Whether the participants started antibiotics after consulting and being prescribed by a physician or not
Continuation of the antibiotics	Whether the participants completed the full course of the antibiotics or discontinued
Previous hospitalisation history	Whether the patients were admitted to the hospital within the last six months of sampling
Outcome	Whether the patients were discharged or dead

Figure 2.11 Data collection during sampling.

2.7 Transfer of biological specimens from Bangladesh to the UK

Clinical isolates, rectal swabs, and farm samples collected using Amies charcoal transport swabs (COPAN Diagnostics, VWR, UK) were transported to the UK in UN3373 containers (UN3373, Lelystad, the Netherlands) with appropriate labelling and proper documentation. The cryo-boxes containing flies and water samples were placed in UN3373 containers and transported using dry ice.

2.8 Isolation and identification of bacteria

After receiving the samples at my laboratory at the University of Oxford, clinical isolates from UTIs and SSIs were subcultured onto chromogenic UTI agar with vancomycin (10 mg/L) (Liofilchem, Roseto, Italy). Swab samples were initially plated onto chromogenic UTI agar containing vancomycin (10 mg/L), and then green colonies were picked and subcultured to obtain pure isolates. The growth conditions used for all strains were overnight incubation aerobically at 37°C. Fly samples were diluted 1:2000 (999.5 µl of sterile fresh Luria broth + 0.5 µl of growth from cryotube) in an eppendorf tube, and 100 µl from the tube was spread into chromogenic UTI agar with vancomycin (10 mg/L) using a spreader. In the case of water, 100 µl of the sample from the cryotube was transferred and spread into the chromogenic UTI agar plate with vancomycin. In both cases, the plates were incubated at 37°C overnight. Green colonies were picked and subcultured for the purification of colonies. Bacteria were isolated by colony colour and morphology on chromogenic UTI agar plates. The species were identified by Matrix-Assisted Laser Desorption/Ionisation–Time of Flight Mass Spectrometry, MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). A colony of each isolate from the pure culture was picked up with a sterile wooden stick/toothpick. The colony was spread in two spots of MSP 96 ceramic target (Bruker Daltonik GmbH, Germany) and covered with 1µL of HCCA matrix for MALDI-TOF MS (Bruker Daltonik GmbH, Germany) and left to dry at room temperature. The MALDI-TOF MS was performed using a Micro-flex LT instrument (Bruker Daltonik, Germany) operated in the linear positive ion mode (mass range 2–20 kDa) using Flex Control 3.3 software (Bruker Daltonik, Germany). The MALDI-TOF results were printed, and the isolates were labelled accordingly. All confirmed isolates were cryopreserved at –80 °C using cryopreservation storage beads (Technical Service Consultants Ltd, Lancashire, UK). WGS subsequently confirmed *Klebsiella* isolates identified by MALDI-TOF MS at the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA), and only WGS-confirmed *Klebsiella* isolates were included for analysis.

2.9 Determination of phenotypic resistance patterns

Minimum inhibitory concentration (MIC) was determined to assess the phenotypic resistance of *K. pneumoniae* and related species according to the latest guidelines outlined by European Committee on Antimicrobial Susceptibility Testing (EUCAST) (v.12.0) (EUCAST, 2022). The same interpretive cut-offs were applied uniformly across all isolates, irrespective of sample sources. The agar dilution method was implemented to determine the MIC of clinically relevant antimicrobials (Andrews, 2001). Sixteen antimicrobials (amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftazidime-avibactam, cefotaxime, cefepime, imipenem, meropenem, ciprofloxacin, levofloxacin, aztreonam, amikacin, gentamicin, tigecycline, fosfomycin, and colistin) underwent MIC testing in this study (Table 2.4).

Table 2.4 Details of antimicrobials and inhibitors used for susceptibility testing.

Name	Abbreviation	Breakpoint	Manufacturer	Solvent	Diluent
Amikacin	AMK	(S≤8, R>8)	Sigma-Aldrich, Missouri, USA	Water	Water
Amoxicillin-clavulanate (4:1)	AMC	(S≤8, R>8)	Sigma-Aldrich, Missouri, USA	DMSO+ NaHCO3 /Water	Water
Aztreonam	ATM	(S≤1, R>4)	Sigma-Aldrich, Missouri, USA	NaHCO3	Water
Cefepime	FEP	(S≤1, R>4)	Thermo Fisher Scientific Inc., MA, USA	Water	Water
Cefotaxime	CTX	(S≤1, R>2)	MedChemExpress, NJ, USA	Water	Water
Ceftazidime	CAZ	(S≤1, R>4)	MedChemExpress, NJ, USA	NaHCO3	Water
Ceftazidime-avibactam	CZA	(S≤8, R>8)	MedChemExpress, NJ, USA	NaHCO3 + Water	Water
Ciprofloxacin	CIP	(S≤0.25, R>0.5)	Sigma-Aldrich, Missouri, USA	1 ml HCL + Water	Water
Colistin	CST	(S≤2, R>2)	Sigma-Aldrich, Missouri, USA	Water	Water

Table continued.

Name	**Abbreviation**	**Breakpoint**	**Manufacturer**	**Solvent**	**Diluent**
Fosfomycin-Glucose-6-Phosphate	FOS	(S≤8, R>8)	Sigma-Aldrich, Missouri, USA	Water	Water
Gentamicin	GEN	(S≤2, R>2)	Sigma-Aldrich, Missouri, USA	Water	Water
Imipenem	IPM	(S≤2, R>4)	Sigma-Aldrich, Missouri, USA	$NaHCO3$	Water
Levofloxacin	LVX	(S≤0.5, R>1)	Sigma-Aldrich, Missouri, USA	Water	Water
Meropenem	MEM	(S≤2, R>8)	Sigma-Aldrich, Missouri, USA	Water	Water
Piperacillin-tazobactam	TZP	(S≤8, R>8)	LKT Laboratories, MN, USA	DMSO + $NaHCO3$	Water
Tigecycline	TGC	(S≤0.5, R>0.5)	Sigma-Aldrich, Missouri, USA	Water	Water

S, sensitive; R, resistant.

Mueller Hinton agar plates containing antibiotics of interest ranging from 0.06 µg/ml to 64 µg/ml were prepared, and 10^4 CFU/spot of each strain of up to 96 bacterial colonies per plate were applied by multi-point inoculator. The plates were then incubated overnight at 37ºC. The lowest concentration of the plate showing no growth was the MIC value of the strain. AST were interpreted according to EUCAST clinical breakpoints or epidemiological cut-off (ECOFF) value (EUCAST, 2022). *K. pneumoniae* ATCC 700603 strain was used as the reference strain for determining MIC values. All antibiotics were prepared to a concentration of 2560 mg/L (stock A), with serial dilution to reach the desired concentration (Table 2.5). Stock solutions were prepared using the following formula:

$$\frac{1000}{\text{Potency x 10}} \text{ x } V \text{ x } C = W$$

P = potency given by the manufacturer (µg/mg)

V = volume required (mL)

C = final concentration of solution (multiples of 1000) (mg/L)

W = weight of antibiotic in mg to be dissolved in volume V (mL).

Table 2.5 Serial dilution of antimicrobials to reach the desired concentration.

Stock solution	**Serial dilution**	**Concentration (µg/ml)**
Stock A (2560 mg/L)	880 µl of stock A in 35 ml agar	64
	440 µl of stock A in 35 ml agar	32
	220 µl of stock A in 35 ml agar	16
	110 µl of stock A in 35 ml agar	8
Stock B (80 mg/L: 500 µl of stock A+15.5 ml solvent)	1.75 ml of stock B in 35 ml agar	4
	880 µl of stock A in 35 ml agar	2
	440 µl of stock A in 35 ml agar	1
	220 µl of stock A in 35 ml agar	0.5
	110 µl of stock A in 35 ml agar	0.25
Stock C (2.5 mg/L: 500 µl stock B + 15.5 ml solvent)	1.75 ml of stock B in 35 ml agar	0.125
	880 µl of stock A in 35 ml agar	0.06

2.10 Whole genome sequencing

2.10.1 DNA extraction

QIAcube automated extraction system (Qiagen, Hilden, Germany) was used for extracting DNA. For DNA extraction, firstly, I revived my isolates from -80°C by culturing them on UTI chromogenic agar at 37°C overnight. Then, a pure isolated bacterial colony was subcultured into an eppendorf containing 2 ml of fresh Luria broth and incubated for five hours at 37°C in a shaking incubator. After five hours of incubation, 18 µl of chloramphenicol (prepared by adding 18 mg of chloramphenicol to 1ml freshly prepared 70% ethanol) was added to each eppendorf and again set in the shaking incubator for another hour. After six hours, the culture was centrifuged at 13,000 rpm for 10 minutes. The supernatant was then discarded, and the pellet was used for DNA extraction using QIAcube (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The extracted DNA was preserved at -20°C until use.

2.10.2 DNA quantification

The resulting genomic DNA was quantified using Qubit 3.0 (Thermo Fisher Scientific, Waltham, USA). For each standard sample and DNA sample, 200 µl of working solution was prepared by diluting the Qubit reagent 1:200 in Qubit buffer. Assay tubes for standards were prepared by mixing 10 µl of the standard and 190 µl of the working solution. From each DNA sample, 2 µl of DNA was added into 198 µl working solution to measure the DNA concentration. Following 2 minutes of incubation at room temperature, assay tubes were inserted in the Invitrogen Qubit Fluorometer (Thermo Fisher Scientific, Waltham, USA) to take the readings.

2.10.3 Short read sequencing

For my study isolates, short-read sequencing was performed by MicrobesNG (Birmingham, UK) using the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) with an intended sequencing depth of approximately 30x coverage per isolate. Prior to shipment, isolates were revived from -80°C and cultured on UTI chromogenic agar plates at 37°C. After overnight incubation, a single colony from an agar plate was mixed with 200 µl of 1x PBS, of which 100 µl was added to 10 ml of sterile Luria broth. The bacterial cells in Luria broth were incubated at 37°C in a shaking incubator for 6 hours to obtain an optical density (OD600) of 1. The harvested bacterial cells in broth were then centrifuged at 8000 rcf for 3 minutes. After discarding the supernatant, the bacterial pellet was washed twice with 1 ml PBS. The pellet was resuspended with 0.5 ml of 1x DNA Shield buffer, provided by the sequencing service (https://microbesng.com/). The bacterial cells in DNA Shield buffer were transferred into a 2 ml screw cap tube, labelled and then shipped for sequencing to MicrobesNG (Birmingham, UK). For each isolate, sample information and the expected species were uploaded to the MicrobesNG online system. MicrobesNG generated paired-end Illumina reads and performed primary read-level and sample-level quality control (QC).

2.10.4 Long read sequencing

Long-read sequencing was performed on the PromethION platform (Oxford Nanopore Technologies, Oxford, UK). Genomic libraries for long read sequencing were prepared using Rapid Barcoding Kit 96

V14Q20+ Kit14 (SQK-RBK114.96) and barcoded DNA loaded onto R10.4.1 flow cell (FLO-PRO114M) following the manufacturer's protocol. Sequencing was run on PromethION 2 Solo using MinKNOW Software (24.02.8), producing raw signal data in pod5 format.

2.11 Bioinformatic analysis

2.11.1 Assembly and quality assessment workflow of sequencing data

Bioinformatic processing followed a structured workflow comprising read-level QC, de novo assembly, and assembly-level QC. Illumina paired-end reads were generated by MicrobesNG (Birmingham, UK) to an expected sequencing coverage (depth and breadth) of 30x per isolate. MicrobesNG performed initial read processing and QC, including adapter removal and quality trimming using Trimmomatic. Post-trimming assessment was performed using MicrobesNG in-house scripts that incorporated bwa-mem, SAMtools, and BedTools to summarise read- and mapping-based metrics.

To verify sample identity and assess potential contamination or mixed cultures, MicrobesNG generated taxonomic composition summaries using Kraken, reporting the proportion of unclassified reads and the most abundant and second-most abundant taxa at family and genus levels, as well as the most abundant species. The report additionally included the proportion of reads assigned to *Escherichia coli* as a specific indicator of potential non-target signal. Assembly-level QC metrics were calculated using QUAST, and MicrobesNG flagged samples with atypical GC% and/or assembly size relative to the declared taxon. In addition, 16S rRNA gene sequences detected in assemblies were compared against the SILVA 16S database to provide an independent check of sample taxonomy.

For Illumina short reads, additional read-level QC was performed using FastQC (v.0.11.9) and read were assembled into contigs (.fasta) using Shovill, which incorporated adapter trimming, assembly with SPAdes/SKESA/Megahit, and correction of minor assembly errors (v.1.1.0-foss-2018b-Python-2.7.15). Assembly metrics were evaluated using Quast (v.2.1), including total assembly length, number of contigs, N50, GC%, and related statistics. The de novo assemblies were then annotated using Bakta (v.1.9.3).

Assemblies were considered consistent with *K. pneumoniae* when overall genome size (approximately 5.5 Mbp) and GC content (around 57.08%) aligned with expectations for the species and when taxonomic profiling (Kraken) and in-house species confirmation on assembled contigs (KmerFinder v.3.0.2) supported *K. pneumoniae* as the dominant organism. Assemblies that were inconsistent with expected *Klebsiella* genome characteristics (e.g., unusually large/small genome size, atypical GC%, or extreme fragmentation, high contig counts, very low N50) were flagged and reviewed alongside Kraken taxonomic profiles and provider QC outputs. Where Kraken and/or provider QC indicated substantial proportions of non-*Klebsiella* reads, or where other QC indicators suggested mixed species, isolates were treated as potentially contaminated and excluded from *K. pneumoniae*-specific downstream comparative analyses unless single-colony purification and re-sequencing yielded taxonomically consistent reads and assemblies.

For long-read sequencing, the raw data (.pod5) were processed using the Dorado basecaller (v.0.7.2) to obtain raw reads in FASTQ format. Then duplex basecalling was performed using dna_r10.4.1_e8.2_400bps_sup@v4.2.0 model. The reads were assembled into contigs using Flye (v.2.9.4), followed by assembly polishing using Medaka (v.1.12.0). Where both data types were available, Unicycler (v.0.5.0) was used to yield hybrid assemblies integrating both short reads (.fastq) and long reads (.fastq) data to improve assembly contiguity and plasmid resolution.

2.11.2 Genomic characterisation of assemblies

Whole-genome assemblies were analysed using a combination of pipelines and databases from the Centre for Genomic Epidemiology (CGE, https://www.genomicepidemiology.org) and other curated resources. The de novo assemblies were subjected to downstream analysis using an in-house pipeline that incorporated KmerFinder (v.3.0.2) for species identification on assembled contigs, pubMLST database for multilocus sequence typing (MLST) and ABRicate against curated databases including ResFinder (v.4.6.0) and CARD (Comprehensive Antibiotic Resistance Database, v.4.0.1) for AMR genes screening, VFDB (Virulence Finder Data Base) (v.3.2.0) for detecting virulence-associated genes, and PlasmidFinder (v.2.1.1) to screen plasmid replicon types. Additionally, Kleborate (v.3.0) was used to determine virulence and AMR profiles, while Kaptive was used to identify surface antigens (K and O antigen loci) of *K. pneumoniae* (Wyres *et al.,* 2016; Lam *et al.,* 2021).

2.12 Phylogenetic analysis

To investigate evolutionary relationships among the *Klebsiella* isolates, a core-genome phylogeny was inferred using a mapping-based workflow followed by tree inference and visualisation. Read mapping, variant calling, and generation of a pseudogenome alignment from high-quality positions were performed using Bactmap (v.1.0.0). The aligned sequences were then processed with the VeryFastTree (v.4.0), an optimised variant of the FastTree algorithm, to construct an approximately maximum-likelihood (ML) phylogenetic tree.

VeryFastTree was selected due to its scalability and computational efficiency, enabling the analysis of large bacterial genomic datasets with reduced run time (Piñeiro & Pichel, 2024). The tree file generated was subsequently uploaded to the Interactive Tree of Life (iTOL, v.6) platform for visualisation. iTOL was employed to annotate and customise the phylogenetic tree, including the integration of metadata such as sample origin, AMR profiles, and other epidemiological characteristics.

In addition to the core genome ML phylogeny, allele-based relatedness was summarised using minimum spanning trees constructed from core genome MLST profiles. These minimum spanning trees were used to visualise clustering patterns and the distribution of STs and species across the dataset. For the ST-focused analysis, a core genome MLST-based minimum spanning tree (“GrapeTree”) was generated and annotated using ReporTree (web-based pipeline) to visualise the distribution of isolates across STs and related clusters (Mixão *et al*., 2023). Another minimum spanning tree was constructed from the core loci and annotated using GrapeTree to summarise inter-species structure (Zhou *et al*., 2018).

For fine-scale genetic distance estimation, SNP (single-nucleotide polymorphism) calling was performed using Snippy (v.4.4.5), followed by recombination removal using Gubbins (v.2.3.4) (Croucher *et al*., 2015), and pairwise SNP calculation using pairsnp (v.0.0.7) (GitHub, 2018). SNP-based network graphs were deployed to plot putative transmission and epidemiological links using

Cytoscape (v.3.10.1) with the clusters having the following criteria: 1) contained isolates that differed by ≤30 SNPs, 2) contained clinical isolates, and 3) consisted of at least five isolates (Stimson *et al*., 2019; Azarian & Black, 2024).

2.13 Plasmid analysis and visualisation

Plasmid assemblies and annotations were performed using Geneious Prime (v.2025.2.2, Biomatters Ltd.), which enabled the detection of plasmid backbones, AMR genes, and associated mobile elements. Plasmid-based clustering was performed using plasmid replicon presence–absence profiles identified by PlasmidFinder. Replicon data were encoded as binary variables, and pairwise dissimilarities between isolates were calculated using Jaccard distance (Jaccard, 1901; Legendre & Legendre, 2012). Hierarchical clustering was conducted using average linkage to assess similarity in plasmid content across isolates. To compare plasmid sequences, the BLAST Ring Image Generator (BRIG, v.0.95) (Alikhan *et al*., 2011) was used, providing visual insights into sequence homology and structural differences. High-resolution linear plasmid maps were then generated and refined with EasyFig (v.2.2.5), a genome visualisation platform, to highlight regional similarities across plasmids.

2.14 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (v.30.0). Associations between categorical variables were assessed using the Chi-square (χ^2) test, Fisher's exact test, where appropriate. Univariate logistic regression analysis was used to estimate odds ratios and 95% confidence intervals. For comparisons involving outcomes expressed as counts with varying denominators across groups, log-linear Poisson regression models incorporating a log offset were applied to estimate prevalence ratios (PRs) and corresponding 95% confidence intervals. Where applicable, multivariate models with nested effects were applied to account for clustering of isolates nested within higher-level groupings. Statistical significance was defined as $p<0.05$.

Chapter 3

A One Health Approach to Investigate the Prevalence of *Klebsiella pneumoniae* in Mymensingh District of Bangladesh

3.1 Introduction

Klebsiella pneumoniae is a prominent member of Gram-negative Enterobacterales responsible for a wide range of infections in humans, animals, and the environment, making it a significant public health concern. It is typically an opportunistic hospital-associated pathogen causing pneumonia, urinary tract infections (UTIs), wound infections and bloodstream infections (BSIs), particularly in neonates and immunocompromised patients (Lam *et al.,* 2021). It can act as an aggressive pathogen, causing severe CAIs, including soft tissue infections, pyogenic liver abscesses and meningitis, especially in diabetic and alcoholic patients (Stojowska-swędrzyńska *et al.,* 2021). *K. pneumonia*e can cause diseases in farm animals, such as mastitis in cattle, endometritis, infertility in the mare, and upper respiratory tract infections in the broiler (Zheng *et al.,* 2022; Song *et al.,* 2023; Alchalaby *et al.,* 2024; Scholtz *et al.,* 2024). This bacterium is frequently found in water, soil, sewage, and hospital wastewater, likely owing to contamination that contributes to its environmental persistence (Denissen *et al.,* 2022).

K. pneumoniae exhibits a complex transmission cycle across humans, animals, and the environment (Wyres & Holt, 2018). Human-to-human transmission primarily occurs in healthcare settings through direct contact (e.g., contaminated hands of healthcare personnel) or indirect contact (via fomites, medical devices, and hospital surfaces) (Podschun & Ullmann, 1998). Contaminated meat and dairy products may serve as foodborne transmission routes, leading to human colonisation and infections. Farmers, veterinarians, and animal handlers are at higher risk of exposure due to close contact with colonised animals (Zong *et al.*, 2021). Contaminated water sources, particularly those affected by hospital or agricultural runoff, insects, and flies, can serve as a medium for the dissemination of antimicrobial-resistant strains (Hassan *et al.,* 2021; Zhang *et al.,* 2023; Cook *et al.,* 2025). Biofilms formed on medical equipment, water pipes, and wastewater treatment facilities enhance the survival and transmission potential of this bacterium (Shankar *et al.*, 2019). *K. pneumoniae* generally shows a higher transmission rate compared to *Escherichia coli*, particularly in healthcare environments, due to biofilm formation and environmental persistence (Wyres & Holt, 2018). The presence of mobile genetic elements (e.g., plasmids, integrons, transposons) allows horizontal transfer of resistance genes between environmental, animal, and human strains (Peirano *et al.*, 2020). The emergence of MDR and hypervirulent strains of *K. pneumoniae* has further compounded its threat (Wyres *et al.,* 2020). The transmission of *K. pneumoniae* across humans, animals, and environmental reservoirs makes it a key pathogen within the One Health framework (Wyres & Holt, 2018).

In Bangladesh, the prevalence of *K. pneumoniae* infections has been a growing concern, with limited data available on its distribution across different ecosystems. Several studies have highlighted the prevalence of *K. pneumoniae* in different regions of Bangladesh. For instance, a study conducted in Chattogram reported a significant presence of MDR *K. pneumoniae* in clinical isolates (Tanni *et al.,* 2021). A recent study from Dhaka found a high prevalence of CR-KP in both human stool samples and household water sources, indicating potential environmental reservoirs (Kar *et al.,* 2024). These findings underscore the widespread nature of *K. pneumoniae* across various settings in Bangladesh.

Mymensingh district, situated in the northern part of the country, offers a unique environment to investigate the prevalence of *K. pneumoniae* due to its dynamic interplay between humans, livestock, and the environment. Mymensingh city exhibits a high population density, with approximately 44,458 individuals per square kilometre, making it one of the most densely populated cities in Bangladesh, serviced by a single government tertiary care hospital. As of the 2022 census, Mymensingh District in Bangladesh has a population of 5,899,005 with a population density of about 1,400 people per square kilometre (Mymensingh - Wikipedia). Mymensingh is a notable hub for poultry farming in Bangladesh, encompassing both small-scale backyard hobby farms to large commercial enterprises. Studies indicate that in selected areas of Mymensingh, the average household rears approximately 10.4 chickens and 9.95 ducks (Alam *et al.,* 2014). The district is also prominent in dairy farming activities (Rahman *et al.,* 2020). As a region with significant agricultural activity, particularly in poultry and dairy farming, close human-animal-environment interactions may contribute to the spread and persistence of *K. pneumoniae* (Khan *et al.,* 2020; Nahar *et al.,* 2022; Supty *et al.,* 2023). Furthermore, the overuse of antibiotics in human healthcare and animal husbandry exacerbates the risk of resistance development (Noman *et al.,* 2024). Considering the facts, this chapter aims to adopt a One Health approach to investigate the prevalence of *K. pneumoniae* in Mymensingh district. Adopting a One Health approach, which integrates human, animal, and environmental health, offers a comprehensive framework to understand this issue, which will help to track AMR trends and inform strategies for effective surveillance and intervention in the region.

The specific objectives for this chapter are as follows:

1. To estimate the prevalence of *K. pneumoniae* from various sample sources and to investigate whether the prevalence significantly varies across these sources.
2. To investigate whether the prevalence of *K. pneumoniae* from individual sample sources significantly varies between different sampling locations.
3. To examine the variation in the prevalence of *K. pneumoniae* from individual sample sources across different sampling periods.

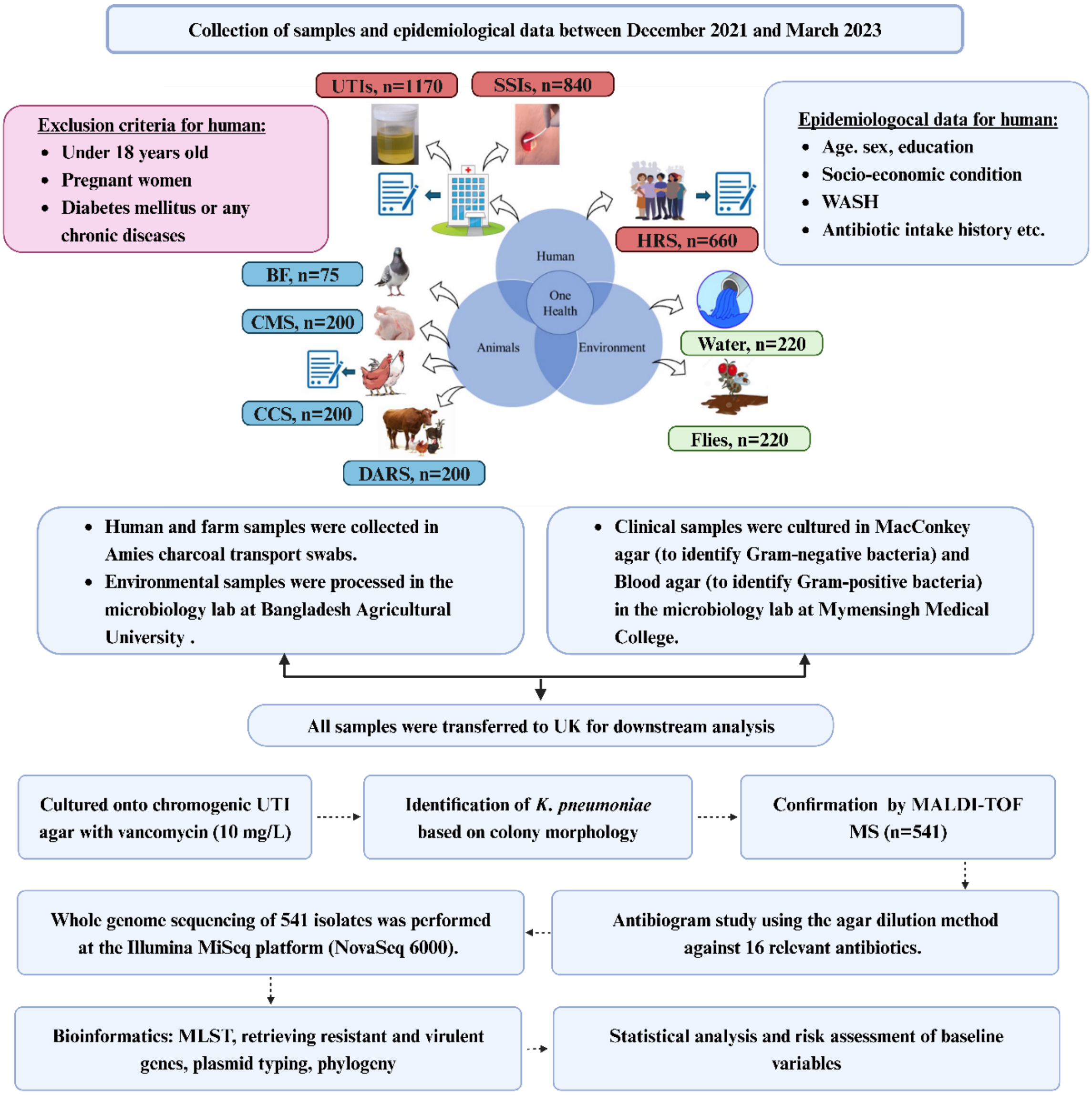
3.2 Results

3.2.1 Prevalence of *K. pneumoniae* in various samples

A total of 3759 samples were collected from humans (rectal swabs from healthy human volunteers, urine from outpatients with suspected UTIs, and wound swabs from patients with SSIs admitted to the ward), farms (CCS, CMS, DARS, BF) and the environment (water, flies) in Mymensingh, Bangladesh, from December 2021 to March 2023. The study outline is illustrated in Figure 3.1. The overall prevalence of *K. pneumoniae* isolated from various samples in the study area was 14.4 % (541/3759), with the highest prevalence (36.4%, 80/220) in flies (Table 3.1).

Table 3.1 Prevalence of *K. pneumoniae* from various sample sources.

Sample source	**Number of samples**	***K. pneumoniae* positive**	**Prevalence**
Water	220	25	11.4%
Flies	220	80	36.4%
Chicken cloacal swabs, CCS	200	62	31.0%
Chicken meat swabs, CMS	200	54	27.0%
Domestic animal rectal swabs, DARS	200	15	7.5%
Bird faeces, BF	75	13	17.3%
Urinary tract infections, UTIs	1170	14	1.2%
Surgical site infections, SSIs	814	93	11.4%
Human rectal swabs, HRS	660	185	28.0%
Total	**3759**	**541**	**14.4%**



Collection of samples and epidemiological data between December 2021 and March 2023

Exclusion criteria for human:

- Under 18 years old
- Pregnant women
- Diabetes mellitus or any chronic diseases

Epidemiologocal data for human:

- Age. sex, education
- Socio-economic condition
- WASH
- Antibiotic intake history etc.

- Human and farm samples were collected in Amies charcoal transport swabs.
- Environmental samples were processed in the microbiology lab at Bangladesh Agricultural University .

- Clinical samples were cultured in MacConkey agar (to identify Gram-negative bacteria) and Blood agar (to identify Gram-positive bacteria) in the microbiology lab at Mymensingh Medical College.

All samples were transferred to UK for downstream analysis

Cultured onto chromogenic UTI agar with vancomycin (10 mg/L)

Identification of *K. pneumoniae* based on colony morphology

Confirmation by MALDI-TOF MS (n=541)

Antibiogram study using the agar dilution method against 16 relevant antibiotics.

Whole genome sequencing of 541 isolates was performed at the Illumina MiSeq platform (NovaSeq 6000).

Bioinformatics: MLST, retrieving resistant and virulent genes, plasmid typing, phylogeny

Statistical analysis and risk assessment of baseline variables

Figure 3.1 Schematic flow diagram of the study.

N, number; HRS, human rectal swabs; UTIs, urinary tract infections; SSIs, surgical site infections; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; WASH, water, sanitation and hygiene; MALDI-TOF MS, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; MLST, multi-locus sequence typing.

In addition to estimating prevalence, differences in *K. pneumoniae* positivity across various sample sources were evaluated using a log-linear Poisson regression model with a log offset for the total number of samples tested per source, thereby accounting for unequal sampling denominators. Using BF as the reference category, *K. pneumoniae* positivity was significantly higher in flies (prevalence ratio, PR, 2.10; 95% CI: 1.17–3.77; *p*=0.013). Although elevated prevalence ratios were also observed for CCS (PR, 1.79; 95% CI: 0.98–3.25) and HRS (PR, 1.62; 95% CI: 0.92–2.84), these associations did not reach conventional levels of statistical significance (Table 3.2).

Table 3.2 Log-linear (Poisson) regression analysis of *K. pneumoniae* positivity across different sample sources.

Sample source	***K. pneumoniae* positive (n = 541)**	***K. pneumoniae* negative (n = 3218)**	***p*-value**	**PR**	**95% CI**
Water (n=220)	25 (4.6%)	195 (6.1%)	0.217	0.66	0.34 – 1.28
Flies (n=220)	80 (14.8%)	140 (4.4%)	0.013	2.1	1.17 – 3.77
CCS (n=200)	62 (11.5%)	138 (4.3%)	0.057	1.79	0.98 – 3.25
CMS (n=200)	54 (10.0%)	146 (4.5%)	0.151	1.56	0.85 – 2.85
DARS (n=200)	15 (2.8%)	185 (5.7%)	0.027	0.43	0.21 – 0.91
BF (n=75)	13 (2.4%)	62 (1.9%)	—	—	—
UTIs (n=1170)	14 (2.6%)	1156 (35.9%)	<0.001	0.07	0.03 – 0.15
SSIs (n=814)	93 (17.2%)	721 (22.4%)	0.159	0.66	0.37 – 1.18
HRS (n=660)	185 (34.2%)	475 (14.8%)	0.094	1.62	0.92 – 2.84

A log-linear Poisson regression model with a log offset for the total number of samples tested per source was used to estimate prevalence ratios (PRs) of *K. pneumoniae* positivity across sample sources. Observed counts and percentages are presented for descriptive purposes. Bird faeces (BF) was used as the reference category; therefore, PRs, confidence intervals (CI) and *p*-values are not shown for this group. Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$. n, number; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs.

3.2.2 Prevalence of *K. pneumoniae* in various sampling locations

Samples were collected from five different subdistricts in the Mymensingh district of Bangladesh. Clinical samples were collected only from Mymensingh Medical College Hospital (MMCH). Farm samples were collected from a broiler and a layer farm in each selected subdistrict, whereas human and environmental samples were collected within a 10 km radius of the selected poultry farms and the MMCH. The distribution of *K. pneumoniae* in different sampling sites isolated from various sources is presented in a tabular form (Table 3.3).

Using log-linear Poisson regression models with offsets for the number of samples tested, spatial variation in *K. pneumoniae* positivity was evaluated for flies, CCS and CMS across different subdistricts. Within each sample source, Gauripur was used as the reference subdistrict for estimation of prevalence ratios. Other sample sources, including BF, DARS, water and HRS, were not included in spatial regression analyses due to sparse counts, zero events in multiple subdistricts, or non-comparable sampling frameworks. Clinical samples (UTIs and SSIs) were also excluded from the analysis since they were collected only from MMCH in the Mymensingh Sadar subdistrict. In case of flies, *K. pneumoniae* positivity was significantly higher in Mymensingh Sadar (33/60, 55.0%) (p=0.002). For CCS, *K. pneumoniae* positivity was significantly higher in Trishal (18/40, 45.0%) (p=0.032) (Table 3.4).

Table 3.3 Distribution of *K. pneumoniae* across selected subdistricts with respective sampling sites from different sources.

Attributes	**Subdistricts with respective sampling sites in each subdistrict**										
	Mymensingh Sadar			**Muktagachha**		**Trishal**		**Gauripur**		**Tarakanda**	
Source	MMCH	MyB	MyL	MkB	MkL	TrB	TrL	GB	GL	TaB	TaL
Water (n=25)	6	4	–	3	3	5	–	2	2	–	–
Flies (n=80)	12	11	10	4	3	5	5	5	12	5	8
CCS (n=62)	–	9	4	13	2	10	8	3	6	4	3
CMS (n=54)	–	5	6	–	2	6	9	7	7	9	3
DARS (n=15)	–	2	3	2	1	–	2	1	2	1	1
BF (n=13)	–	8	–	–	3	–	2	–	–	–	–
UTIs (n=14)	14	–	–	–	–	–	–	–	–	–	–
SSIs (n=93)	93	–	–	–	–	–	–	–	–	–	–
HRS (n=185)	15	19	20	12	13	20	18	13	19	18	18
Kp positive	140	58	43	34	27	46	44	31	48	37	33
Total Sample	2084	207	160	160	169	160	168	165	160	164	162
Prevalence, %	6.7	28.0	26.9	21.3	16.0	28.8	26.2	18.8	30.0	22.6	20.4

n, number; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs; MMCH, Mymensingh Medical College Hospital; MyB, Mymensingh broiler; MyL, Mymensingh layer; MkB, Muktagachha broiler; MkL, Muktagachha layer; TrB, Trishal broiler; TrL, Trishal layer; GB, Gauripur broiler; GL, Gauripur layer; TaB, Tarakanda broiler; TaL, Tarakanda layer farms.

Table 3.4 Log-linear (Poisson) regression analysis of *K. pneumoniae* positivity across different subdistricts for selected sample sources.

Sample source	**Subdistrict**	***K. pneumoniae* (positive/total, %)**	**PR**	**95% CI**	**p-value**
Flies	Mymensingh Sadar	33/60 (55.0)	2.04	1.29 – 3.23	0.002
	Muktagachha	7/40 (17.5)	0.41	0.19 – 0.90	0.026
	Trishal	10/40 (25.0)	0.62	0.32 – 1.21	0.162
	Tarakanda	13/40 (32.5)	0.86	0.47 – 1.57	0.617
	Gauripur (Ref.)	17/40 (42.5)	1	—	—
CCS	Mymensingh Sadar	13/40 (32.5)	1.18	0.64 – 2.17	0.593
	Muktagachha	15/40 (37.5)	1.42	0.79 – 2.58	0.245
	Trishal	18/40 (45.0)	1.85	1.06 – 3.25	0.032
	Tarakanda	7/40 (17.5)	0.78	0.37 – 1.64	0.516
	Gauripur (Ref.)	9/40 (22.5)	1	—	—
CMS	Mymensingh Sadar	11/40 (27.5)	0.79	0.42 – 1.48	0.46
	Muktagachha	2/40 (5.0)	0.16	0.04 – 0.68	0.012
	Trishal	15/40 (37.5)	1.07	0.61 – 1.90	0.812
	Tarakanda	12/40 (30.0)	0.86	0.47 – 1.57	0.631
	Gauripur (Ref.)	14/40 (35.0)	1	—	—

Log-linear Poisson regression models with a log offset for the total number of samples tested per subdistrict were fitted separately for flies, CCS (chicken cloacal swabs), and CMS (chicken meat swabs) to estimate prevalence ratios (PRs) of *K. pneumoniae* positivity across five different subdistricts. Within each sample source, Gauripur was used as the reference (Ref.) subdistrict. Positivity is presented as the number of *K. pneumoniae*–positive samples divided by the total number tested, with percentages shown in parentheses. Sample sources with sparse counts or non-comparable sampling frameworks were excluded from spatial modelling. Statistical significance was defined as $p<0.05$.

Study participants with suspected UTIs (n=1170) were from various areas within (n=1150) and outside (n=20) the Mymensingh district. Among the study areas, the highest number of participants were from Mymensingh Sadar subdistrict (53.9%, 631/1170), followed by Trishal (8.1%, 95/1170), Tarakanda (6.4%, 75/1170), Gauripur (4.4%, 51/1170) and Muktagachha (3.7%, 43/1170). Of the 14 patients with confirmed UTIs by *K. pneumoniae*, 12 were from the Mymensingh Sadar subdistrict, while one was from Trishal and one from Tarakanda. When comparing patients from different subdistricts, the odds of *K. pneumoniae* positivity among patients with suspected UTIs was significantly higher among those from Mymensingh Sadar (OR, 5.21; 95% CI: 1.16–23.36, *p*=0.031) (Table 3.5). Out of 814 hospitalised patients with SSIs, *K. pneumoniae* was recovered from the wound swabs of 93 patients, of which 92.5% (86/93) were considered as hospital-acquired infections (samples were collected after 48 hours of hospital admission).

Table 3.5 Univariate logistic regression analysis for odds of *K. pneumoniae* positivity across patients suspected of UTIs from different subdistricts.

Subdistrict	***K. pneumoniae* positive (n=14)**	***K. pneumoniae* negative (1156)**	***p*-value**	**OR**	**95% CI**
Mymensingh Sadar (n=631)	12 (85.7%)	619 (53.5%)	0.031	5.205	1.160 - 23.361
Trishal (n=95)	1 (7.1%)	94 (8.1%)	0.893	0.869	0.112 - 6.716
Tarakanda (n=75)	1 (7.1%)	74 (6.4%)	0.910	1.125	0.145 - 8.715

Univariate logistic regression was used to examine associations between the subdistrict of residence and *K. pneumoniae* positivity among individuals with suspected UTIs. Odds ratios (ORs) with 95% confidence intervals (CIs) represent relative odds of *K. pneumoniae* detection within this clinical cohort. Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$.

3.2.3 Prevalence of *K. pneumoniae* in different sampling periods

Samples were collected from farms, human volunteers, and the environment every four months during the 16-month sampling period. In contrast, hospital samples were collected continuously throughout this period. To observe the effect of seasonal variation on prevalence, the sampling period was divided into four quarters, each lasting four months (Table 3.6).

Table 3.6 Sampling periods with respect to months and seasons of the year.

Sampling quarter	**Month and year**	**Season**
Quarter 1 (Q–1)	December 2021 – January 2022	Winter
	February 2022 – March 2022	Spring
Quarter 2 (Q–2)	April 2022 – May 2022	Summer
	June 2022 – July 2022	Monsoon
Quarter 3 (Q–3)	August 2022 – September 2022	Autumn
	October 2022 – November 2022	Late autumn
Quarter 4 (Q–4)	December 2022 – January 2022	Winter
	February 2023 – March 2023	Spring

The average climatic parameters of Mymensingh during different months of the year were retrieved from Weather Atlas (https://www.weather-atlas.com/en/bangladesh/mymensingh-climate, last updated on 30 Jan 2024). Weather data of the Mymensingh region shows that April is the warmest month, with the highest average high temperature of 33.4°C, while January is the coldest month, with the lowest average low temperature of 11°C (Figure 3.2). June is the wettest month, recording the highest precipitation at 469 mm, while December is the driest month, receiving only 2 mm of rainfall (Figure 3.3). Regarding relative humidity, June and August have the highest levels at 75%, whereas March experiences the lowest relative humidity at 32% (Figure 3.4). Analysis according to the sampling periods shows that during the months of December, January, March, and April, which encompass winter and spring, the average temperature, rainfall, and humidity remain low. April and May comprise summer when the average temperature reaches its peak and precipitation humidity starts rising. Then, the monsoon remains in June and July, when the highest precipitation occurs, and the temperature remains warmer. The months of August, September, October and November comprise autumn and late autumn. During August and September, average temperature, precipitation and humidity remain higher and feel warmer. However, temperature, precipitation, and humidity start to decline during late autumn.

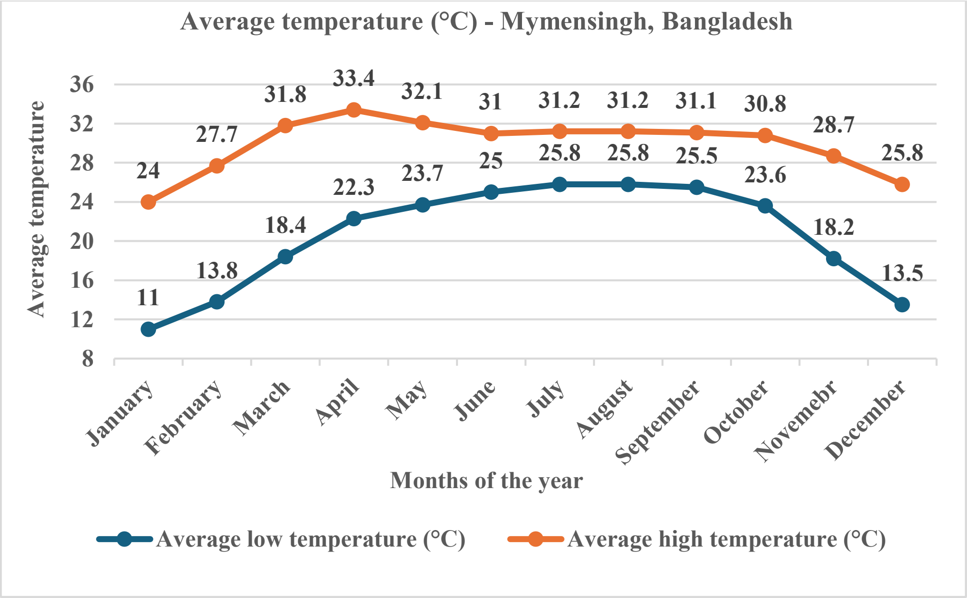


Figure 3.2 The average temperature of Mymensingh, Bangladesh. This line graph shows the average low and high temperatures throughout the year in Mymensingh, Bangladesh.

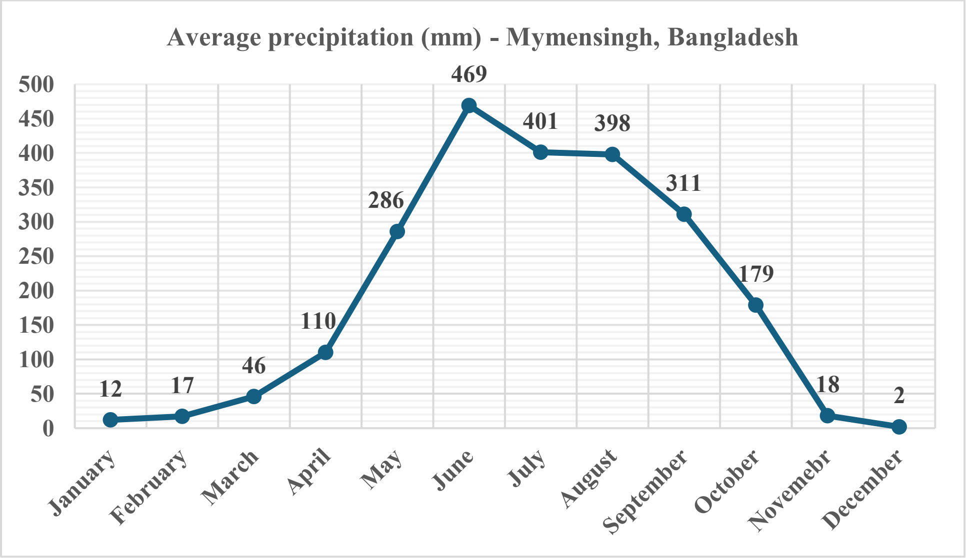


Figure 3.3 The average precipitation in Mymensingh, Bangladesh. This line graph shows the average rainfall in different months in Mymensingh, Bangladesh.

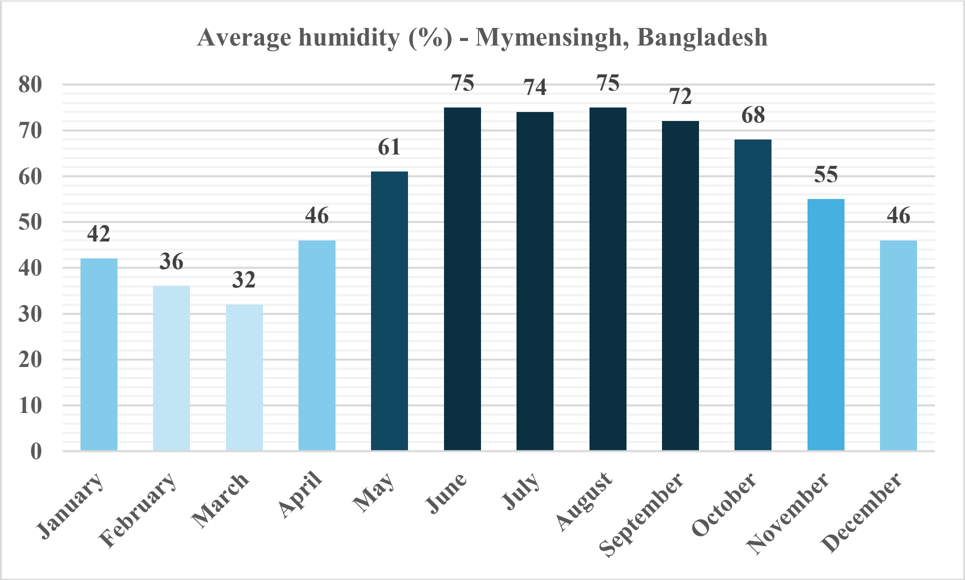


Figure 3.4 The average humidity in Mymensingh, Bangladesh. The bar chart shows the monthly average humidity level in Mymensingh, Bangladesh.

Quarterly analysis of *K. pneumoniae* isolated from various samples across different sampling quarters showed overall higher prevalence in the second (17.3%, 179/1034) and third (16.4%, 177/1080) quarter samples compared to the first (13.0%, 96/741) and fourth (9.8%, 89/904) quarter samples (Figure 3.5).

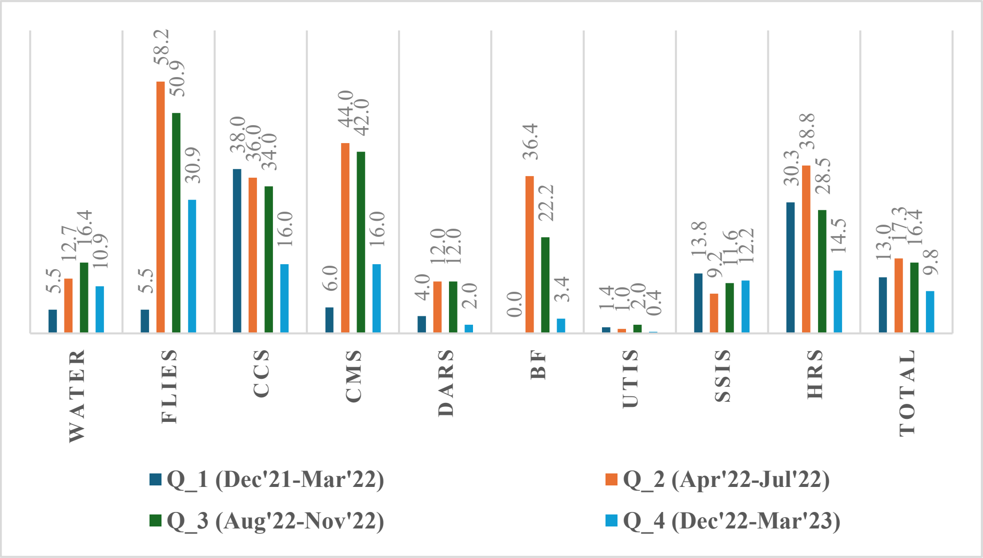


Figure 3.5 Prevalence of *K. pneumoniae* isolated from various sources across different sampling quarters. CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal

swabs; Q-1, Quarter-1 (December 2021 – March 2022); Q2, Quarter-2 (April 2022 – July 2022); Q3, Quarter-3 (August 22 – November 2022); Q4, Quarter-4 (December 2022 – March 2023).

Temporal variation in *K. pneumoniae* positivity by sample source was evaluated using log-linear Poisson regression models with offsets, with the December–March period (Quarter 1 and Quarter 4) as the reference category (Table 3.7). For flies, *K. pneumoniae* positivity was significantly higher during Quarter 2 (32/55, 58.2%; PR, 3.20; 95% CI: 1.90–5.30; $p<0.001$) and Quarter 3 (28/55, 50.9%; PR, 2.80; 95% CI: 1.70–4.60; $p<0.001$) compared with the December–March period. Similarly, CMS exhibited significantly higher positivity during Quarter 2 (22/50, 44.0%; PR, 4.00; 95% CI: 2.10–7.60; $p<0.001$) and Quarter 3 (21/50, 42.0%; PR, 3.80; 95% CI: 2.00–7.20; $p<0.001$) relative to the December–March period. Among HRS, *K. pneumoniae* positivity was significantly higher in Quarter 2 (64/165, 38.8%; PR, 1.73, 95% CI: 1.28–2.33, $p<0.001$), whereas the increase observed in Quarter 3 (47/165, 28.5%) did not reach statistical significance (PR, 1.27; 95% CI: 0.93–1.74; $p=0.13$). No significant differences in *K. pneumoniae* positivity across sampling quarters were detected for CCS or SSI samples.

Table 3.7 Log-linear (Poisson) regression analysis of *K. pneumoniae* positivity across different sampling quarters in various sample sources.

Sample source	**Sampling quarter**	**Positivity (positive/total, %)**	**PR**	**95% CI**	***p*-value**
Flies	Quarter 1+ Quarter 4	20/110 (18.2)	1	—	—
	Quarter 2	32/55 (58.2)	3.2	1.90 – 5.30	<0.001
	Quarter 3	28/55 (50.9)	2.8	1.70 – 4.60	<0.001
CCS	Quarter 1+ Quarter 4	27/100 (27.0)	1	—	—
	Quarter 2	18/50 (36.0)	1.33	0.78 – 2.27	0.29
	Quarter 3	17/50 (34.0)	1.26	0.73 – 2.18	0.4
CMS	Quarter 1+ Quarter 4	11/100 (11.0)	1	—	—
	Quarter 2	22/50 (44.0)	4	2.10 – 7.60	<0.001
	Quarter 3	21/50 (42.0)	3.8	2.00 – 7.20	<0.001
HRS	Quarter 1+ Quarter 4	74/330 (22.4)	1	—	—
	Quarter 2	64/165 (38.8)	1.73	1.28 – 2.33	<0.001
	Quarter 3	47/165 (28.5)	1.27	0.93 – 1.74	0.13
SSIs	Quarter 1+ Quarter 4	36/282 (12.8)	1	—	—
	Quarter 2	18/196 (9.2)	0.72	0.42 – 1.22	0.22
	Quarter 3	39/336 (11.6)	0.91	0.57 – 1.44	0.69

Sampling quarters were grouped into a December–March period (Quarter 1 and Quarter 4) and compared with individual non–December–March quarters (Quarter 2 and Quarter 3) to account for shared seasonal characteristics. Log-linear Poisson regression models with a log offset for the number of samples tested were used to estimate prevalence ratios (PRs) of *K. pneumoniae* positivity, with the December–March period used as the reference category. Positivity is presented as the number of *K. pneumoniae*–positive samples divided by the total number tested, with percentages shown in parentheses. Sample sources with sparse counts across sampling quarters (water, domestic animal rectal swabs, bird faeces and urinary tract infections) were excluded. CCS, chicken cloacal swabs; CMS, chicken meat swabs; HRS, human rectal swabs; SSIs, surgical site infections. Statistical significance was defined as $p<0.05$.

3.3 Discussion

K. pneumoniae can be a commensal or potential pathogen and survive a wide range of ecological niches, including humans, animals, water, soil and plants (Wyres & Holt, 2018). The rise in prevalence of *K. pneumoniae* in clinical and non-clinical settings has become a global concern in recent years. In Bangladesh, *K. pneumoniae* prevalence has been mostly studied within healthcare settings, focusing on AMR (Khan *et al.,* 2018; Rahman *et al.,* 2018; Mahbub, 2020; Okanda *et al.,* 2021; Tanni *et al.,* 2021; Hussain *et al.,* 2023; Rahman *et al.,* 2023; Kawser *et al.,* 2025). No comprehensive data is available on the prevalence of *K. pneumoniae* based on the One Health approach in Bangladesh. In this study, the overall prevalence of *K. pneumoniae* isolated from various samples was 14.4 % (541/3759). Studies have reported that the prevalence of CR-KP in India and China ranges from 1% to 22% (Tesfa *et al.,* 2022). A similar study from Pakistan reported the prevalence of *K. pneumoniae* at 15.7% (120/775) from human, animal and environmental sources (Aslam *et al.,* 2022).

In this study, the prevalence of *K. pneumoniae* was found to be 28.0% (185/660) in healthy human volunteers (Table 3.1). The national AMR surveillance data in Bangladesh showed the prevalence of *Klebsiella* spp. at 42.9% from rectal swabs (AMR dashboard Bangladesh). Kar *et al.* reported the carriage of *K. pneumoniae* at 81% (21/26) from stool samples in healthy human adults in Dhaka, Bangladesh (Kar *et al.,* 2024). The differences in carriage might be due to the sampling criteria and the type of sample used, such as stool or rectal swab. In our study, the human volunteers were apparently healthy without any chronic diseases, and the samples were rectal swabs rather than stool samples. Since rectal swabs collect a smaller quantity of faecal material compared to stool samples, this could lead to a lower detection rate, and *K. pneumoniae* may also not be evenly distributed in the intestinal tract (Calderon-Gonzalez *et al.,* 2023). However, research suggested that rectal swabs provide a practical and reliable alternative to stool samples for detecting *K. pneumoniae* colonisation and surveillance purposes (Lerner *et al.,* 2013; Bassis *et al.,* 2015). A log-linear Poisson regression analysis revealed a higher association of *K. pneumoniae* positivity with HRS (Table 3.2). *K. pneumoniae* is a commensal organism in the human gut, which may contribute to higher isolation (Calderon-Gonzalez *et al.,* 2023). Analysis of *K. pneumoniae* prevalence in HRS among various sampling locations did not find any significant differences. In HRS, the prevalence was significantly higher in the second quarter samples, which might be due to seasonal variation (Table 3.7). Several studies observed fluctuations in the incidence of Gram-negative bacterial infections, particularly in relation to temperature, humidity, and other seasonal factors (Al-Hasan *et al.,* 2009; Eber *et al.,* 2011).

In my study, the prevalence of *K. pneumoniae* was 1.2% (14/1170) in patients with UTIs at the outpatient service of MMCH. The national AMR surveillance data in Bangladesh showed the prevalence of *K. pneumoniae* in urine was 12.7%, whereas the prevalence in MMCH was 7.6% (AMR Bangladesh – dashboard.iedcr.gov.bd). A study in Dhaka documented that the prevalence of community-acquired UTIs was 20% (920 of 4,500), whereas *Klebsiella* spp. was 12.1% among the causative pathogens (Islam *et al.,* 2022). Another study from community settings in different regions of India reported that 10.1% (250/2459) of urine samples were positive for bacteriuria, where 68% (170/250) were *E. coli* and 17.6% (44/250) were *K. pneumoniae* (Mohapatra *et al.,* 2022). The differences in prevalence might be due to sampling fractions, inclusion and exclusion criteria (such as participants aged 18 or over, absence of any chronic diseases), patient types (inpatient and/or outpatient) and regional variation (Saperston *et al.,* 2014; Kaye *et al.,* 2024; Sahoo *et al.,* 2025). The odds of *K. pneumoniae* positivity in UTIs showed a significantly higher association with outpatients from Mymensingh Sadar subdistrict in Mymensingh (Table 3.5). This might be due to the highest number of study participants and the highest population density in the Mymensingh Sadar (Mymensingh district, Banglapedia). Studies have shown that higher population density is linked to an increased risk of MDR UTIs (Casey *et al.,* 2021). *K. pneumoniae* prevalence across different sampling quarters showed higher prevalence in the third quarter samples during autumn and late autumn (Figure 3.5). Previous studies reported a higher frequency of *K. pneumoniae* in urine specimens during summer and autumn, which supports the findings of this study (Kito *et al.,* 2021).

In the present study, the prevalence of *K. pneumoniae* was 11.4% (93/814) in hospitalised patients of SSIs at MMCH, where 92.5% (86/93) of the samples were considered HAIs. A recent study conducted across selected tertiary care hospitals in Bangladesh reported that *K. pneumoniae* accounted for 20.9%

of SSIs (Harun *et al.,* 2025). Another study focusing on abdominal SSIs found *K. pneumoniae* in 11.8% of cases, which is consistent with the findings of the present study (Khan *et al.,* 2022).

Data revealed a higher association of *K. pneumoniae* positivity with CCS (Table 3.2), whereas the prevalence was 31% (62/200). Saha *et al.* found a similar prevalence (33.3%) of *K. pneumoniae* in CCS from a commercial poultry farm in Narsingdi, Bangladesh (Saha *et al.,* 2023). A significantly higher prevalence of *K. pneumoniae* was found in CCS from the Trishal subdistrict (Table 3.4). Several factors such as poor sanitation, high stock density, insufficient waste management, contamination of feed and water sources, and extensive use of antibiotics both for treatment and prophylaxis, may contribute to increased colonisation in chickens (Hayati *et al.,* 2019). Although researchers found higher isolation rates during warmer seasons (Tsitsos *et al.,* 2025), no significant difference in *K. pneumoniae* positivity in CCS was observed across different sampling quarters in this study (Table 3.7). The prevalence in the first, second and third quarter samples was almost similar (Figure 3.5). It is challenging to conclusively determine any factors for such findings and establish definitive patterns.

In the present study, the prevalence of *K. pneumoniae* in CMS was 27% (54/200). In a recent study in 13 subdistricts of Sylhet, Bangladesh, the prevalence of *K. pneumoniae* was detected at 34.74 % (99/285) in retail poultry meat, supporting the findings of the present study (Tanni *et al.,* 2025). During slaughter and processing, *K. pneumoniae* present in the gastrointestinal tract of chickens can contaminate the meat. Improper handling, inadequate hygiene practices, and cross-contamination in processing facilities can further spread the bacteria to meat products. Research has shown that *K. pneumoniae* can persist throughout the poultry production chain, from farm to retail meat products (Mourão *et al.,* 2024). The prevalence of *K. pneumoniae* in CMS was significantly higher during the second and third quarter sampling (Table 3.7) when the average temperature ranged from 31 to 33.4°C, favouring the growth of the bacterium (Eber *et al.,* 2011; Kito *et al.,* 2021).

The prevalence of *K. pneumoniae* was 17.3% (13/75) in the faeces of free-flying birds around the poultry farms. Specific data on the prevalence of *K. pneumoniae* in free-flying birds is limited in Bangladesh. A study that examined faecal samples from wild birds found that 34.6% of the samples harboured ESBL-producing bacteria, with *K. pneumoniae* constituting 8.3% of these isolates (Saeed *et al.,* 2023). Another study reported a 12.2% occurrence of *K. pneumoniae* in wild animals, including birds (Chiaverini *et al.,* 2022), supporting the findings of the present study. A higher prevalence was observed in the second quarter samples during the warmest months with higher precipitation and humidity (Figure 3.5), which is consistent with the findings of previous research (Tsitsos *et al.,* 2025).

The prevalence of *K. pneumoniae* in DARS was 7.5% (15/200). Data on the prevalence of *K. pneumoniae* in rectal swabs of domestic animals in Bangladesh is currently limited. A systematic review and meta-analysis assessed the overall prevalence of ESBL-producing bacteria among animals in India. The study reported an overall ESBL prevalence of 9% in animals, with *K. pneumoniae* accounting for 11% of these isolates (Kuralayanapalya *et al.,* 2019). These findings support the findings of the present study. Although no significant association was observed across different sampling periods, the prevalence was higher during the second and third sampling periods, which coincided with the warmer months.

Water samples were collected from various sources, including drains, community sewer lines, ponds, and rivers near the farms and the hospital. The prevalence of *K. pneumoniae* was 11.4% (25/220) in water from the study area in Mymensingh. A study conducted in Dhaka reported the presence of *K. pneumoniae* in 64% (21 out of 33) of household tap water samples, while 85% (23 out of 27) were in standing water sources, such as ponds and lakes (Kar *et al.,* 2024). Other research identified *K. pneumoniae* in 11.2% of bacterial isolates from hospital wastewater (Addae-Nuku *et al.,* 2022), which is consistent with our results. A study conducted in India also reported that 14.3% of water samples collected from various farms and slaughterhouses were positive for *K. pneumoniae* (Kalla & Paila, 2023). The presence of *K. pneumoniae* in water samples might be due to the contamination of surrounding water bodies with farm wastes and human sewage. While no significant associations were found across the sampling periods, higher prevalence rates were observed in the second (summer, monsoon) and third quarter (autumn, and late autumn) samples (Figure 3.5).

Flies had the highest prevalence of *K. pneumoniae* (36.4%, 80/220) among the various samples collected in this study (Table 3.1). Currently, there is a lack of specific data on the prevalence of *K. pneumoniae* in flies in Bangladesh. However, studies from other regions have demonstrated that flies can act as vectors for various pathogenic bacteria, including *K. pneumoniae* (Hassan *et al.,* 2021; Yin *et al.,* 2022). Ranjbar *et al.* reported 11.3% carriage of *K. pneumoniae* in captured house flies from selected provinces of Iran (Ranjbar *et al.,* 2016). However, another study from India isolated 36.7% of *Klebsiella* spp. from hospital flies, similar to our findings (Fotedar *et al.,* 1992). The highest prevalence in flies indicates environmental contamination and potential environmental reservoirs, which may contribute to its environmental dissemination and possible transmission to humans and animals (Mourão *et al.,* 2024; Cook *et al.,* 2025). In this study, the prevalence of *K. pneumoniae* was significantly higher in Mymensingh Sadar. The poor waste management system in the city can be a plausible reason. Poor waste management can lead to the accumulation of organic waste in the environment, which can harbour bacteria like *K. pneumoniae*, and flies can act as mechanical vectors of this organism through regurgitation, defecation and shedding from the bodily surface (Cook *et al.,* 2025). Additionally, their ability to travel between 5–7 km makes them a feasible spreader of this bacterium (Onwugamba *et al.,* 2020). Analysis across different sampling periods revealed a significantly higher prevalence in summer and monsoon (second quarter), which aligns with previous reports indicating an increased percentage of MDR Enterobacterales isolation from arthropods in summer (Hassan *et al.,* 2021).

My study found that the overall prevalence of *K. pneumoniae* was higher in the second (17.3%, 179/1034) and third (16.4%, 177/1080) quarter samples compared to the first (13.0%, 96/741) and fourth (9.8%, 89/904) quarter samples. Higher prevalence in the second and third-quarter samples might be due to warmer temperatures and higher humidity, which is supported by findings from previous research (Anderson *et al.,* 2008; Kito *et al.,* 2021). Additionally, higher precipitation and flooding can contribute to an increased prevalence of *K. pneumoniae* infections since floodwaters can contain high levels of sewage, hospital waste, and agricultural runoff, which may carry *K. pneumoniae* from human and animal sources to the environment (Aguilar-Salazar *et al.,* 2023).

The findings of this chapter indicate that *K. pneumoniae* is widely distributed across various sources (humans, livestock, and environmental reservoirs) in the study area. This distribution reflects the interconnectivity between these domains and reinforces the need for a One Health approach to address the prevalence and AMR concerns associated with *K. pneumoniae*.

Chapter 4

Investigating the Epidemiology of Antimicrobial Resistance in *Klebsiella pneumoniae* at the Human–Animal–Environment Interface in Bangladesh

4.1 Introduction

Antimicrobial resistance has been recognised as an emerging global health crisis jeopardising a century of medical progress. The estimated number of deaths caused by AMR is projected to be at least 10 million annually by 2050, disproportionately affecting LMICs (Naghavi *et al.,* 2024). Amid the global surge in AMR, *K. pneumoniae* represents a serious public health threat, driven by its capacity to acquire resistance to multiple antibiotic classes, thereby limiting treatment options and increasing both morbidity and mortality (Jin *et al.,* 2021; Gao *et al.,* 2025). The WHO 2024 Bacterial Priority Pathogens List designates 3GC-resistant and CR-KP as critical threats, placing them in a priority pathogen group that requires urgent research and intervention (WHO BPPL, 2024).

K. pneumoniae serves as a major conduit for transferring AMR genes among *Klebsiella* species and other Enterobacterales across clinical and non-clinical settings. This is driven by its frequent association with plasmids and mobile genetic elements rich in resistance determinants, highlighting its critical role in the dissemination and evolution of AMR (Wyres & Holt, 2018). Since the initial detection in 1982, around 200 ESBL variants of *K. pneumoniae* have been identified, many of which have since been transferred to *E. coli*. Additionally, the plasmid-mediated spread of carbapenemase-encoding genes in clinical settings further exacerbates the situation (Wilson et al., 2018). Plasmids play a central role in the dissemination of AMR in *K. pneumoniae*, harbouring multiple AMR genes and leading to MDR phenotypes. Many diverse plasmids have been sequenced from *K. pneumoniae*, with IncFIIK and IncFIBK being the most prevalent. In *K. pneumoniae*, most AMR genes are carried on IncF (FII, FIA, FIB), IncN, IncR, IncX3, IncAC/2 and IncHI1B plasmid replicons (large conjugative and self-transmissible), and a few are on small Col plasmids (mobile but not self-transmissible) (Wyres *et al.,* 2020). Some plasmid replicons are found to be associated with particular resistance genes. For instance, the IncFIIK and IncFIBK plasmids are frequently linked to ESBL genes, notably $bla_{\text{CTX–M–15}}$, whereas the IncX3 plasmid is prominently associated with carbapenemase genes, such as bla_{NDM}, bla_{KPC}, and $bla_{\text{OXA–181}}$ (Guo *et al.,* 2022; Pankok *et al.,* 2022).

K. pneumoniae infections are caused by diverse clones that are geographically widespread. There are hundreds of distinct phylogenetic lineages or clones within the *K. pneumoniae* population (Wyres *et al.,* 2020). Certain STs such as ST11, ST14, ST15, ST48, ST147, ST231 and ST307 are recognised as MDR clones or high-risk clones (resistant to at least three classes of antibiotics and often associated with hospital outbreaks) while others such as ST23, ST65, ST 86, ST268 are hypervirulent clones (presence of enhanced virulent factors and often associated with CAIs) (Zhu *et al.,* 2021; Hussain *et al.,* 2023). Some STs cause localised problems, such as MDR clones ST70 and ST323, that caused outbreaks in Kilifi, Kenya and Melbourne, Australia, whereas ST258/512, alongside ST11, ST14, ST15, ST17/20, ST37, ST101, ST147 and ST307, are considered global problem clones (resistant to 3GCs and/or carbapenems and widely distributed). There is also a significant geographical difference in the global distribution of these STs. For instance, ST258/512 is common in Europe and America, whereas ST11 and ST23 are mostly found in the Asia-Pacific region (Wyres *et al.,* 2020). Previous studies in Bangladesh reported high-risk MDR clones (ST11, ST14, ST15, ST307, ST231 and ST147) from clinical infections (Farzana *et al*., 2020; Hussain *et al.,* 2023).

ESBL and CR-KP are endemic in the Indian subcontinent (e.g., India, Bangladesh, Pakistan, and Sri Lanka) and recovered from humans, food animals and the environment (Kumarasamy *et al.,* 2010; Farzana *et al.,* 2013; Islam *et al.,* 2013; Begum & Shamsuzzaman, 2016; Mohanty *et al.,* 2017; Khan *et al.,* 2017; Nahid *et al.*, 2017; Wyres & Holt, 2018). However, information regarding the prevalence of *K. pneumoniae* producing ESBLs and carbapenemase, as well as their molecular epidemiological features, is limited from Bangladesh (Khan *et al.,* 2018). *K. pneumoniae* harbouring CTX–M–1, CTX–M–15, NDM–1, OXA–48, OXA–181, and *mcr–8.1* genes were isolated from clinical isolates and hospitalised patients in Mymensingh, Chittagong, and Dhaka, Bangladesh (Khan *et al.*, 2018; Farzana *et al.*, 2020; Safain *et al.,* 2021; Tanni *et al.,* 2021). Research focusing on poultry farms in Bangladesh identified MDR *K. pneumoniae* strains resistant to multiple antibiotic classes, including carbapenems and ESBLs (Saha *et al.*, 2023; Munim *et al.*, 2024). A study in Dhaka detected *K. pneumoniae* in both human faecal samples and environmental water sources, with a notable proportion exhibiting multidrug resistance, including resistance to carbapenems (Kar *et al.*, 2024). This highlights the role of environmental reservoirs in the transmission dynamics of AMR pathogens. In countries like Bangladesh, where human and animal cohabitation are common, it is more likely to promote the dissemination of antibiotic resistance from humans to animals and vice versa (Swarthout *et al.,* 2022). The interconnectedness of human, animal, and environmental health in Bangladesh necessitates a comprehensive understanding of the epidemiological trends of AMR in *K. pneumoniae*. The existing data on the abundance, distribution, and transmission of AMR strains and genes within and between humans, animal farms, and environmental settings are piecemeal, thereby presenting a knowledge gap.

To address this knowledge gap, this study deployed a comprehensive One Health sampling strategy to investigate the molecular epidemiology of AMR in *K. pneumoniae* circulating at the human-animal-environment interface from Mymensingh, Bangladesh. This chapter aims to explore the following specific objectives:

1. To determine the prevalence of dominant resistance mechanisms in *K. pneumoniae* at the human-animal-environment interface in Mymensingh, Bangladesh.
2. To assess the prevalent resistant genes associated with the predominant resistance mechanisms in *K. pneumoniae* in Bangladesh.
3. To evaluate whether the clonal dissemination of AMR in *K. pneumoniae* can be explained through a One Health perspective.
4. To assess the potential role of plasmids in *K. pneumoniae* regarding the dissemination of resistance at the human-animal-environment interface.

4.2 Results

4.2.1 AMR profile of *K. pneumoniae*

K. pneumoniae isolates (n=541) recovered from various sample sources were subjected to antimicrobial susceptibility testing against 16 clinically relevant antibiotics using the agar dilution method. Antibiogram revealed that *K. pneumoniae* showed overall higher resistance to fosfomycin (62.8%, 340/541), ciprofloxacin (47.5%, 257/541), levofloxacin (46%, 249/541), amoxicillin–clavulanic acid (36.2%, 196/541), and higher susceptibility to colistin (3.1%, 17/541), ceftazidime–avibactam (3.5%, 19/541), meropenem (4.6%, 25/541) and imipenem (5.0%, 27/541) (Table 4.1).

Isolates from water samples showed higher resistance to fosfomycin (60.0%, 15/25), ciprofloxacin (40%, 10/25), levofloxacin (40%, 10/25), and amoxicillin–clavulanic acid (36.0%, 9/25), as well as similar non-susceptibility (4%, 1/25) to piperacillin–tazobactam, ceftazidime–avibactam, imipenem, meropenem, and 8% (2/25) were resistant to colistin. 47.5% (38/80), 43.8% (35/80), 38.8% (31/80), and 28.8% (23/80) of isolates from flies showed resistance to fosfomycin, ciprofloxacin, levofloxacin, and amoxicillin–clavulanic acid, respectively. All *K. pneumoniae* from flies were susceptible to ceftazidime–avibactam, imipenem and meropenem.

K. pneumoniae isolated from CCS showed higher resistance to ciprofloxacin (88.7%, 55/62), levofloxacin (87.1%, 54/62), fosfomycin (69.4%, 43/62), amoxicillin–clavulanic acid (66.1%, 41/62), gentamicin (56.5%, 35/62), ceftazidime (45.2%, 28/62), and tigecycline (45.2%, 28/62). In case of CMS, higher resistance to fosfomycin (90.7%, 49/54), ciprofloxacin (53.7%, 29/54), and levofloxacin (50.0%, 27/54) was observed. Isolates from DARS and BF showed higher resistance to fosfomycin, at 46.7% and 30.8%, respectively. All isolates from CCS, CMS, DARS and BF were sensitive to ceftazidime–avibactam, imipenem and meropenem.

Around 90% of *K. pneumoniae* from SSIs were resistant to amoxicillin–clavulanic acid, cefotaxime, ceftazidime, cefepime, aztreonam, ciprofloxacin, levofloxacin and fosfomycin, whereas resistance to gentamicin, amikacin, piperacillin–tazobactam, tigecycline, imipenem, meropenem and ceftazidime–

avibactam was 61.3%, 51.6%, 54.8%, 26.9%, 26.9%, 25.8% and 19.4%, respectively. All *K. pneumoniae* isolates recovered from patients with SSIs were susceptible to colistin (Table 4.1). *K. pneumoniae* from UTIs were resistant to amoxicillin–clavulanic acid (50.0%, 7/14), ciprofloxacin (50.0%), ceftazidime (42.9%), aztreonam (42.9%), cefotaxime (35.7%), cefepime (35.7%) and levofloxacin (21.4%). All isolates from UTIs were susceptible to piperacillin–tazobactam, ceftazidime–avibactam, amikacin, gentamicin, imipenem, meropenem and colistin. *K. pneumoniae* isolated from HRS of apparently healthy human volunteers showed high resistance to fosfomycin (51.9%), whereas showed 100% susceptibility to ceftazidime–avibactam and meropenem.

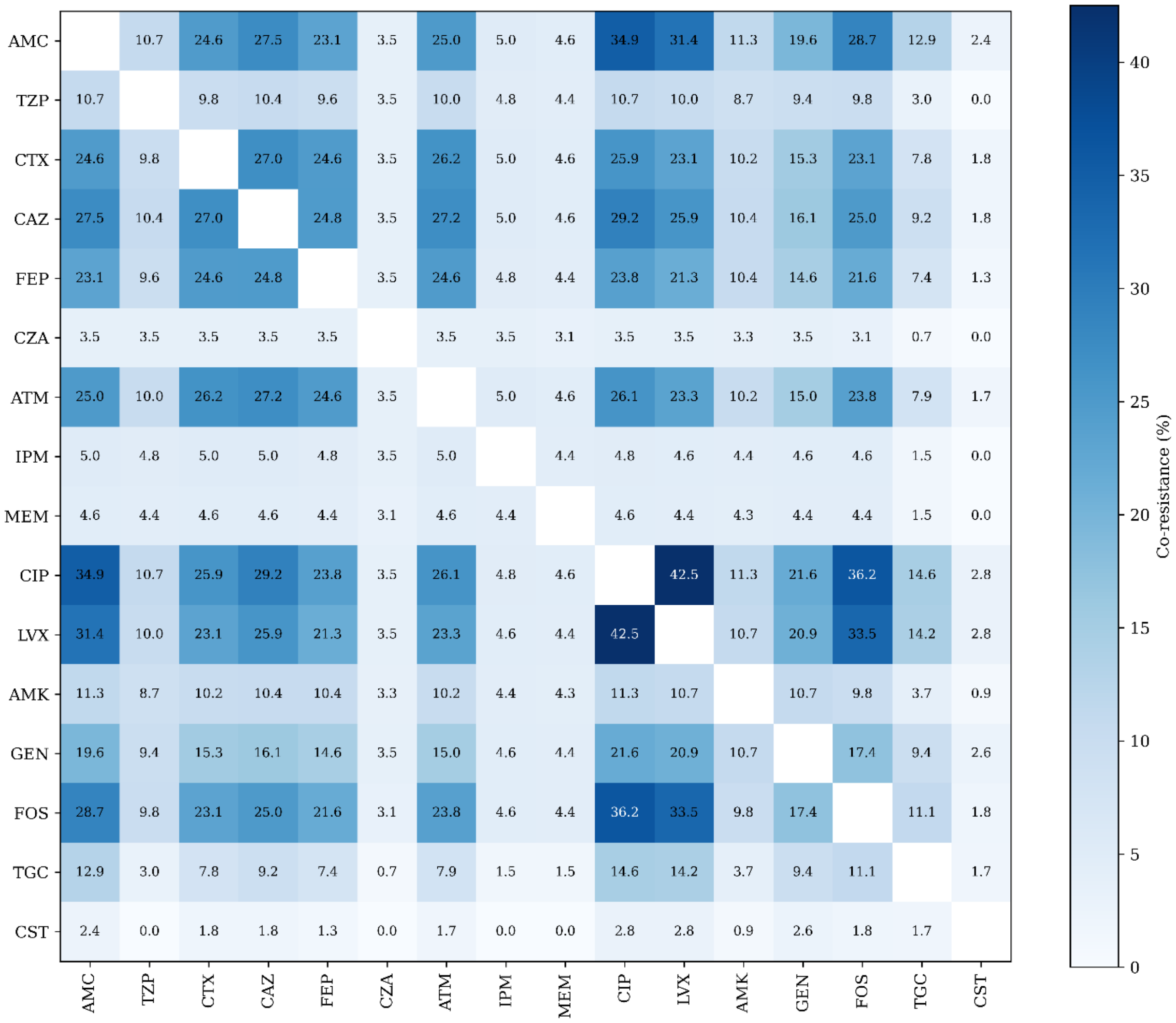
To investigate resistance beyond individual antibiotic resistance frequencies, isolate-level co-resistance patterns among *K. pneumoniae* isolates (n=541) were examined using a pairwise co-resistance heatmap based on antimicrobial susceptibility data (Figure 4.1). The highest co-resistance was observed between ciprofloxacin and levofloxacin, detected in 42.5% isolates. Ciprofloxacin and levofloxacin also showed strong co-resistance with fosfomycin. Simultaneous resistance to ciprofloxacin and fosfomycin was observed in 36.2% of isolates, while levofloxacin–fosfomycin co-resistance occurred in 33.5% of isolates. Co-resistance between cefotaxime and ceftazidime was observed in 27.0% of isolates, while concurrent resistance to cefotaxime and cefepime occurred in 24.6% of isolates. Similarly, co-resistance between ceftazidime and aztreonam was detected in 27.2% of isolates. Amoxicillin–clavulanic acid also showed frequent co-resistance with cefotaxime (24.6%), ceftazidime (27.5%) and cefepime (23.1%).

Concurrent resistance to gentamicin and ciprofloxacin was detected in 21.6% of isolates, whereas co-resistance between amikacin and ciprofloxacin was lower, occurring in 11.3% of isolates. Tigecycline exhibited limited co-resistance with most antibiotics (percentages typically below 10%). Imipenem and meropenem showed minimal co-resistance with other antibiotics, with concurrent resistance generally below 5–10% across antibiotic pairs. Similarly, colistin and ceftazidime–avibactam exhibited very low co-resistance frequencies (typically <5%) (Figure 4.1).

Table 4.1 Antibiotic susceptibility patterns of *K. pneumoniae* (n=541) isolated from various samples.

Attributes	**Resistance to respective antibiotics, n (%)**															
Sample sources	**AMC**	**TZP**	**CTX**	**CAZ**	**FEP**	**CZA**	**ATM**	**IPM**	**MEM**	**CIP**	**LVX**	**AMK**	**GEN**	**FOS**	**TGC**	**CST**
Water (n=25)	9 (36.0)	1 (4.0)	5 (20.0)	6 (24.0)	3 (12.0)	1 (4.0)	5 (20.0)	1 (4.0)	1 (4.0)	10 (40.0)	10 (40.0)	1 (4.0)	8 (32.0)	15 (60.0)	5 (20.0)	2 (8.0)
Flies (n=80)	23 (28.8)	3 (3.8)	6 (7.5)	10 (12.5)	5 (6.3)	0 (0)	5 (6.3)	0 (0)	0 (0)	35 (43.8)	31 (38.8)	5 (6.3)	10 (12.5)	38 (47.5)	7 (8.8)	1 (1.3)
CCS (n=62)	41 (66.1)	2 (3.2)	21 (33.9)	28 (45.2)	18 (29.0)	0 (0)	23 (37.1)	0 (0)	0 (0)	55 (88.7)	54 (87.1)	5 (8.1)	35 (56.5)	43 (69.4)	28 (45.2)	9 (14.5)
CMS (n=54)	17 (31.5)	0 (0)	7 (13.0)	10 (18.5)	7 (13.0)	0 (0)	8 (14.8)	0 (0)	0 (0)	29 (53.7)	27 (50.0)	0 (0)	4 (7.4)	49 (90.7)	11 (20.4)	1 (1.9)
DARS (n=15)	1 (6.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (13.3)	2 (13.3)	0 (0)	0 (0)	7 (46.7)	1 (6.7)	1 (6.7)
BF (n=13)	0 (0)	0 (0)	2 (15.4)	2 (15.4)	1 (7.7)	0 (0)	2 (15.4)	0 (0)	0 (0)	3 (23.1)	2 (15.4)	0 (0)	1 (7.7)	4 (30.8)	0 (0)	1 (7.7)
SSIs (n=93)	83 (89.2)	51 (54.8)	90 (96.8)	90 (96.8)	88 (94.6)	18 (19.4)	90 (96.8)	25 (26.9)	24 (25.8)	89 (95.7)	81 (87.1)	48 (51.6)	57 (61.3)	83 (89.2)	25 (26.9)	0 (0)
UTIs (n=14)	7 (50.0)	0 (0)	5 (35.7)	6 (42.9)	5 (35.7)	0 (0)	6 (42.9)	0 (0)	0 (0)	7 (50.0)	3 (21.4)	0 (0)	0 (0)	5 (35.7)	1 (7.1)	0 (0)
HRS (n=185)	15 (8.1)	1 (0.5)	10 (5.4)	12 (6.5)	7 (3.8)	0 (0)	9 (4.9)	1 (0.5)	0 (0)	27 (14.6)	39 (21.1)	2 (1.1)	3 (1.6)	96 (51.9)	3 (1.6)	2 (1.1)
Total (n=541)	**196 (36.2)**	**58 (10.7)**	**146 (27.0)**	**164 (30.3)**	**134 (24.8)**	**19 (3.5)**	**148 (27.4)**	**27 (5.0)**	**25 (4.6)**	**257 (47.5)**	**249 (46.0)**	**61 (11.3)**	**118 (21.8)**	**340 (62.8)**	**81 (15.0)**	**17 (3.1)**

Values in parentheses indicate row percentage. The heatmap was generated according to the resistance percentage. N, number; AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime–avibactam; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin; AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline; CST, Colistin. The colour scale from white to red indicates the lowest to the highest resistance percentage in a row.



	AMC	TZP	CTX	CAZ	FEP	CZA	ATM	IPM	MEM	CIP	LVX	AMK	GEN	FOS	TGC	CST
AMC		10.7	24.6	27.5	23.1	3.5	25.0	5.0	4.6	34.9	31.4	11.3	19.6	28.7	12.9	2.4
TZP	10.7		9.8	10.4	9.6	3.5	10.0	4.8	4.4	10.7	10.0	8.7	9.4	9.8	3.0	0.0
CTX	24.6	9.8		27.0	24.6	3.5	26.2	5.0	4.6	25.9	23.1	10.2	15.3	23.1	7.8	1.8
CAZ	27.5	10.4	27.0		24.8	3.5	27.2	5.0	4.6	29.2	25.9	10.4	16.1	25.0	9.2	1.8
FEP	23.1	9.6	24.6	24.8		3.5	24.6	4.8	4.4	23.8	21.3	10.4	14.6	21.6	7.4	1.3
CZA	3.5	3.5	3.5	3.5	3.5		3.5	3.5	3.1	3.5	3.5	3.3	3.5	3.1	0.7	0.0
ATM	25.0	10.0	26.2	27.2	24.6	3.5		5.0	4.6	26.1	23.3	10.2	15.0	23.8	7.9	1.7
IPM	5.0	4.8	5.0	5.0	4.8	3.5	5.0		4.4	4.8	4.6	4.4	4.6	4.6	1.5	0.0
MEM	4.6	4.4	4.6	4.6	4.4	3.1	4.6	4.4		4.6	4.4	4.3	4.4	4.4	1.5	0.0
CIP	34.9	10.7	25.9	29.2	23.8	3.5	26.1	4.8	4.6		42.5	11.3	21.6	36.2	14.6	2.8
LVX	31.4	10.0	23.1	25.9	21.3	3.5	23.3	4.6	4.4	42.5		10.7	20.9	33.5	14.2	2.8
AMK	11.3	8.7	10.2	10.4	10.4	3.3	10.2	4.4	4.3	11.3	10.7		10.7	9.8	3.7	0.9
GEN	19.6	9.4	15.3	16.1	14.6	3.5	15.0	4.6	4.4	21.6	20.9	10.7		17.4	9.4	2.6
FOS	28.7	9.8	23.1	25.0	21.6	3.1	23.8	4.6	4.4	36.2	33.5	9.8	17.4		11.1	1.8
TGC	12.9	3.0	7.8	9.2	7.4	0.7	7.9	1.5	1.5	14.6	14.2	3.7	9.4	11.1		1.7
CST	2.4	0.0	1.8	1.8	1.3	0.0	1.7	0.0	0.0	2.8	2.8	0.9	2.6	1.8	1.7	

Figure 4.1 Isolate-level co-resistance heatmap of *K. pneumoniae* (n=541). The heatmap illustrates pairwise co-resistance patterns among all *K. pneumoniae* isolates based on isolate-level antimicrobial susceptibility data. Each cell represents the percentage of isolates simultaneously resistant to the corresponding pair of antibiotics. Darker shades indicate higher frequencies of co-resistance. Diagonal cells were masked to highlight pairwise co-resistance patterns. AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime–avibactam; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin; AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline; CST, Colistin.

Sample source specific multivariate regression analysis demonstrated that *K. pneumoniae* isolates from CCS were significantly associated with resistance to ciprofloxacin (88.7%, 55/62), levofloxacin (87.1%, 54/62), fosfomycin (69.4%, 41/62) and gentamicin (56.5%, 35/62), whereas CMS isolates showed strong association with levofloxacin (50.0%, 27/54) and fosfomycin (90.7%, 49/54) resistance (p<0.05). Isolates from SSIs exhibited very high resistance frequencies (≥87%) across multiple antibiotic classes, including ciprofloxacin, levofloxacin, fosfomycin, cefotaxime, ceftazidime, aztreonam and cefepime (p<0.05) (Table 4.2).

Out of 541 *K. pneumoniae*, 122 (22.6%) were MDR across the samples, indicating resistance to at least one agent in three or more antimicrobial categories, including cephalosporins (cefotaxime, ceftazidime, cefepime), carbapenems (imipenem, meropenem), aminoglycosides (amikacin, gentamicin), quinolones (ciprofloxacin, levofloxacin), tetracyclines (tigecycline), and polymyxins (colistin) (Figure 4.2). Additionally, 16 (3.0%) of these isolates were extensively drug–resistant (XDR), meaning they were non-susceptible to at least one agent in all categories except for one or two categories. MDR *K. pneumoniae* were commonly found in SSIs (73.1%, 68/93), followed by CCS (50%, 31/62), water (24.0%, 6/25), CMS (11.1%, 6/54), flies (8.8%, 7/80), BF (7.7%, 1/13) and HRS (1.6%, 3/185). No MDR *K. pneumoniae* was found in DARS and UTIs in this study. The occurrence of XDR *K. pneumoniae* was 9.7% in SSIs, followed by 4.8% in CCS, 4.0% in water, 1.9% in CMS and 1.1% in HRS. No XDR *K. pneumoniae* was detected in flies, DARS, BF and UTIs.

Table 4.2 Association between sample source and antibiotic resistance in *K. pneumoniae*.

Sample source	**Antibiotics**	**Resistant / total (n/N)**	**Resistance (%)**	**Adjusted OR**	**95% CI**	***p*-value**
CCS	CIP	55 / 62	88.7	26.19	5.78 – 118.68	<0.001
	LVX	54 / 62	87.1	37.13	6.92 – 199.13	<0.001
	FOS	43 / 62	69.4	5.09	1.39 – 18.60	0.014
	GEN	35 / 62	56.5	15.56	1.90 – 127.14	0.011
CMS	LVX	27 / 54	50.0	5.5	1.11 – 27.19	0.037
	FOS	49 / 54	90.7	22.05	4.95 – 98.29	<0.001
SSIs	CIP	89 / 93	95.7	74.17	14.48 – 379.85	<0.001
	LVX	81 / 93	87.1	37.13	7.32 – 188.36	<0.001
	FOS	83 / 93	89.2	18.68	4.85 – 71.91	<0.001
	GEN	57 / 93	61.3	19	2.37 – 152.42	0.006
	CTX	90 / 93	96.8	165	24.79 – 1098.28	<0.001
	CAZ	90 / 93	96.8	165	24.79 – 1098.28	<0.001
	ATM	90 / 93	96.8	165	24.79 – 1098.28	<0.001
	FEP	88 / 93	94.6	211.2	22.71 – 1964.41	<0.001

CCS, chicken cloacal swabs; CMS, chicken meat swabs; SSIs, surgical site infections; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; ATM, Aztreonam; CIP, Ciprofloxacin; LVX, Levofloxacin; GEN, Gentamicin; FOS, Fosfomycin. Associations were assessed using multivariate logistic regression models fitted with generalised estimating equations and a binomial distribution with logit link. Isolate ID was specified as the clustering variable to account for repeated antimicrobial susceptibility measurements within the same isolate (nested effects). For each antibiotic, the sample source was included as the explanatory variable, with all other sample sources combined as the reference category. The table presents only statistically significant associations ($p<0.05$). Crude resistance frequencies are shown as resistant/total isolates (n/N, %) for descriptive purposes, whereas odds ratios (OR) and 95% confidence intervals (CI) represent adjusted estimates from the multivariate models.

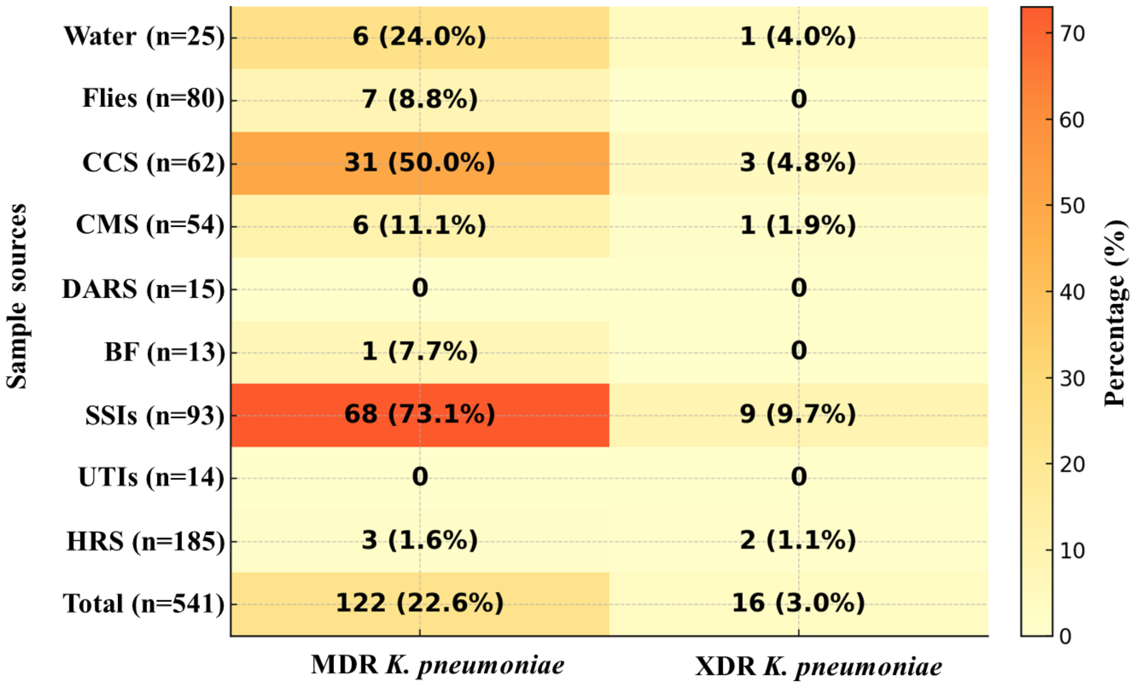


Figure 4.2 Heatmap shows the distribution of MDR and XDR *K. pneumoniae* across various samples. MDR, multidrug–resistant; XDR, extensively drug–resistant; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs.

In this study, *K. pneumoniae* isolates were classified into five phenotypic resistance groups (Group A–E) based on susceptibility to major antibiotic classes, including third-generation cephalosporins (3GCs), carbapenems, aminoglycosides, quinolones and colistin. The phenotypic resistance groups were defined to summarise phenotypic resistance patterns across major antibiotic classes. The groups are as follows:

- Group **A**: 3GC sensitive, carbapenem sensitive (regardless of aminoglycosides, quinolones and colistin susceptibility)
- Group **B**: 3GC-resistant, carbapenem sensitive (regardless of aminoglycosides, quinolones and colistin susceptibility)
- Group **C**: carbapenem-resistant, but susceptible to at least one major non β-lactam class (aminoglycosides or fluoroquinolones) (regardless of colistin susceptibility)

- Group **D**: carbapenem, aminoglycoside and quinolone-resistant (regardless of colistin susceptibility)
- Group **E**: carbapenem, aminoglycoside, quinolone and colistin-resistant.

3GC resistance refers to resistance to either cefotaxime or ceftazidime. Carbapenem resistance refers to resistance to either imipenem or meropenem. Aminoglycoside resistance refers to resistance to either amikacin or gentamicin, and quinolone resistance pertains to ciprofloxacin or levofloxacin.

Based on this classification, 69.7% (377/541) of *K. pneumoniae* isolates were sensitive to both 3GC and carbapenems (Group A), followed by 25.1% (136/541) that were resistant to 3GC but sensitive to carbapenems (Group B) (Figure 4.3). Carbapenem-resistant phenotypes with susceptibility to either aminoglycosides or quinolones were uncommon, with only 0.4% (2/541) of isolates classified as Group C. Additionally, 4.8% (26/541) of the isolates were resistant to carbapenems, quinolones and aminoglycosides (Group D). No isolates were found in group E (carbapenem, aminoglycoside, quinolone and colistin-resistant).

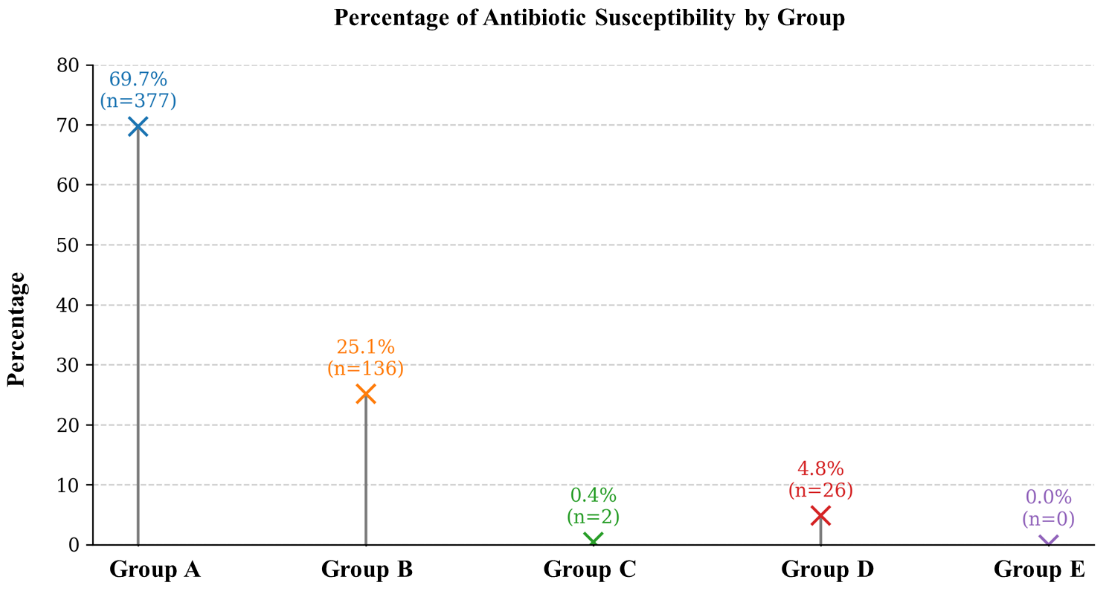


Figure 4.3 Percentages of *K. pneumoniae* isolates classified into phenotypic resistance groups based on susceptibility to major antibiotic classes. Group A: 3GC-sensitive, carbapenem-sensitive; B: 3GC-resistant, carbapenem-sensitive; C: Carbapenem–resistant but susceptible to at least one major

non-β-lactam class; D: Carbapenem, aminoglycoside and quinolone-resistant and E: Carbapenem, aminoglycoside, quinolone and colistin-resistant.

The phenotypic resistance pattern of *K. pneumoniae* isolated from five different subdistricts showed that 51.9% (125/241) of the isolates from Mymensingh Sadar were sensitive to both 3GC and carbapenems (Group A), while more than 80% of isolates from Muktagachha, Trishal, Gauripur and Tarakanda were susceptible to these antibiotics (Table 4.3 and Figure 4.4). 3GC-resistant (Group B) *K. pneumoniae* were more prevalent (36.9%) in Mymensingh Sadar, followed by 18.6% in Tarakanda, 16.4% in Muktagachha, 15.6% in Trishal and 12.7% in Gauripur. Only one isolate from Gauripur and one from Mymensingh Sadar were classified as Group C (Carbapenem–resistant but susceptible to at least one major non-β-lactam class). All *K. pneumoniae* (10.8%, 26/241) that belonged to Group D (resistant to carbapenems, quinolones, and aminoglycosides) were isolated only from Mymensingh Sadar.

Table 4.3 Distribution of phenotypic resistance groups of *K. pneumoniae* across different subdistricts.

Subdistricts	**Phenotypic resistance pattern in *K. pneumoniae* (n = 541)**			
	Group A	**Group B**	**Group C**	**Group D**
Mymensingh Sadar (n=241)	125 (51.9%)	89 (36.9%)	1 (0.4%)	26 (10.8%)
Muktagachha (n=61)	51 (83.6%)	10 (16.4%)	–	–
Trishal (n=90)	76 (84.4%)	14 (15.6%)	–	–
Gauripur (n=79)	68 (86.1%)	10 (12.7%)	1 (1.3%)	–
Tarakanda (n=70)	57 (81.4%)	13 (18.6%)	–	–

Values in parentheses indicate row percentage. Group A: 3GC-sensitive, carbapenem-sensitive; B: 3GC-resistant, carbapenem-sensitive; C: Carbapenem–resistant but susceptible to at least one major non-β-lactam class; and D: Carbapenem, aminoglycoside and quinolone-resistant.

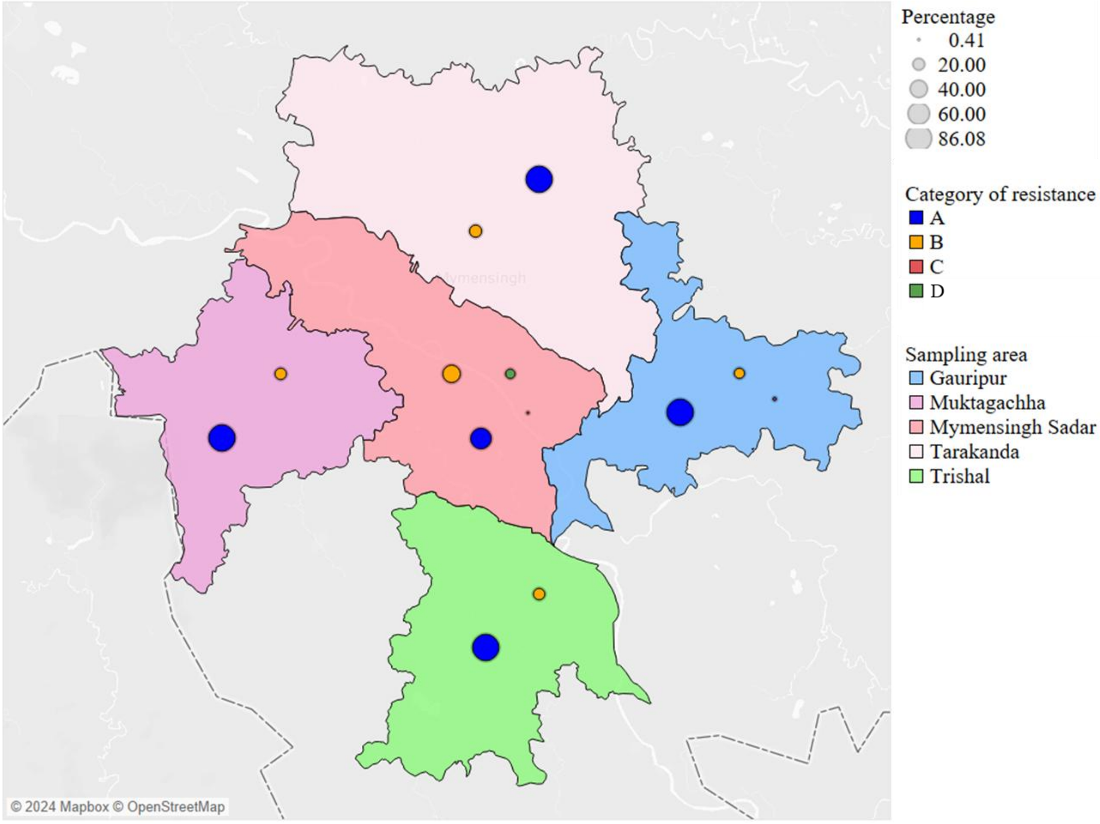


Figure 4.4 Distribution of phenotypic resistance groups of *K. pneumoniae* in different subdistricts. Different colours of the circles represent various categories of resistance, and the sizes indicate the percentage of the resistant isolates. Group A: 3GC-sensitive, carbapenem-sensitive; B: 3GC-resistant, carbapenem-sensitive; C: Carbapenem–resistant but susceptible to at least one major non-β-lactam class; and D: Carbapenem, aminoglycoside and quinolone-resistant.

Furthermore, sampling location-specific multivariate logistic regression analyses demonstrated that *K. pneumoniae* isolates recovered from MMCH were significantly associated with resistance to multiple antibiotics after multivariate adjustment compared with isolates from other sampling locations ($p<0.05$) (Table 4.4).

Table 4.4 Association between sample location and antibiotic resistance in *K. pneumoniae*.

Sampling location	**Antibiotic**	**Resistant / total (n/N)**	**Resistance (%)**	**Adjusted OR**	**95% CI**	***p*-value**
MMCH	AMC	96 / 140	68.6	4.58	1.99 – 10.54	<0.001
	TZP	52 / 140	37.1	17.73	2.35 – 133.85	0.005
	CTX	101 / 140	72.1	17.48	5.74 – 53.21	<0.001
	CAZ	102 / 140	72.9	13.96	5.00 – 38.98	<0.001
	FEP	98 / 140	70.0	15.75	5.19 – 47.82	<0.001
	ATM	102 / 140	72.9	18.12	5.95 – 55.21	<0.001
	CIP	106 / 140	75.7	3.79	1.69 – 8.48	0.001
	LVX	92 / 140	65.7	3.03	1.36 – 6.77	0.007
	AMK	49 / 140	35.0	5.03	1.45 – 17.37	0.011
	GEN	59 / 140	42.1	4.92	1.63 – 14.81	0.005
	FOS	103 / 140	73.6	2.29	1.03 – 5.11	0.042

MMCH, Mymensingh Medical College Hospital; AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; ATM, Aztreonam; CIP, Ciprofloxacin; LVX, Levofloxacin; AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin. Associations were assessed using multivariate logistic regression models fitted with generalised estimating equations and a binomial distribution with logit link. Isolate ID was specified as the clustering variable to account for repeated antimicrobial susceptibility measurements within the same isolate (nested effects). For each antibiotic, the sampling location was included as the explanatory variable, with all other sampling locations combined as the reference category. The table presents only statistically significant associations ($p<0.05$). Crude resistance frequencies are shown as resistant/total isolates (n/N, %) for descriptive purposes, whereas odds ratios (OR) and 95% confidence intervals (CI) represent adjusted estimates from the multivariate models.

This study focused on 3GC, carbapenem, aminoglycoside and quinolone resistance. The prevalence of 3GC-resistant *K. pneumoniae* was 30.3% (164/541), followed by quinolone resistance at 51.0% (276/541), aminoglycoside resistance at 22.4% (121/541) and carbapenem resistance at 5.2%

(28/541). Pearson's chi–square test revealed that 3GC resistance in *K. pneumoniae* was significantly linked to carbapenem, aminoglycoside and quinolone resistance ($p<0.05$) (Table 4.5).

Table 4.5 Association of 3GC resistance with resistance to other antibiotic classes in *K. pneumoniae*.

Antibiotic classes	**3GC resistant (n=164)**	**3GC sensitive (n=377)**	***p*–value**	**OR**	**95% CI**
Carbapenem resistance (n=28)	28 (17.1%)	0 (0)	<0.001	NA	NA
Aminoglycoside resistance (n=121)	88 (53.7%)	33 (8.8%)	<0.001	12.070	7.537 – 19.330
Quinolone resistance (n=276)	159 (97.0%)	117 (31.0%)	<0.001	70.667	28.259 – 176.712

Values in parentheses indicate column percentage. n, number; NA, not applicable. Statistical significance was set at $p<0.05$.

Minimum inhibitory concentration (MIC) values were analysed for the antibiotics tested. Ranges of MIC values of all antimicrobials against *K. pneumoniae* are presented in Figure 4.5. All MIC values were interpreted using EUCAST (v.12.0) breakpoints for *K. pneumoniae*, and the same interpretive cut-off values were applied to isolates across all sample sources. MIC values for amoxicillin–clavulanic acid ranged from 1 to >64. Other antibiotics exhibited the following ranges: piperacillin–tazobactam (0.06 to >64), cefotaxime (0.06 to >64), ceftazidime (0.06 to >64), ceftazidime–avibactam (0.06 to >64), aztreonam (0.06 to >64), imipenem (0.06 to >64), meropenem (0.06 to >64), amikacin (0.5 to >64), gentamicin (0.25 to >64), ciprofloxacin (0.06 to >64), levofloxacin (0.06 to >64), fosfomycin (0.06 to >64), colistin (0.06 to >64) and tigecycline (0.25 to 8).

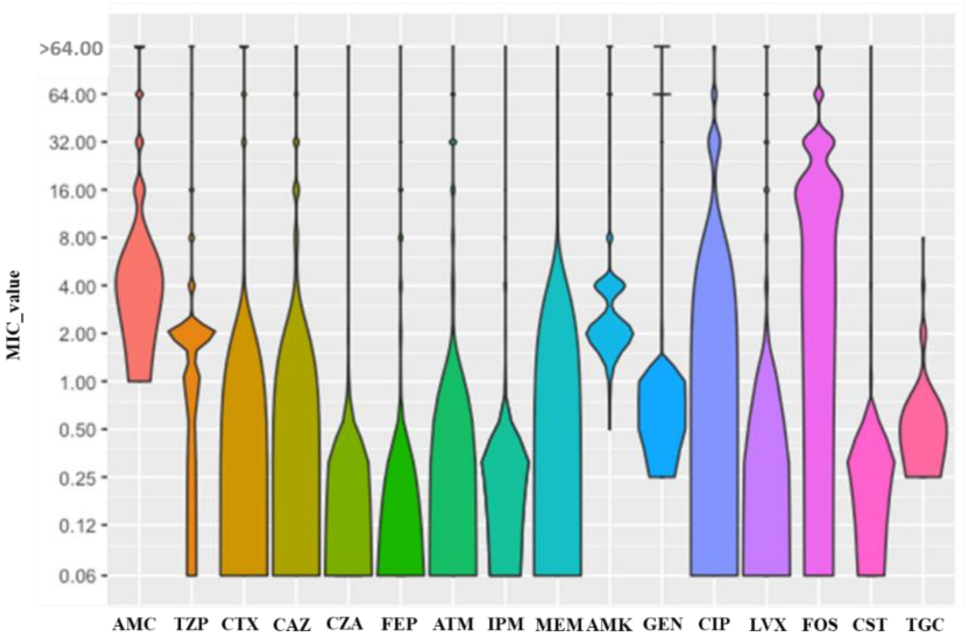


Figure 4.5 Violin plot shows the MIC ranges of different antibiotics against *K. pneumoniae*. AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; CZA, Ceftazidime–avibactam; FEP, Cefepime; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; AMK, Amikacin; GEN, Gentamicin; CIP, Ciprofloxacin; LVX, Levofloxacin; FOS, Fosfomycin; CST, Colistin; TGC, Tigecycline. MIC values were interpreted using EUCAST v.12.0 breakpoints for *K. pneumoniae*; the same cut-off values were applied across all sample sources.

MIC50 (the MIC at which ≥50% of isolates are inhibited) and MIC90 (the MIC at which ≥90% of isolates are inhibited) values of different antibiotics for *K. pneumoniae* isolated from various sample types are summarised in Table 4.6 and 4.7. MIC50 values (mg/L) exceeded the resistance breakpoints for amoxicillin–clavulanic acid (64), piperacillin–tazobactam (16), cefotaxime (>64), ceftazidime (32), cefepime (16), aztreonam (32), amikacin (64), gentamicin (64) and fosfomycin (16) in *K. pneumoniae* from SSIs. Higher values for amoxicillin–clavulanic acid (16), ciprofloxacin (32), levofloxacin (32), gentamicin (64) and fosfomycin (32) were found in isolates from CCS. In case of other samples, MIC50 values were lower than the breakpoints except for fosfomycin.

Table 4.6 MIC50 values of different antibiotics for *K. pneumoniae* from various sample types.

Antibiotics (Breakpoint)	**MIC50 values (mg/L) to respective antibiotics**								
	Water n=25	**Flies n=80**	**CCS n=62**	**CMS n=54**	**DARS n=15**	**BF n=13**	**UTIs n=14**	**SSIs n=93**	**HRS n=185**
AMC (S≤8, R>8)	8	4	16	4	4	4	8	64	4
TZP (S≤8, R>8)	2	2	2	2	2	1	1	16	2
CTX (S≤1, R>2)	0.06	0.06	0.25	0.06	0.06	0.06	0.06	>64	0.06
CAZ (S≤1, R>4)	0.25	0.25	1	0.25	0.25	0.25	0.25	32	0.25
FEP (S≤1, R>4)	0.06	0.06	0.125	0.06	0.06	0.06	0.06	16	0.06
CZA (S≤8, R>8)	0.25	0.125	0.25	0.125	0.125	0.25	0.125	0.5	0.125
ATM (S≤1, R>4)	0.06	0.06	0.125	0.06	0.06	0.06	0.06	32	0.06
IPM (S≤2, R>4)	0.25	0.25	0.25	0.25	0.25	0.25	0.125	0.25	0.25
MEM (S≤1, R>8)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.125	0.06
CIP (S≤0.25, R>0.5)	0.06	0.06	32	0.5	0.06	0.06	0.25	4	0.06
LVX (S≤0.5, R>1)	0.125	0.125	32	0.5	0.06	0.06	0.25	1	0.06
AMK (S≤8, R>8)	2	4	4	4	2	2	4	64	2
GEN (S≤2, R>2)	1	1	64	1	1	0.5	0.5	64	0.5
FOS (S≤8, R>8)	16	16	32	32	16	8	8	16	16
TGC (S≤0.5, R>0.5)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
CST (S≤2, R>2)	0.25	0.125	0.25	0.25	0.25	0.25	0.25	0.25	0.25

n, number; S, sensitive; R, resistant; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs; AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime–avibactam; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin;

AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline; CST, Colistin. MIC values were interpreted using EUCAST (v.12.0) breakpoints for *K. pneumoniae*; the same cut-off values were applied across all sample sources.

For MIC90, values exceeded the resistance breakpoints for all antimicrobials in isolates from SSIs, except for tigecycline (2) and colistin (0.5). On the contrary, MIC90 of tigecycline (4) and colistin (4) exceeded the cut-offs in CCS. In isolates from UTIs, a rise in MIC90 values (mg/L) was observed for amoxicillin–clavulanic acid (16), cefotaxime (64), ceftazidime (16), aztreonam (16), and fosfomycin (32). The higher MIC90 values were found in CCS and CMS isolates, except for ceftazidime–avibactam (0.5), imipenem (0.25), and meropenem (0.06). In water isolates, MIC90 values (mg/L) were higher for amoxicillin–clavulanic acid (>64), cefotaxime (16), ceftazidime (64), aztreonam (8), ciprofloxacin (32), levofloxacin (>64), gentamicin (>64), fosfomycin (32) and tigecycline (2). *K. pneumoniae* from flies showed higher values for amoxicillin–clavulanic acid (64), ciprofloxacin (32), levofloxacin (8), gentamicin (32) and fosfomycin (32). MIC90 values of different antibiotics in *K. pneumoniae* from DARS, BF and HRS were lower than the breakpoint, except for fosfomycin.

Table 4.7 MIC90 values of different antibiotics for *K. pneumoniae* from various sample types.

Antibiotics (Breakpoint)	**MIC90 values (mg/L) to respective antibiotics**								
	Water n=25	**Flies n=80**	**CCS n=62**	**CMS n=54**	**DARS n=15**	**BF n=13**	**UTIs n=14**	**SSIs n=93**	**HRS n=185**
AMC (S≤8, R>8)	>64	64	>64	64	8	8	16	>64	8
TZP (S≤8, R>8)	8	4	8	4	2	2	2	>64	2
CTX (S≤1, R>2)	16	0.5	32	16	0.06	16	64	>64	0.5
CAZ (S≤1, R>4)	64	4	32	16	0.25	8	16	>64	0.5
FEP (S≤1, R>4)	4	0.25	8	8	0.06	0.25	8	>64	0.06
CZA (S≤8, R>8)	0.5	0.25	0.5	0.5	0.25	0.25	0.25	>64	0.25
ATM (S≤1, R>4)	8	0.25	16	16	0.125	2	16	64	0.125
IPM (S≤2, R>4)	0.5	0.25	0.25	0.25	0.25	0.25	0.25	16	0.5
MEM (S≤1, R>8)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	32	0.06
CIP (S≤0.25, R>0.5)	32	32	64	32	0.5	0.5	1	>64	0.5
LVX (S≤0.5, R>1)	>64	8	>64	64	1	1	1	64	1
AMK (S≤8, R>8)	4	4	8	4	4	4	4	>64	4
GEN (S≤2, R>2)	>64	32	>64	1	1	1	1	>64	1
FOS (S≤8, R>8)	32	32	>64	64	64	16	32	64	32
TGC (S≤0.5, R>0.5)	2	0.5	4	2	0.5	0.5	0.5	2	0.5
CST (S≤2, R>2)	0.5	0.25	4	0.25	0.25	0.5	0.5	0.5	0.5

n, number; S, sensitive; R, resistant; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs; AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime–avibactam; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin;

AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline; CST, Colistin. MIC values were interpreted using EUCAST (v.12.0) breakpoints for *K. pneumoniae*; the same cut-off values were applied across all sample sources.

4.2.2 Linkage between phenotypic resistance and genotypically determined ARGs for 3GC, carbapenem, aminoglycoside and quinolone resistance in *K. pneumoniae*

A total of 131 distinct ARGs were identified from 541 *K. pneumoniae* isolates. The frequency of various resistant genes found in *K. pneumoniae* is presented in Figure 4.6. Data showed that $bla_{\text{TEM-1B}}$ was the most common among the β-lactam genes, present in 25.0% (135/541) of isolates, whereas $bla_{\text{CTX–M–15}}$ was the most predominant ESBL gene, detected in 23.5% (127/541). Among the carbapenem resistance alleles, $bla_{\text{NDM–5}}$ was the most common (n=14), followed by $bla_{\text{NDM–1}}$ (n=4), $bla_{\text{NDM–4}}$ (n=1), $bla_{\text{OXA–232}}$ (n=7), and $bla_{\text{OXA–181}}$ (n=5). The combinations of carbapenem alleles recovered were: $bla_{\text{NDM–5}}$ + $bla_{\text{OXA–181}}$ (n=3), $bla_{\text{NDM–5}}$ + $bla_{\text{OXA–232}}$ (n=1) and $bla_{\text{NDM–4}}$ + $bla_{\text{OXA–181}}$ (n=1). For colistin resistance, *mcr–8.1* was the most frequently found *mcr* gene in *K. pneumoniae* isolates (n=9), followed by *mcr–1.1* (n=2). A single isolate was recovered with a combination of *mcr1.1* and *mcr8.1* genes from HRS. The *oqxA* (n=528) and *oqxB* (n=525) were the most frequently found quinolone-resistant genes, followed by *qnrS1* (n=157) and *qnrB1* (n=67). For aminoglycoside resistance, *aph(6)–Id* (n=98), *aph(3")–Ib* (n=93), *aac(6')–Ib* (n=86), *aadA2* (n=84), *aac(3)–IIa* (n=55) were the prevalent resistant genes. The *tet(A)* was the most frequently identified tetracycline-resistant gene, found in 32.5% (176/541) of the isolates.

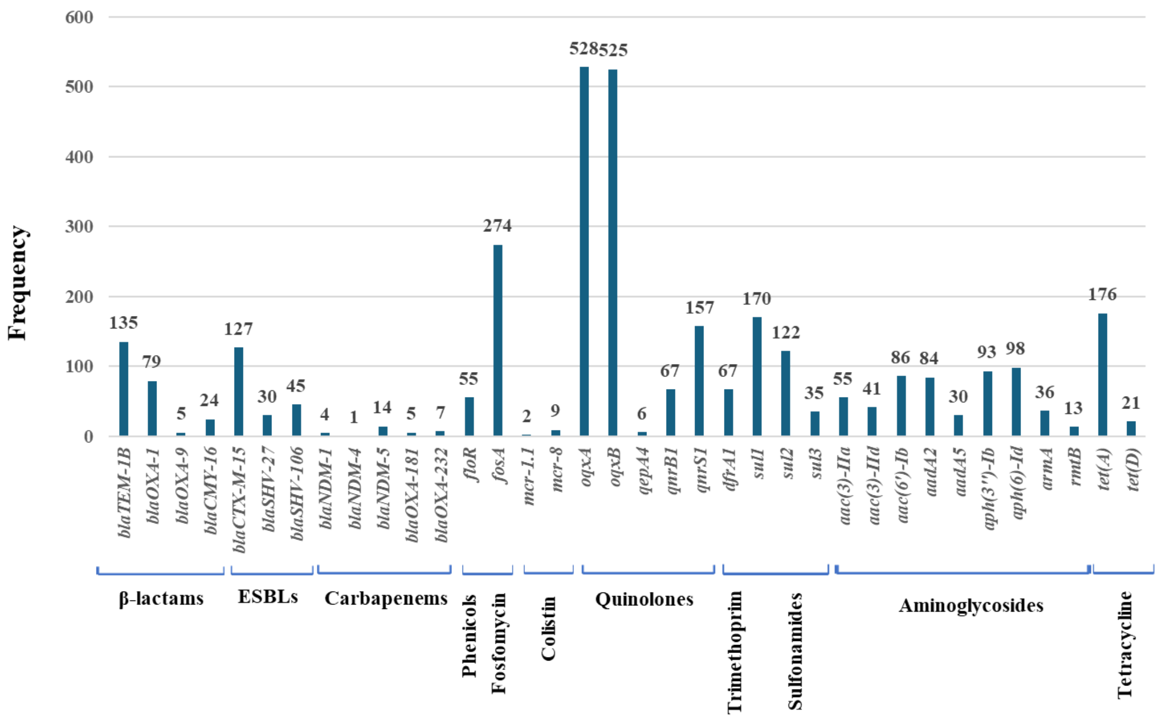


Figure 4.6 Distribution of ARGs in *K. pneumoniae* (n=541).

The associations of 3GC, carbapenem, aminoglycoside, and quinolone-resistant *K. pneumoniae* with their respective ARGs were examined. The ARGs were considered for the analysis only if the frequency of any gene exceeded 10. Results showed a significant association of *bla*$_{\text{CTX–M–15}}$ (77.4%, 127/164) with 3GC resistance, followed by *bla*$_{\text{NDM–5}}$ (50.0%, 14/28) with carbapenem resistance, *aac(6')–Ib* (59.5%, 72/121), *aph(6)–Id* (51.2%, 62/121) with aminoglycoside resistance and *qnrS1* (55.4%, 153/276), *qnrB1* (23.9%, 66/276) with quinolone resistance in *K. pneumoniae* from various samples (p<0.05) (Table 4.8).

Table 4.8 Linkage between phenotypic resistance and genotypically determined ARGs in *K. pneumoniae*.

ARGs pertinent to phenotypic resistance	**3GC-resistant (n=164)**	**3GC-sensitive (n=377)**	***p*–value**	**OR**	**95% CI**
bla$_{\text{CTX–M–15}}$ (n=127)	127 (77.4%)	0 (0)	–	NA	NA
	Carbapenem-resistant (n=28)	**Carbapenem-sensitive (n=513)**	***p*–value**	**OR**	**95% CI**
bla$_{\text{NDM–5}}$ (n=14)	14 (50.0%)	0 (0)	–	NA	NA
	Aminoglycoside-resistant (n=121)	**Aminoglycoside-sensitive (n=420)**	***p*–value**	**OR**	**95% CI**
aac(6')–Ib (n=86)	72 (59.5%)	14 (3.3%)	<0.001	42.612	22.364 – 81.192
aph(6)–Id (n=98)	62 (51.2%)	36 (8.6%)	<0.001	11.209	6.841 – 18.365
	Quinolone-resistant (n=276)	**Quinolone-sensitive (n=265)**	***p*–value**	**OR**	**95% CI**
qnrS1 (n=157)	153 (55.4%)	4 (1.5%)	<0.001	81.165	29.397 – 224.095
qnrB1 (n=67)	66 (23.9%)	1 (0.4%)	<0.001	82.971	11.421 – 602.779

Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$. NA, not applicable. ARGs that had a significantly higher association with respective antibiotic classes are listed in the table.

ESBL gene *bla*$_{\text{CTX–M–15}}$ was found across all the sample types except for DARS whereas carbapenem-resistant genes (*bla*$_{\text{NDM–1}}$, *bla*$_{\text{NDM–4}}$, *bla*$_{\text{NDM–5}}$, *bla*$_{\text{OXA–181}}$ and *bla*$_{\text{OXA–232}}$) were mostly limited to SSIs (Figure 4.7). However, *bla*$_{\text{NDM–5}}$ was found in a single isolate from hospital drainage water. Colistin-resistant genes (*mcr-1.1* and *mcr-8.1*) were found in water, flies, CCS, CMS and HRS but not in clinical samples and DARS. Aminoglycoside (*aac(6')–Ib*, *aph(6)–Id*) and fluoroquinolone–resistant genes (*qnrS1*, *qnrB1*) were also present in *K. pneumoniae* from different sources (Figure 4.7).

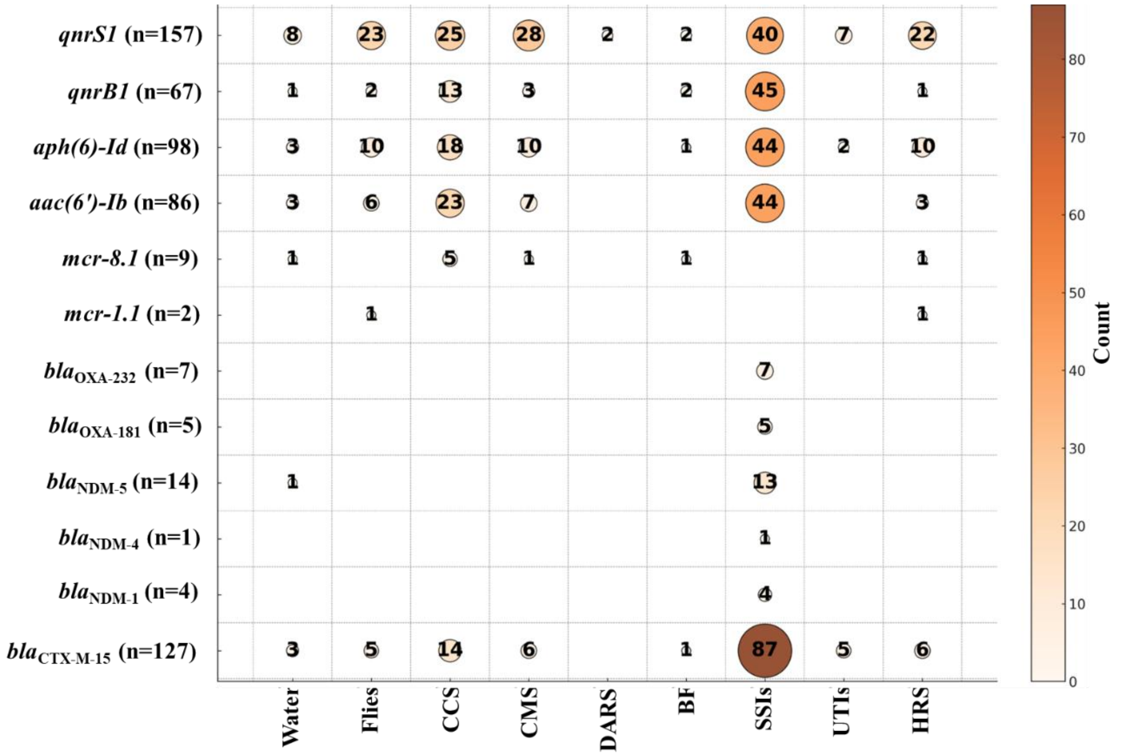


Figure 4.7 Bubble chart showing the distribution of different ARGs in *K. pneumoniae* recovered from various sources. Values in the boxes indicate the frequency of respective ARGs. The colour gradient from white to deep orange indicates a progression from lower to higher values.

This study also examined the associations of various prevalent ARGs with 3GC and carbapenem–resistant *K. pneumoniae* (Table 4.9 and 4.10). Aminoglycoside–resistance genes (*aac(3)–IIa*, *aac(3)–IId*, *aac(6')–Ib*, *aadA2*, *aadA5*, *aph(3'')–Ib*, *aph(6)–Id*, *armA*, *rmtB*), β–lactamase gene *bla*$_{\text{TEM–1B}}$, carbapenem resistance gene *bla*$_{NDM–5}$, fluoroquinolone–resistance genes (*qnrB1*, *qnrS1*), sulfonamide– and trimethoprim–resistance genes (*sul1, sul2, sul3* and *dfrA1*), and tetracycline resistance genes (*tet(A)* and *tet(D)*) were significantly correlated to 3GC-resistant *K. pneumoniae* (p<0.05). In carbapenem–resistant *K. pneumoniae*, significant associations were found with aminoglycoside–resistance genes (*aac(3)–IIa*, *aac(6')–Ib*, *aadA2*, *aadA5*, *armA*, *rmtB*), β–lactamase gene *bla*$_{\text{TEM–1B}}$, ESBL gene *bla*$_{\text{CTX–M–15}}$, fluoroquinolone–resistance gene *qnrB1* and sulfonamide–resistance gene *sul1*($p<0.05$).

Table 4.9 Distribution of ARGs between 3GC-resistant and sensitive *K. pneumoniae*.

ARGs	**3GC-resistant (n=164)**	**3GC-sensitive (n=377)**	***p*–value**	**OR**	**95% CI**
aac(3)–IIa	51 (31.1%)	4 (1.1%)	<0.001	42.086	14.887 – 118.979
aac(3)–IId	19 (11.6%)	22 (5.8%)	0.020	2.114	1.111 – 4.024
aac(6')–Ib	67 (40.9%)	19 (5.0%)	<0.001	13.015	7.459 – 22.708
aadA2	55 (33.5%)	29 (7.7%)	<0.001	6.055	3.678 – 9.969
aadA5	25 (15.2%)	5 (1.3%)	<0.001	13.381	5.023 – 35.645
aph(3'')–Ib	76 (46.3%)	17 (4.5%)	<0.001	18.289	10.289 – 32.508
aph(6)–Id	76 (46.3%)	22 (5.8%)	<0.001	13.936	8.213 – 23.648
armA	35 (21.3%)	1 (0.3%)	<0.001	102.016	13.837 – 752.143
bla$_{\text{NDM–5}}$	14 (8.5%)	0 (0)	–	NA	NA
bla$_{\text{TEM–1B}}$	106 (64.6%)	29 (7.7%)	<0.001	21.931	13.356 – 36.012
dfrA1	53 (32.3%)	14 (3.7%)	<0.001	12.380	6.619 – 23.156
qnrB1	63 (38.4%)	4 (1.1%)	<0.001	58.166	20.678 – 163.620
qnrS1	79 (48.2%)	78 (20.7%)	<0.001	3.563	2.400 – 5.288
rmtB	13 (7.9%)	0 (0)	–	NA	NA
sul1	115 (70.1%)	55 (14.6%)	<0.001	13.740	8.849 – 21.334
sul2	83 (50.6%)	39 (10.3%)	<0.001	8.881	5.656 – 13.944
sul3	20 (12.2%)	15 (4.0%)	<0.001	3.352	1.670 – 6.728
tet(A)	98 (59.8%)	78 (20.7%)	<0.001	5.692	3.818 – 8.486
tet(D)	13 (7.9%)	8 (2.1%)	0.001	3.971	1.613 – 9.775

Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$. NA, not applicable. ARGs that had a significantly higher association with the respective antibiotic class are listed in the table.

Table 4.10 Distribution of ARGs between carbapenem-resistant and sensitive *K. pneumoniae*.

ARGs	**Carbapenem-resistant (n=28)**	**Carbapenem-sensitive (n=513)**	***p*–value**	**OR**	**95% CI**
aac(3)–IIa	9 (32.1%)	46 (9.0%)	<0.001	4.809	2.058 – 11.240
aac(6')–Ib	10 (35.7%)	76 (14.8%)	0.003	3.194	1.420 – 7.184
aadA2	16 (57.1%)	68 (13.3%)	0.003	8.725	3.957 – 19.241
aadA5	4 (14.3%)	26 (5.1%)	0.038	3.122	1.009 – 9.660
armA	9 (32.1%)	27 (5.3%)	<0.001	8.526	3.527 – 20.612
bla$_{\text{CTX–M–15}}$	25 (89.3%)	102 (19.9%)	<0.001	33.578	9.943 – 113.399
bla$_{\text{TEM–1B}}$	17 (60.7%)	118 (23.0%)	<0.001	5.173	2.358 – 11.351
qnrB1	12 (42.9%)	55 (10.7%)	<0.001	6.245	2.809 – 13.886
rmtB	11 (39.3%)	2 (0.4%)	<0.001	165.324	33.977 – 804.427
sul1	22 (78.6%)	148 (28.8%)	<0.001	9.043	3.594 – 22.752

Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$. NA, not applicable. ARGs that had a significantly higher association with the respective antibiotic class are listed in the table.

4.2.3 Clonal distribution and genomic resistance of the *K. pneumoniae* population

A comprehensive insight into the clonal relatedness among the isolates was achieved by combining core–genome alignment, genome–wide SNP analysis, and MLST profiling, where applicable. All 541 *K. pneumoniae* isolated from primary screening were sequenced using the Illumina NovaSeq 6000 platform. Whole-genome sequencing data generated for the 541 *K. pneumoniae* isolates were of consistently good quality. Genome assemblies produced by the MicrobesNG pipeline showed good contiguity, with a median of 34 contigs ≥1,000 bp per genome (interquartile range: 23–51). The median assembled genome size was 5.41 Mb (interquartile range: 5.29–5.53 Mb), and GC content was tightly distributed around a median of 57.3%, consistent with reference *K. pneumoniae* genomes. Assembly continuity was further supported by a median N50 of 464,087 bp, with several assemblies exceeding 1 Mb. No ambiguous bases (Ns) were detected in any assembly. Collectively, these metrics indicate that sequencing and assembly quality were sufficient to support robust core-genome alignment, SNP-based phylogenetic inference, MLST assignment, and detection of antimicrobial resistance genes. The assembly quality metrics for the isolates are provided in the Supplementary Table S1 (Appendix F). The latest ABRicate database was used to screen ARGs. The PubMLST database and Kleborate (v.3.0, a dedicated genotypic tool for the *K. pneumoniae* species complex) were used to identify species, sequence types (STs), and acquired ARGs (Wyres et al., 2016; Lam et al., 2021).

Data revealed a high level of diversity in STs for *K. pneumoniae* across different sample sources. In total, 260 different STs were identified, of which ST37 (n=25), ST490 (n=20), ST15 (n=18), ST147 (n=15), ST48 (n=14), and ST45 (n=12) were the dominant STs, having at least 10 isolates. Twenty-two STs with five or more isolates accounted for 38.3% (207/541) of the isolates, while the remaining 61.7% (334/541) were represented by diverse STs with frequencies ranging from one to four isolates per ST (Figure 4.8).

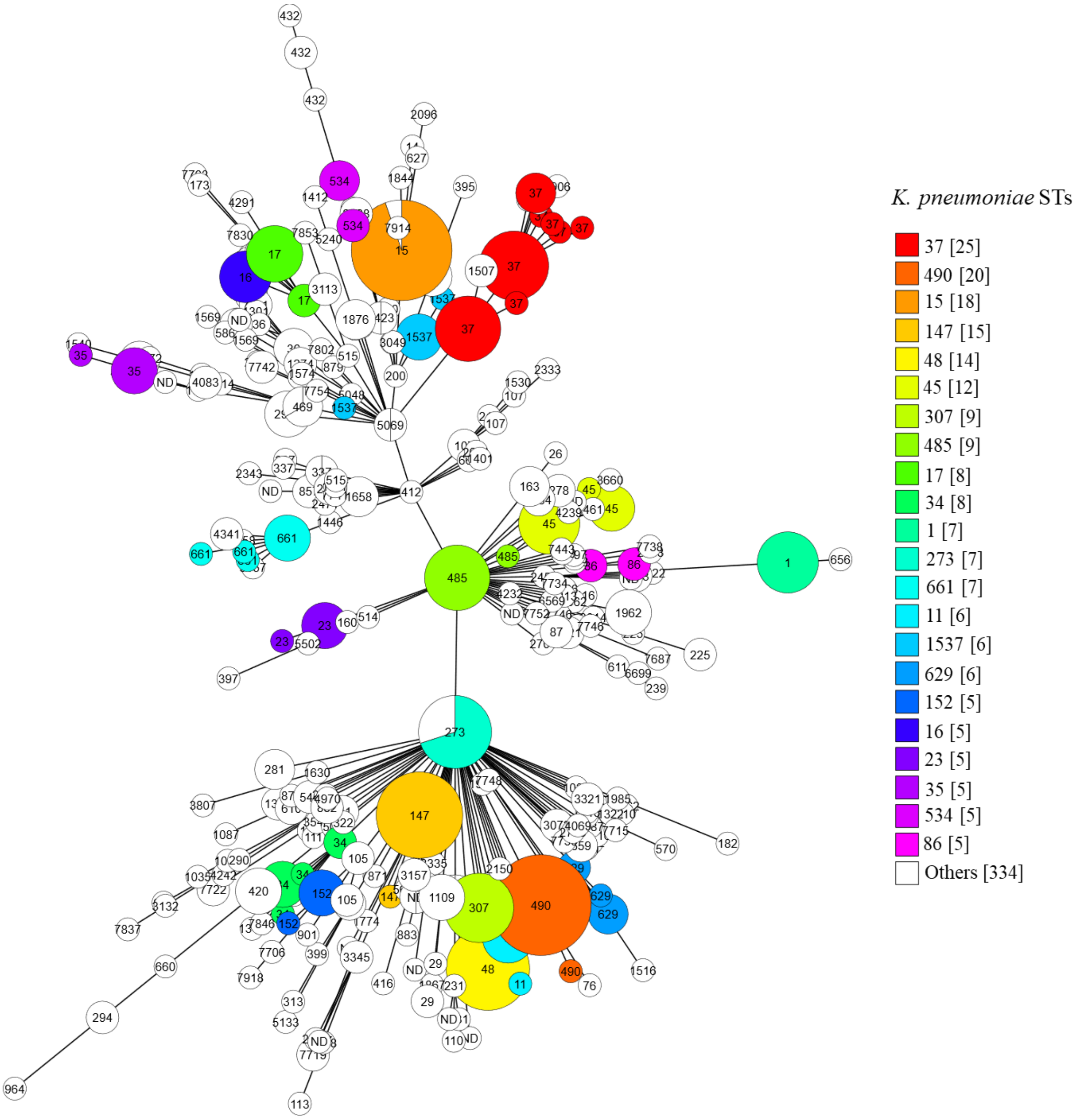


Figure 4.8 Minimum spanning tree showing the distribution of 541 *K. pneumoniae* isolates across different STs. This grape tree was constructed based on core-genome multilocus sequence typing and annotated using the ReporTree (a web-based pipeline) (Mixão *et al.*, 2023). Node colours correspond to individual sequence types (STs), with only STs represented by ≥ 5 isolates shown, thereby highlighting clonal diversity and overall population structure.

The distribution of *K. pneumoniae* STs (with ≥5 isolates) across different sampling sources and sites is presented in Figure 4.9 and 4.10, respectively. All the ST490 and ST48 isolates were limited to MMCH. Among the dominant STs, ST490 and ST48 were found to be significantly associated with SSIs, while ST45 was significantly associated with HRS (Table 4.11).

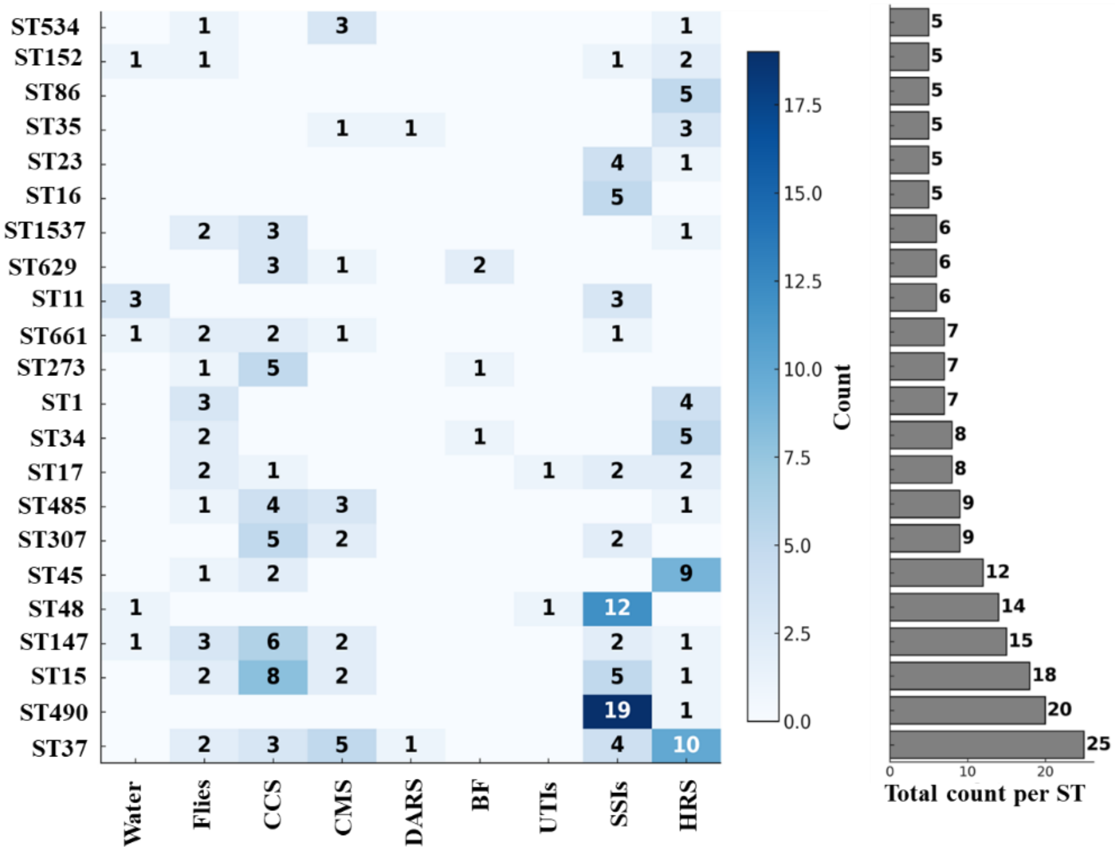
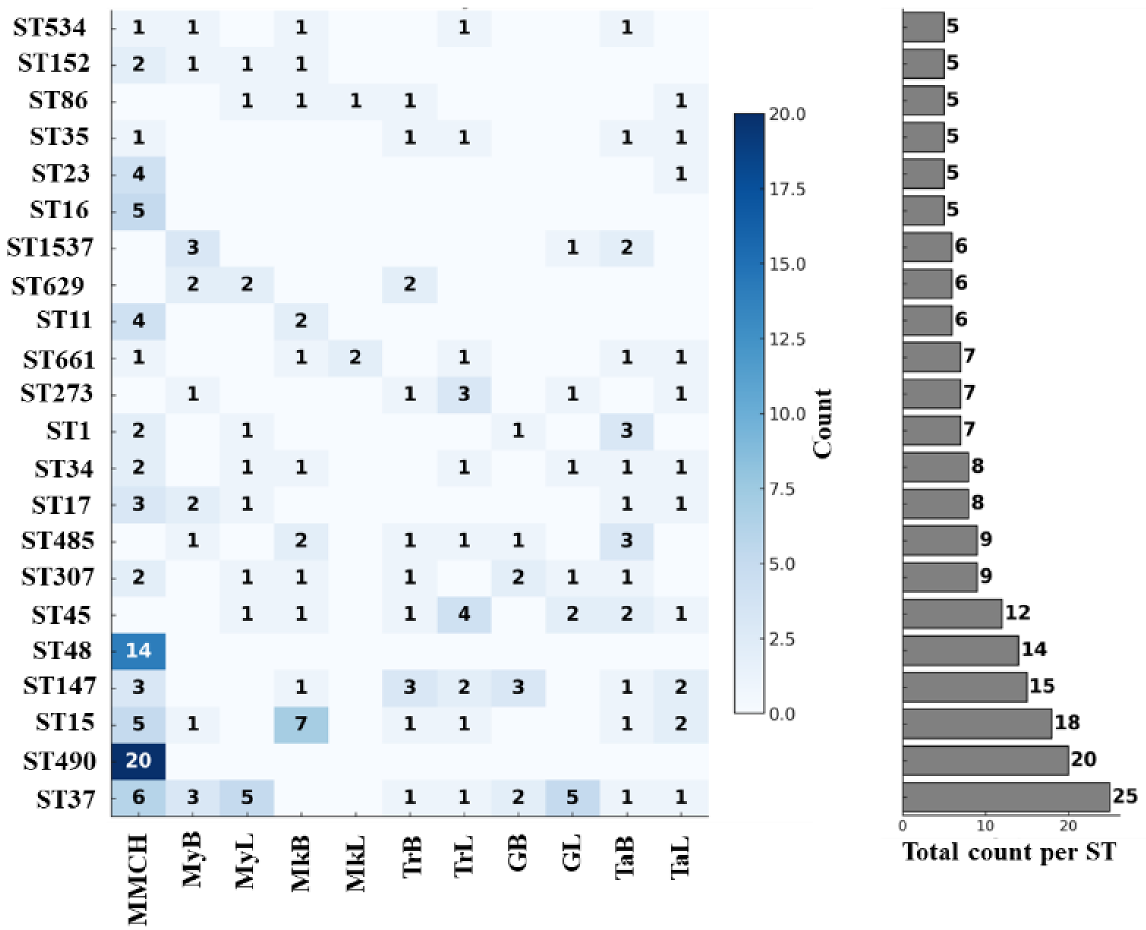


Figure 4.9 Distribution of *K. pneumoniae* STs (having ≥5 isolates) across different sources. CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs. Values in the boxes indicate the frequency of respective STs. The colour gradient from white to deep blue indicates a progression from lower to higher values.



	MMCH	MyB	MyL	MkB	MkL	TrB	TrL	GB	GL	TaB	TaL	Total count per ST
ST534	1	1		1			1			1		5
ST152	2	1	1	1								5
ST86			1	1	1	1					1	5
ST35	1					1	1			1	1	5
ST23	4										1	5
ST16	5											5
ST1537		3							1	2		6
ST629		2	2			2						6
ST11	4			2								6
ST661	1			1	2		1			1	1	7
ST273		1				1	3		1		1	7
ST1	2		1					1		3		7
ST34	2		1	1			1		1	1	1	8
ST17	3	2	1							1	1	8
ST485		1		2		1	1	1		3		9
ST307	2		1	1		1		2	1	1		9
ST45			1	1		1	4		2	2	1	12
ST48	14											14
ST147	3			1		3	2	3		1	2	15
ST15	5	1		7		1	1			1	2	18
ST490	20											20
ST37	6	3	5			1	1	2	5	1	1	25

Figure 4.10 Distribution of *K. pneumoniae* STs (having ≥5 isolates) across different sampling sites. GB, Gauripur broiler; GL, Gauripur layer; MkB, Muktagachha broiler; MkL, Muktagachha layer; MMCH, Mymensingh Medical College Hospital; MyB, Mymensingh broiler; MyL, Mymensingh layer; TaB, Tarakanda broiler; TaL, Tarakanda layer; TrB, Trishal broiler; TrL, Trishal layer farms. Values in the boxes indicate the frequency of respective STs. The colour gradient from white to deep blue indicates a progression from lower to higher values.

Table 4.11 Comparative analysis of *K. pneumoniae* dominant STs with various sample sources.

STs	**SSIs (n=93)**	**Others (n=448)**	***p*–value**
ST490 (n=20)	19 (20.4%)	1 (0.2%)	<0.001
ST48 (n=14)	12 (12.9%)	2 (0.4%)	<0.001
STs	**HRS (n=185)**	**Others (n=356)**	***p*–value**
ST45 (n=12)	9 (4.9%)	3 (0.8%)	0.003

Values in parentheses indicate column percentage. STs, sequence types; ‘Others’ refers to remaining isolates from other sample sources; SSIs, surgical site infections; HRS, human rectal swabs; ‘Others’ meaning remaining isolates from all other sources. Statistical significance was set at $p<0.05$, and only data that were statistically significant are presented in the table.

Kleborate assigns the *K. pneumoniae* isolates four resistance scores (0 to 3): 0 = no ESBL, no carbapenemase, indicating a low level of resistance; 1 = ESBL positive but no carbapenemase, 2 = carbapenemase positive; 3 = Carbapenemase positive with colistin resistance. Typically, an isolate with a score of >0 was considered MDR. Figure 4.11 and 4.12 illustrate the distribution of isolates from various sources and sampling sites, respectively, according to their resistance scores. Regarding the 541 *K. pneumoniae* isolates, 411 (75.9%) belonged to category 0, exhibiting a low level of resistance, 104 (19.2%) carried ESBL genes (category 1), and only 26 (4.8%) carried a carbapenemase gene. No isolates belonged to the highest resistance category 3, which carries both carbapenemase and colistin–resistant genes.

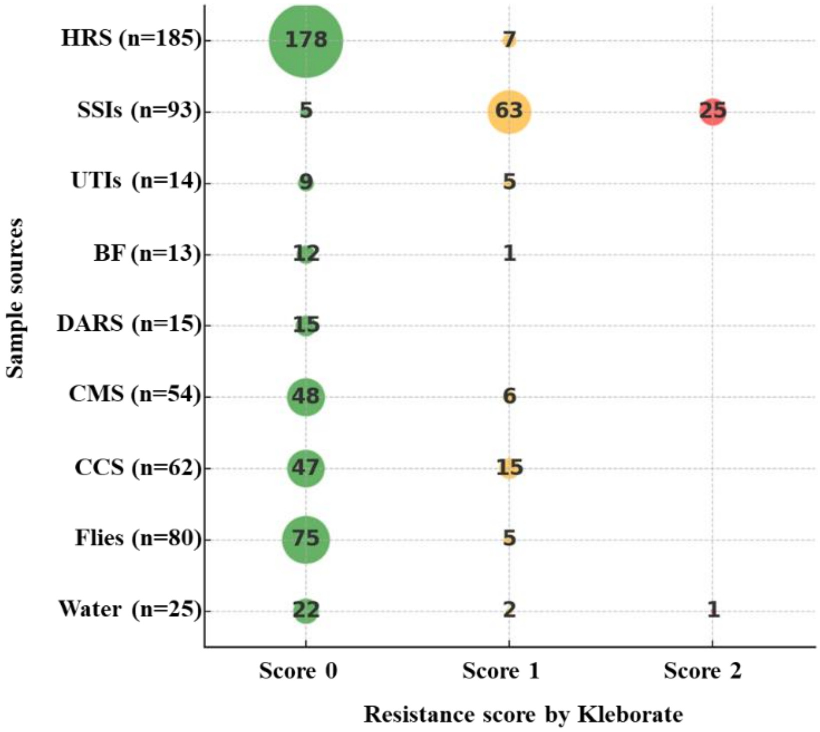


Figure 4.11 Distribution of *K. pneumoniae* from various sources based on their resistance scores. Score 0 = no ESBL, no carbapenemase, indicating a low level of resistance; 1 = ESBL positive but no

carbapenemase, 2 = carbapenemase positive. CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs. Values inside the circles represent the frequency of respective resistance scores, with the size of the circles increasing from smaller to larger as the frequency increases.

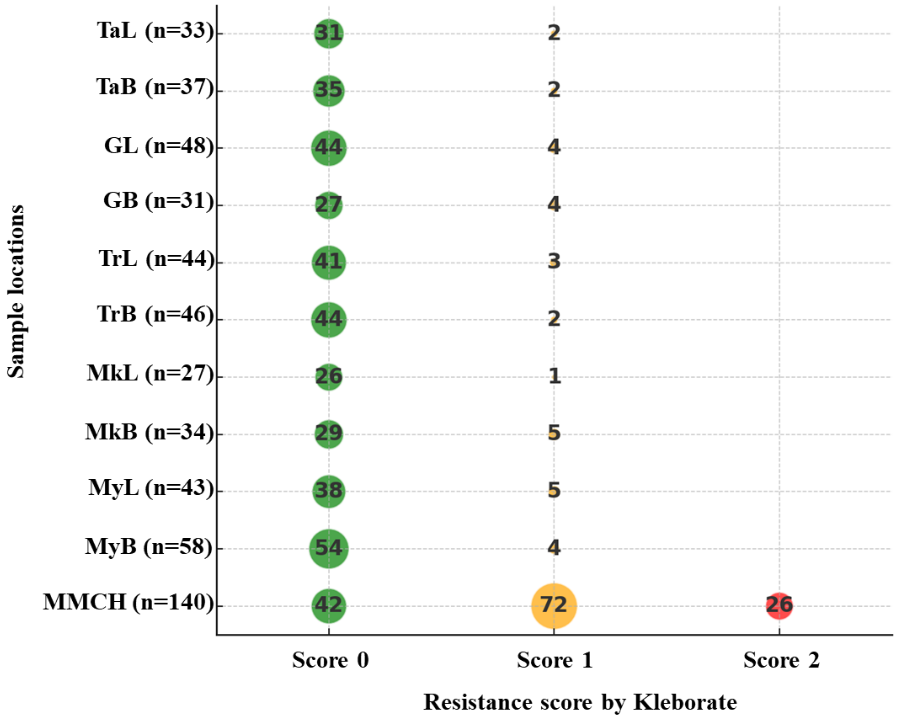


Figure 4.12 Distribution of *K. pneumoniae* from sampling sites based on their resistance scores. Score 0 = no ESBL, no carbapenemase, indicating a low level of resistance; 1 = ESBL positive but no carbapenemase, 2 = carbapenemase positive. MMCH, Mymensingh Medical College Hospital; MyB, Mymensingh broiler; MyL, Mymensingh layer; MkB, Muktagachha broiler; MkL, Muktagachha layer; TrB, Trishal broiler; TrL, Trishal layer; GB, Gauripur broiler; GL, Gauripur layer; TaB, Tarakanda broiler; TaL, Tarakanda layer farms. Values inside the circles represent the frequency of respective resistance scores, with the size of the circles increasing from smaller to larger as the frequency increases.

Of the 26 isolates harbouring carbapenemase genes, all were isolated from the hospital environment (MMCH), where 25 were isolated from SSIs and one from a water sample collected from the hospital drain (Figure 4.12). In the analysis of predominant STs, the Kleborate resistance score indicated that 95% (19/20) of the isolates were MDR, belonging to ST490. This was followed by ST48, with 92.9%

(13/14) isolates, ST37 at 52% (13/25), 46.7% (7/15) from ST147, and 38.9% (7/18) for ST15. All isolates from ST45 exhibited low resistance levels (Figure 4.13).

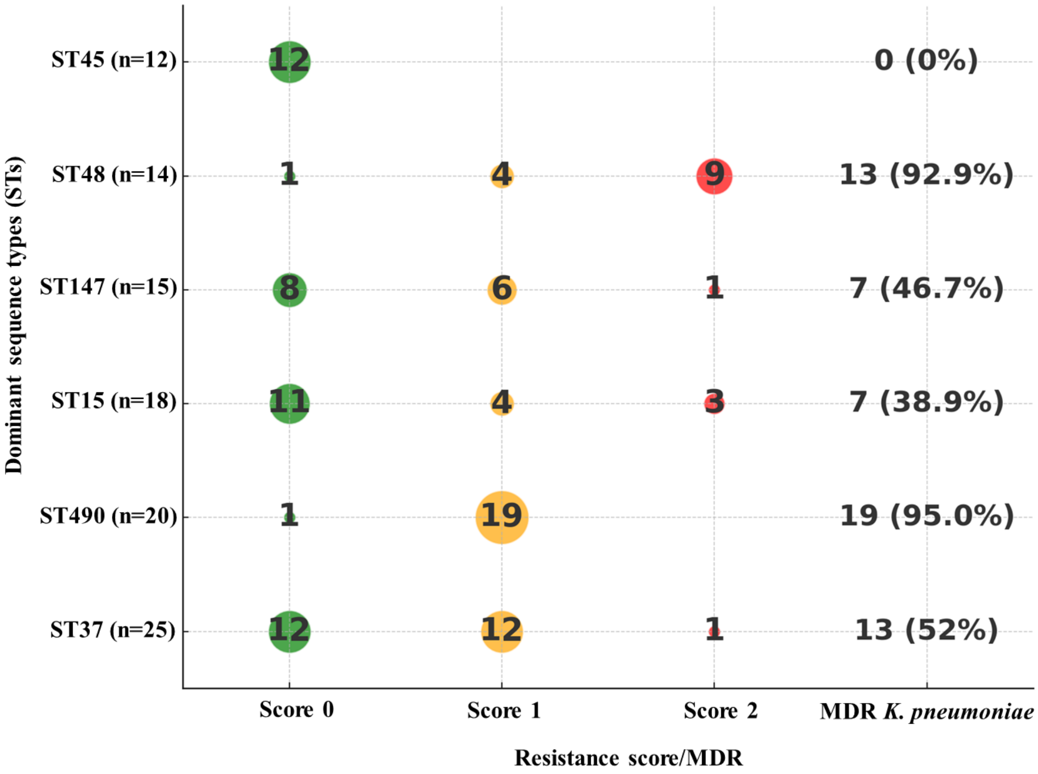


Figure 4.13 Resistance score in *K. pneumoniae* dominant STs. MDR, multidrug-resistant. Score 0 = no ESBL, no carbapenemase, indicating a low level of resistance; 1 = ESBL positive but no carbapenemase, 2 = carbapenemase positive. Isolates with a score of >0 were considered MDR (Kleborate). Values inside the circles represent the frequency of respective resistance scores, with the size of the circles increasing from smaller to larger as the frequency increases.

Univariate logistic regression analyses demonstrated that predominant STs were significantly associated with distinct antibiotic-resistance phenotypes (Figure 4.14). Specifically, ST37, ST490, ST147 and ST48 showed a significant association with 3GC-resistant *K. pneumoniae* (Figure 4.15), whereas ST15 and ST48 were significantly linked to carbapenem resistance (Figure 4.16) ($p<0.05$). Furthermore, ST490, ST15, ST147 and ST48 were each significantly correlated with both aminoglycoside resistance (Figure 4.17) and quinolone resistance (Figure 4.18) ($p<0.05$).

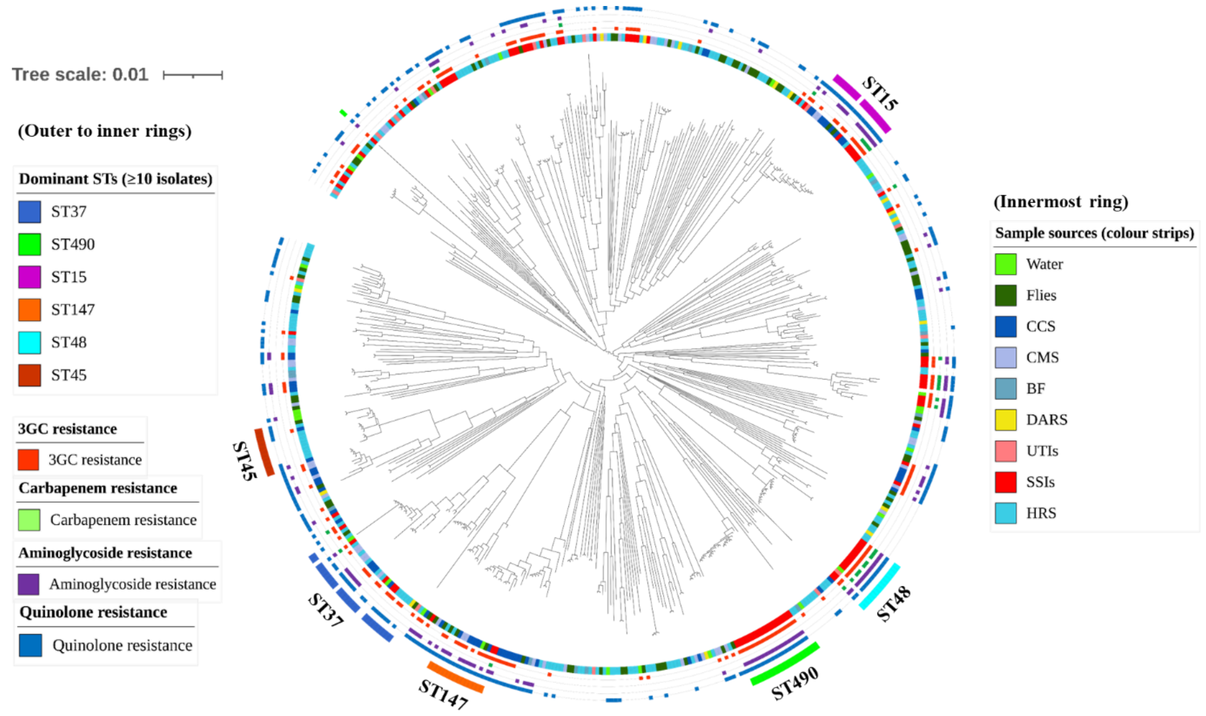


Figure 4.14 Maximum–likelihood (ML) phylogenetic tree of *K. pneumoniae* isolates (n=541) showing the association of the predominant STs of *K. pneumoniae* with distinct antibiotic-resistance phenotypes. The ML tree was constructed based on core-gene alignments (VeryFastTree v.4.0) and visualised in iTOL (v.6), with concentric rings indicating sample sources, resistance profiles to 3GCs, carbapenems, aminoglycosides, and quinolones, and predominant STs.

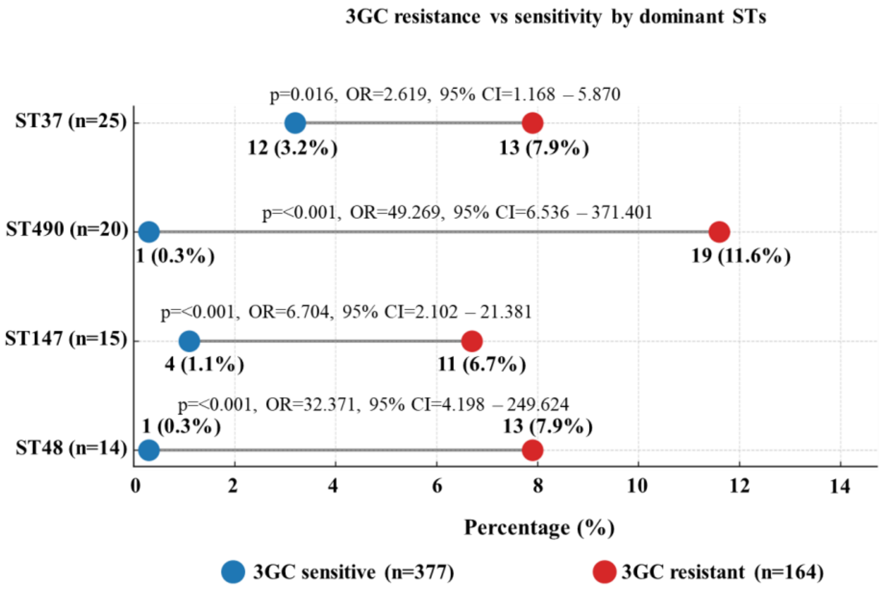


Figure 4.15 Association of 3GC resistance with the dominant STs. Values in parentheses indicate column percentage (percent positive within 3GC-sensitive and 3GC-resistant *K. pneumoniae*). STs,

sequence types. Statistical significance was set at $p<0.05$, and only data that were statistically significant are presented in the figure.

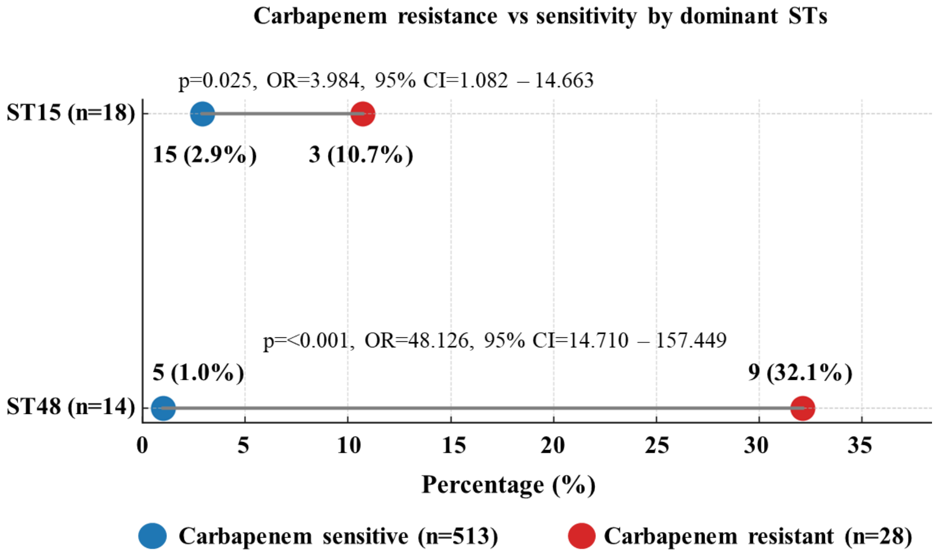


Figure 4.16 Association of carbapenem resistance with the dominant STs. Values in parentheses indicate column percentage (percent positive within carbapenem-sensitive and carbapenem-resistant *K. pneumoniae*). STs, sequence types. Statistical significance was set at $p<0.05$, and only data that were statistically significant are presented in the figure.

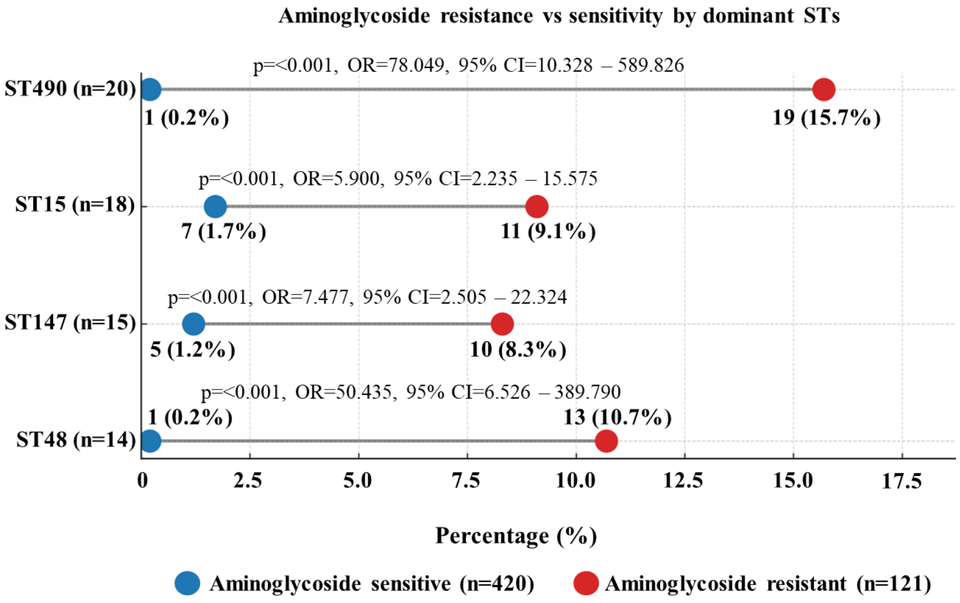


Figure 4.17 Association of aminoglycoside resistance with the dominant STs. Values in parentheses

indicate column percentage (percent positive within aminoglycoside-sensitive and aminoglycoside-resistant *K. pneumoniae*). STs, sequence types. Statistical significance was set at p<0.05, and only data that were statistically significant are presented in the figure.

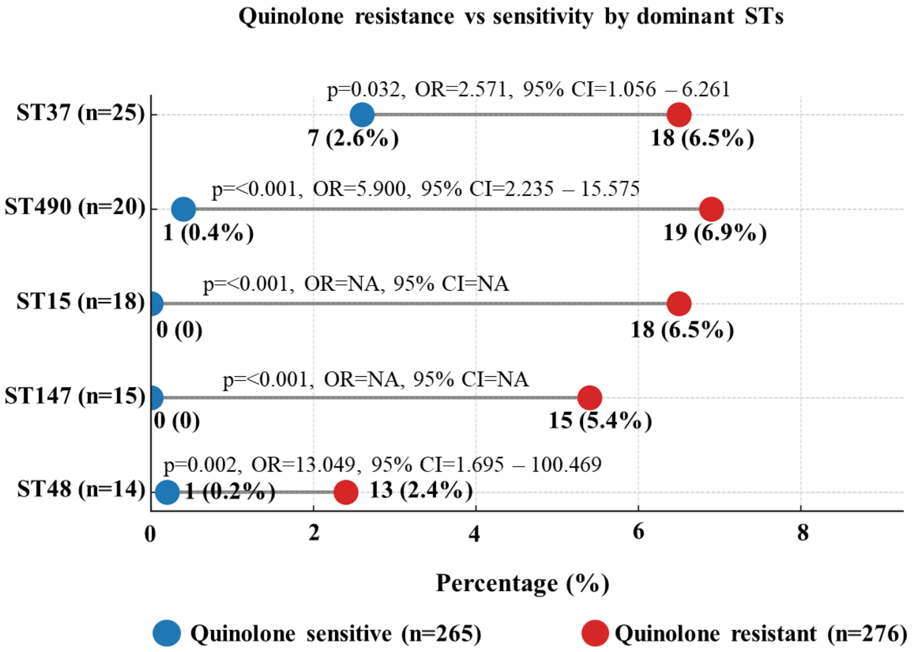


Figure 4.18 Association of quinolone resistance with the dominant STs. Values in parentheses indicate column percentage (percent positive within quinolone-sensitive and quinolone-resistant *K. pneumoniae*). STs, sequence types. Statistical significance was set at p<0.05, and only data that were statistically significant are presented in the figure.

In this study, core-genome SNP analysis was performed following recombination detection and removal using Gubbins. Across the phylogeny, a median of 1,113 single-nucleotide polymorphisms (SNPs) per branch was identified, of which a median of 188 SNPs occurred outside recombination regions and were retained in the clonal frame. The median clonal-frame length was approximately 4.78 Mb, consistent with the assembled genome sizes. Pairwise SNP distances ranged from single-digit values among closely related isolates to several thousand SNPs between unrelated lineages. Several clusters comprising isolates with different MLST profiles were identified that differed by ≤100 SNPs and contained at least five isolates in each cluster. The clusters identified below (**A** to **J**) consisted of isolates from diverse STs, sample sources, sampling periods, locations and differed by ≤100 SNPs: **A.** ST37 (n=10), ST1 (n=7), ST309 (n=1), ST656 (n=1), and ST1507 (n=1); **B.** ST15 (n=16), ST1844 (n=1), and ST7705 (n=1); **C.** ST490 (n=17); **D.** ST48 (n=13); **E.** ST1537 (n=4), ST200 (n=3), ST3049 (n=1), ST4423 (n=1), ST5048 (n=1), ST5069 (n=1), ST7835 (n=1), and ST7875 (n=1); **F.** ST485 (n=8), and ST4232 (n=1); **G.** ST273 (n=3), ST7749 (n=3), ST147 (n=1), and ST1922 (n=1); **H.** ST307 (n=8); **I.** ST11 (n=5), and ST340 (n=1); and **J.** ST16 (n=5) (Figure 4.19).

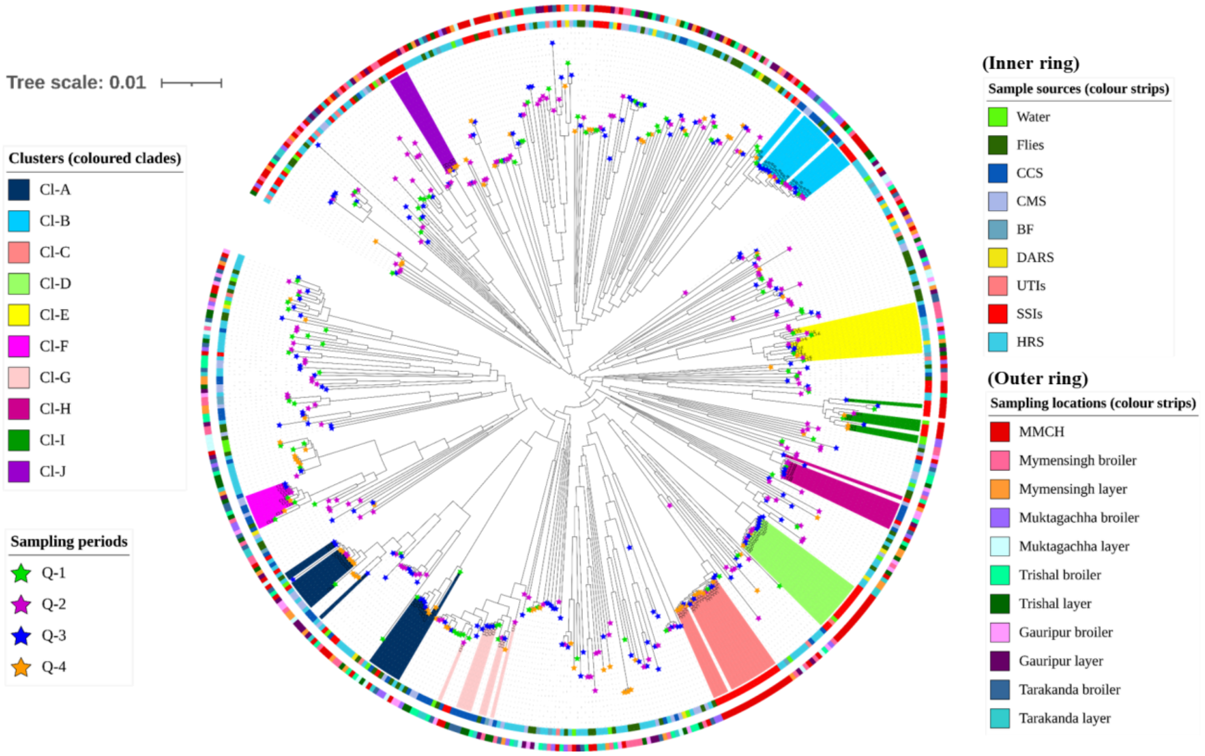


Figure 4.19 ML tree of *K. pneumoniae* clusters, displaying isolates with distinct MLST profiles that differed by no more than 100 SNPs. Isolates in the coloured clusters differ by ≤100 SNPs. The ML tree was constructed based on core-gene alignments (VeryFastTree v.4.0), visualised in iTOL (v.6). SNP calling was performed using Snippy (v.4.4.5), followed by recombination removal using Gubbins (v.2.3.4) and pairwise SNP calculation using pairsnp (v.0.0.7).

4.2.4 Investigating possible transmissibility in putative clonal outbreaks associated with MDR *K. pneumoniae* from SSIs

A combination of genomic approaches and epidemiological data was used to identify putative clonal outbreaks. Cut-offs of zero to 30 SNPs were set to screen for possible transmission among hospitalised patients (Stimson *et al*., 2019; Azarian & Black, 2024). Two putative clonal outbreak clusters belonging to ST490 and ST48 were identified (Figure 4.20). The ward of admission and overlapping of hospital stays of corresponding patients were evaluated to investigate clonal transmission. Genomic assessment revealed that the clusters of MDR *K. pneumoniae* ST490 and ST48 isolates recovered from the corresponding patients differed by ≤10 SNPS, suggesting probable transmissibility between patients and clonal outbreaks. Epidemiological data revealed that the patients had overlapping hospital stays in the surgery, orthopaedics, obstetrics and gynaecology wards of the Surgery Department at MMCH, indicating that the possible transmission was not confined to a single ward.

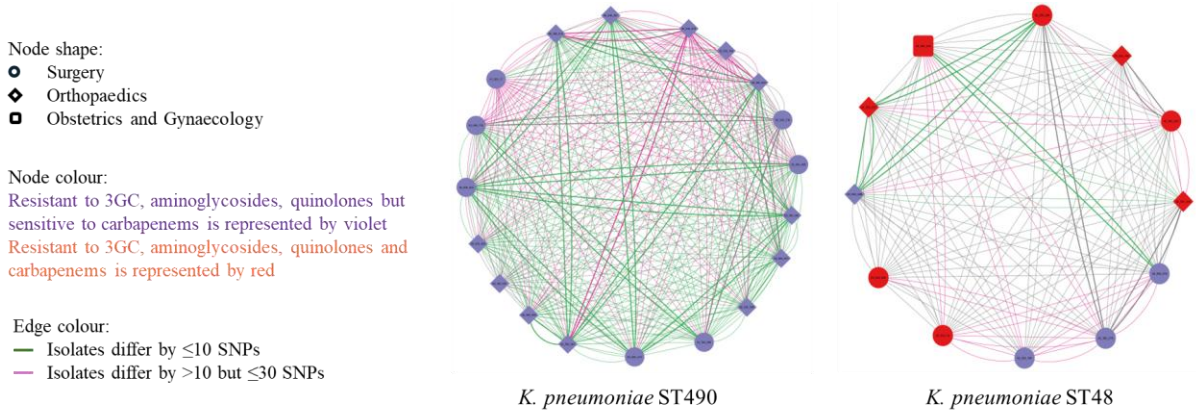


Figure 4.20 Network map showing the probable clonal outbreaks by MDR *K. pneumoniae* ST490 and ST48 at MMCH. The network map was constructed using Cytoscape software (v.3.10.1).

4.2.5 Plasmid diversity in *K. pneumoniae* in this study

To investigate the role of plasmids in *K. pneumoniae* in the dissemination of AMR across the human-animal-environment interface, all 541 isolates underwent short-read sequencing and plasmid profiling against the PlasmidFinder database using BLASTn, providing information on plasmid replicon types. The genomic characterisation of plasmids in this study was conducted through a hybrid assembly approach that integrated both short-read and long-read data. An explanation of the potential plasmid-mediated dissemination of AMR, based on the findings from this hybrid assembly, is presented in Chapter 7.

Out of 541 isolates, 485 showed the presence of at least one type of plasmid replicon. Plasmid replicon typing revealed a diverse array of plasmid replicon markers (n=50) among the isolates. IncFIB(K)Kpn3, Col440I, IncFII(pKP91), ColRNAI, IncR, IncFIA(HI1), Col440II, IncHI1B(pNDM–MAR), IncFIB(pKPHS1) and IncA/C2 were the frequently detected replicon types (with prevalence ≥5%) (Figure 4.21). To further investigate patterns of plasmid similarity among isolates, plasmid-based clustering was performed using data on plasmid replicon presence and absence. The 19 most frequently observed plasmid replicon types were included in the clustering analysis, whereas rare replicons occurring at very low frequencies were excluded to reduce noise. The number of plasmid replicons detected per isolate ranged from 0 to 8, with a median of 3 replicons per isolate (interquartile range: 2–5). Hierarchical clustering based on plasmid replicon presence and absence using Jaccard distance revealed multiple groups of isolates sharing similarity in plasmid profiles (Figure 4.22). While some clusters comprised isolates with relatively homogeneous plasmid content, other clusters exhibited marked heterogeneity. The frequently detected plasmid replicon types were distributed across different types of samples collected in this study, with a higher frequency in *K. pneumoniae* from SSIs and HRS (Table 4.12). IncA/C2 was found only in SSI (96.4%) and fly-derived (3.6%) isolates.

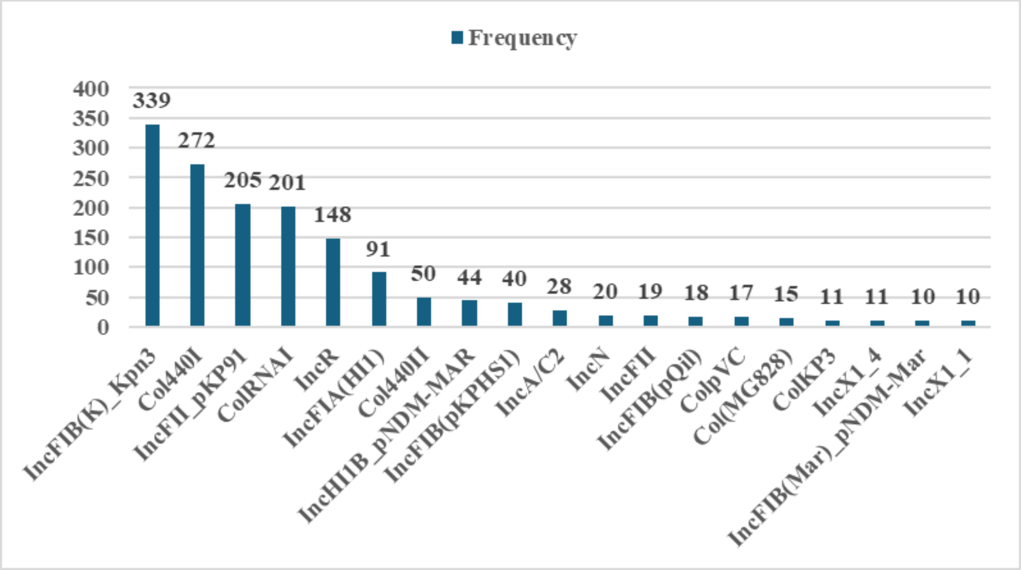


Figure 4.21 Frequency of *K. pneumoniae* plasmid replicon types detected among the isolates. The replicon markers shown are those with a frequency of at least 10, named according to the PlasmidFinder database.

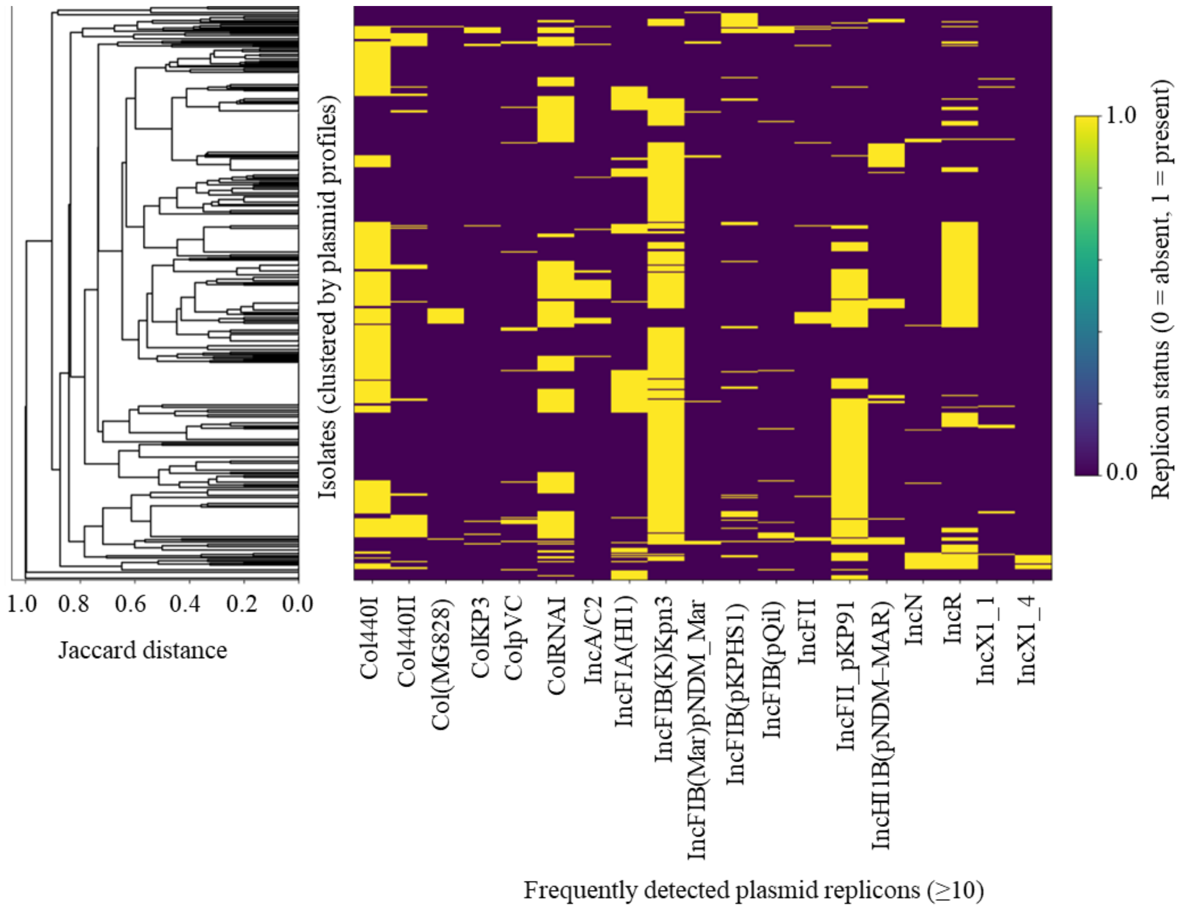


Figure 4.22 Plasmid-based clustering of *K. pneumoniae* isolates using plasmid replicon types. Hierarchical clustering dendrogram and heatmap illustrating similarity in plasmid content among *K. pneumoniae* isolates based on plasmid replicon presence and absence. Clustering was performed using Jaccard distance, which quantifies dissimilarity between binary plasmid replicon profiles, and average linkage. The dendrogram (left) depicts relationships between isolates, with branch length corresponding to the degree of dissimilarity in plasmid content. The heatmap (right) shows plasmid replicon profiles, where rows represent individual isolates ordered according to the dendrogram and columns represent detected plasmid replicon types. Colour intensity indicates plasmid replicon status (0 = absent, 1 = present). Isolates clustering at lower Jaccard distances share more similar plasmid replicon profiles, whereas those clustering at higher distances exhibit greater plasmid heterogeneity.

Table 4.12 Distribution of frequently detected *K. pneumoniae* plasmid replicons across various sources.

Attributes	**Sample sources, n (%)**								
Plasmid replicon type	Water	Flies	CCS	CMS	DARS	BF	UTIs	SSIs	HRS
IncFIB(K)Kpn3 (n=339)	16 (4.7)	53 (15.6)	32 (9.4)	28 (8.3)	7 (2.1)	6 (1.8)	12 (3.5)	62 (18.3)	123 (36.3)
Col440I (n=272)	11 (4.0)	39 (14.3)	34 (12.5)	20 (7.4)	7 (2.6)	4 (1.5)	5 (1.8)	70 (25.7)	82 (30.1)
IncFII_pKP91 (n=205)	8 (3.9)	30 (14.6)	29 (14.1)	20 (9.8)	3 (1.5)	3 (1.5)	8 (3.9)	60 (29.3)	44 (21.5)
ColRNAI (n=201)	11 (5.5)	14 (7.0)	20 (10.0)	23 (11.4)	3 (1.5)	3 (1.5)	6 (3.0)	58 (28.9)	63 (31.3)
IncR (n=148)	11 (7.4)	11 (7.4)	22 (14.9)	19 (12.8)	2 (1.4)	0 (0)	4 (2.7)	44 (29.7)	35 (23.6)
IncFIA(HI1) (n=91)	6 (6.6)	13 (14.3)	12 (13.2)	11 (12.1)	2 (2.2)	3 (3.3)	4 (4.4)	16 (17.6)	24 (26.4)
Col440II (n=50)	1 (2.0)	1 (2.0)	9 (18.0)	4 (8.0)	1 (2.0)	0 (0)	0 (0)	17 (34.0)	17 (34.0)
IncHI1B(pNDM–MAR) (n=44)	1 (2.3)	5 (11.4)	2 (4.5)	2 (4.5)	1 (2.3)	1 (2.3)	1 (2.3)	8 (18.2)	23 (52.3)
IncFIB(pKPHS1) (n=40)	5 (2.0)	6 (15.0)	5 (12.5)	3 (7.5)	0 (0)	1 (2.5)	0 (0)	12 (30.0)	11 (27.5)
IncA/C2 (n=28)	0 (0)	1 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	27 (96.4)	0 (0)

Values in parentheses indicate row percentage. CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs. The heatmap was generated based on the percentage values. The colour scale from white to red indicates the lowest to the highest resistance percentage (from left to right).

A comparative analysis was performed to investigate any link between plasmid replicon types and sample sources. Replicon types with a frequency of at least 10 were included for analysis. The presence of plasmid replicon types Col440I, Col440II, Col(MG828), ColKP3, ColRNAI, IncA/C2, IncFIB(pQil), IncFII, IncFII(pKP91), and IncR was significantly enriched among *K. pneumoniae* isolates recovered from SSIs ($p<0.05$) (Figure 4.23, a). IncFIB(K)Kpn3 and IncHI1B(pNDM–MAR) were significantly correlated to *K. pneumoniae* from HRS ($p<0.05$) (Figure 4.23, b). IncN and IncX1 were linked to CCS, CMS and water-derived isolates ($p<0.05$) (Figure 4.23, c, d and e).

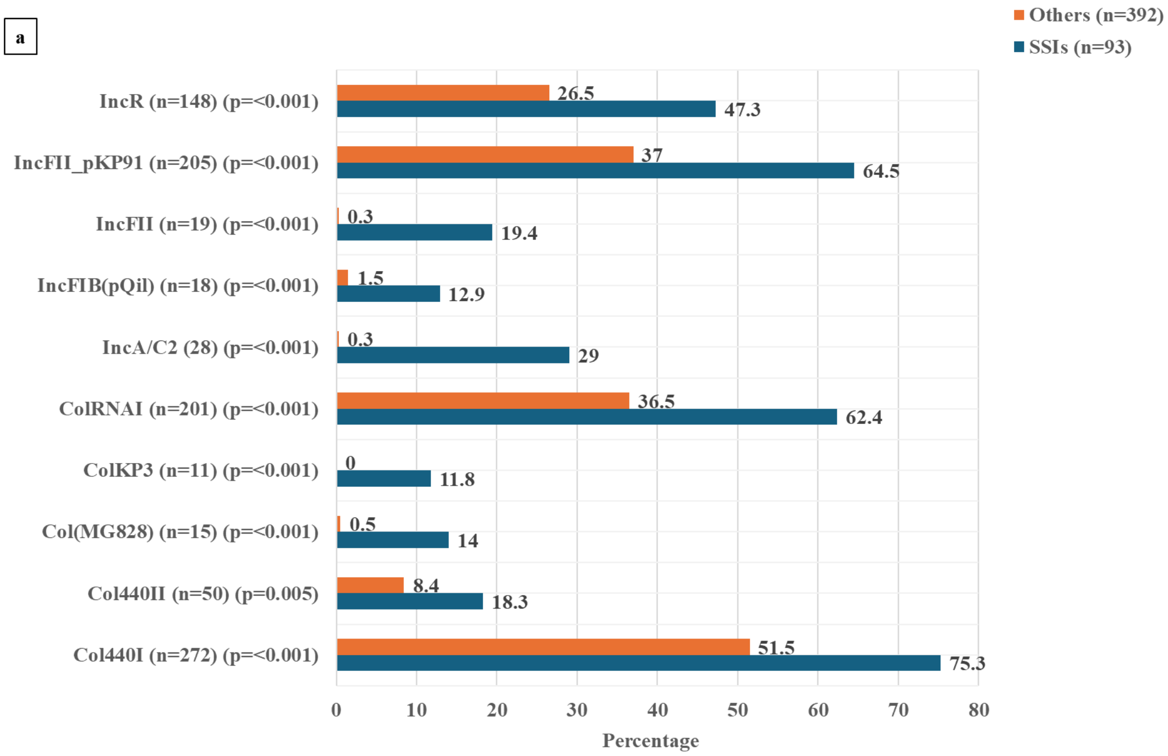


Figure 4.23 Association of *K. pneumoniae* plasmids replicons with various sample sources. **a.** plasmid replicons in SSIs and other samples, **b.** plasmid replicons in HRS and other samples, **c.** plasmid replicons in water and other samples, **d.** plasmid replicons in CCS and other samples, and **e.** plasmid replicons in CMS and other samples. SSIs, surgical site infections; HRS, human rectal swabs; CCS, chicken cloacal swabs; CMS, chicken meat swabs; 'Others' referring to remaining *K. pneumoniae* from all other sources. Statistical significance was set at $p<0.05$, and only data that were statistically significant are presented in the figure.

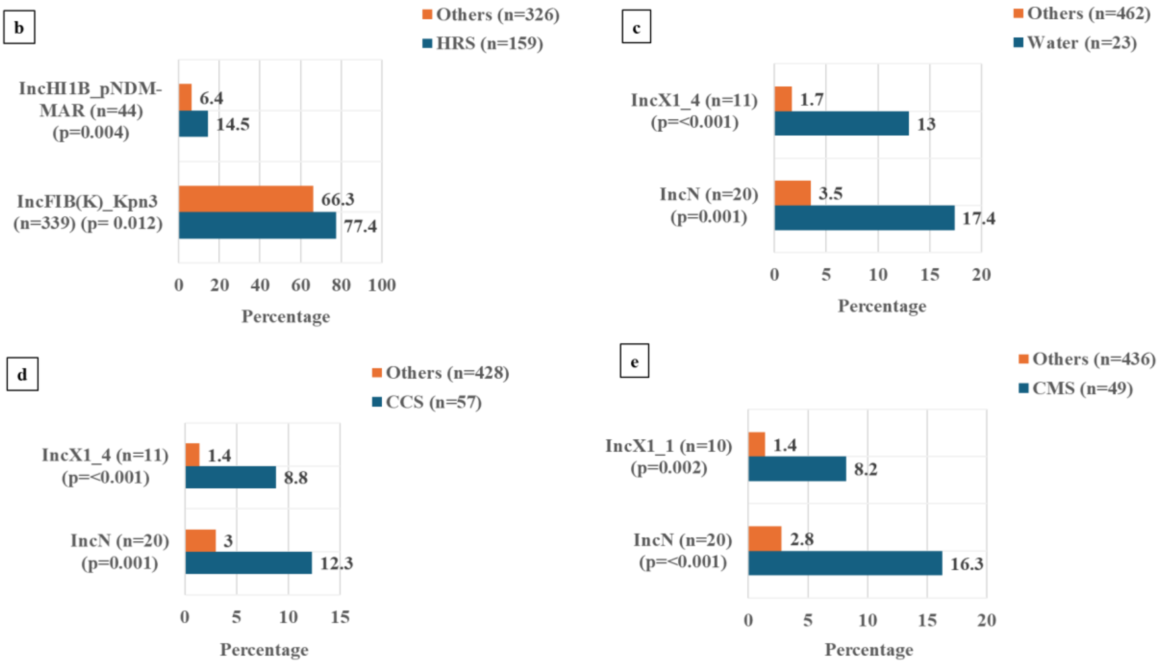


Figure 4.23 Association of *K. pneumoniae* plasmids replicons with various sample sources. **a.** plasmid replicons in SSIs and other samples, **b.** plasmid replicons in HRS and other samples, **c.** plasmid replicons in water and other samples, **d.** plasmid replicons in CCS and other samples, and **e.** plasmid replicons in CMS and other samples. SSIs, surgical site infections; HRS, human rectal swabs; CCS, chicken cloacal swabs; CMS, chicken meat swabs; ‘Others’ referring to remaining *K. pneumoniae* from all other sources. Statistical significance was set at $p<0.05$, and only data that were statistically significant are presented in the figure.

Across the isolates, plasmid–borne resistance genes (*bla*$_{\text{CTX–M–15}}$, *bla*$_{\text{NDM–5}}$, *aac(6')–Ib*, *aph(6)–Id*, *qnrS1*, *qnrB1*) found to be significantly associated with 3GC, carbapenem, aminoglycosides and quinolone resistance in this study, were investigated for any association with specific replicon types. Col440I, Col440II, Col(MG828), ColKP3, ColRNAI, IncA/C2, IncFIB(pQil), IncFII, IncFII(pKP91), IncR replicon markers were found to be significantly related to ESBL gene *bla*$_{\text{CTX–M–15}}$ and carbapenem-resistant gene *bla*$_{\text{NDM–5}}$ ($p<0.05$) (Table 4.13 and 4.14). These plasmids were also associated with *K. pneumoniae* derived from SSIs (Figure 4.22, a).

Table 4.13 Comparative distribution of plasmid replicon types among *bla*$_{\text{CTX–M–15}}$ positive and *bla*$_{\text{CTX–M–15}}$ negative *K. pneumoniae* isolates.

Plasmid replicon	***bla*$_{\text{CTX–M–15}}$ positive (n=127)**	***bla*$_{\text{CTX–M–15}}$ negative (n=358)**	***p*–value**
Col440I (n=272)	89 (70.1%)	183 (51.1%)	<0.001
Col440II (n=50)	20 (15.7%)	30 (8.4%)	0.019
Col(MG828) (n=15)	14 (11.0%)	1 (0.3%)	<0.001
ColKP3 (n=11)	10 (7.9%)	1 (0.3%)	<0.001
ColRNAI (n=201)	76 (59.8%)	125 (34.9%)	<0.001
IncA/C2 (n=28)	26 (20.5%)	2 (0.6%)	<0.001
IncFIB(pQil) (n=18)	13 (10.2%)	5 (1.4%)	<0.001
IncFII (n=19)	18 (14.2)	1 (0.3%)	<0.001
IncFII(pKP91) (n=205)	83 (65.4%)	122 (34.1%)	<0.001
IncR (n=148)	58 (45.7%)	90 (25.1%)	<0.001

Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$.

Table 4.14 Comparative distribution of plasmid replicon types among *bla*$_{\text{NDM–5}}$ positive and *bla*$_{\text{NDM–5}}$ negative *K. pneumoniae* isolates.

Plasmid replicon	***bla*$_{\text{NDM–5}}$ positive (n=14)**	***bla*$_{\text{NDM–5}}$ negative (n=471)**	***p*–value**
Col440I (n=272)	14 (100%)	258 (54.8%)	0.001
Col440II (n=50)	4 (28.6%)	46 (9.8%)	0.023
Col(MG828) (n=15)	9 (64.3%)	6 (1.3%)	<0.001
ColKP3 (n=11)	4 (28.6%)	7 (1.5%)	<0.001
ColRNAI (n=201)	11 (78.6%)	190 (40.3%)	0.004
IncA/C2 (n=28)	5 (35.7%)	23 (4.9%)	<0.001
IncFIB(pQil) (n=18)	2 (14.3%)	16 (3.4%)	0.034
IncFII (n=19)	13 (92.9%)	6 (1.3%)	<0.001
IncFII(pKP91) (n=205)	11 (78.6%)	194 (41.2%)	0.005
IncR (n=148)	11 (78.6%)	137 (29.1%)	<0.001

NA, not applicable. Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$.

Plasmid-borne aminoglycoside resistant genes *aac(6')–Ib* and *aph(6)–Id* were linked to Col440I, ColRNAI, IncA/C2, IncFII_pKP91, IncR, IncX1_1 (p<0.05) (Table 4.15 and 4.16). Quinolone-resistant gene *qnrB1* was significantly associated with Col440I, Col(MG828), ColRNAI, IncFII, IncFII_pKP91, IncR whereas *qnrS1* was associated with Col440II, IncFIA(HI1), IncFII(pKP91), IncR, IncN, IncX1_1, IncX1_4 (p<0.05) (Table 4.17 and 4.18). IncFII(pKP91)and IncR replicons were commonly associated with all the resistant genes (*bla*$_{\text{CTX–M–15}}$, *bla*$_{\text{NDM–5}}$, *aac(6')–Ib* and *aph(6)–Id*, *qnrB1* and *qnrS1*) that were analysed.

Table 4.15 Comparative distribution of plasmid replicon types among *aac(6')–Ib* positive and *aac(6')–Ib* negative *K. pneumoniae* isolates.

Plasmid replicon	***aac(6')–Ib* positive (n=86)**	***aac(6')–Ib* negative (n=399)**	***p*–value**
Col440I (n=272)	59 (68.6%)	213 (53.4%)	0.010
Col(MG828) (n=15)	13 (15.1%)	2 (0.5%)	<0.001
ColRNAI (n=201)	55 (64.0%)	146 (36.6%)	<0.001
IncA/C2 (n=28)	24 (27.9%)	4 (1.0%)	<0.001
IncFII (n=19)	12 (14.0%)	7 (1.8%)	<0.001
IncFII(pKP91) (n=205)	70 (81.4%)	135 (33.8%)	<0.001
IncR (n=148)	55 (64.0%)	93 (23.3%)	<0.001
IncX1_1 (n=10)	6 (7.0%)	4 (1.0%)	<0.001

Values in parentheses indicate column percentage. Statistical significance was set at p<0.05.

Table 4.16 Comparative distribution of plasmid replicon types *aph(6)–Id* positive and *aph(6)–Id* negative *K. pneumoniae* isolates.

Plasmid replicon	***aph(6)–Id* positive (n=97)**	***aph(6)–Id* negative (n=388)**	***p*–value**
Col440I (n=272)	65 (67.0%)	207 (53.4%)	0.015
ColRNAI (n=201)	58 (59.8%)	143 (36.9%)	<0.001
IncA/C2 (n=28)	25 (25.8%)	3 (0.8%)	<0.001
IncFII(pKP91) (n=205)	59 (60.8%)	146 (37.6%)	<0.001
IncR (n=148)	49 (50.5%)	99 (25.5%)	<0.001
IncX1_1 (n=10)	6 (6.2%)	4 (1.0%)	0.001

Values in parentheses indicate column percentage. Statistical significance was set at p<0.05.

Table 4.17 Comparative distribution of plasmid replicon types among *qnrB1* positive and *qnrB1* negative *K. pneumoniae* isolates.

Plasmid replicon	***qnrB1* positive (n=67)**	***qnrB1* negative (n=418)**	***p*–value**
Col440I (n=272)	54 (80.6%)	218 (52.2%)	<0.001
Col(MG828) (n=15)	12 (17.9%)	3 (0.7%)	<0.001
ColRNAI (n=201)	51 (76.1%)	150 (35.9%)	<0.001
IncFII (n=19)	11 (16.4%)	8 (1.9%)	<0.001
IncFII(pKP91) (n=205)	51 (76.1%)	154 (36.8%)	<0.001
IncR (n=148)	34 (50.7%)	114 (27.3%)	<0.001

Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$.

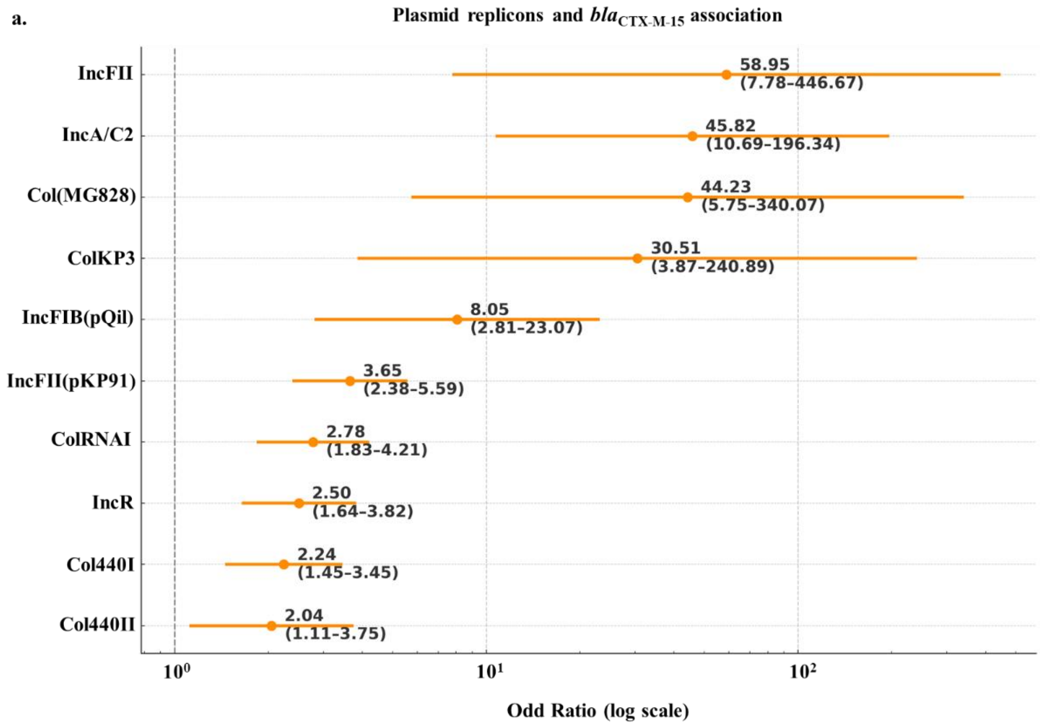
Table 4.18 Comparative distribution of plasmid replicon types among *qnrS1* positive and *qnrS1* negative *K. pneumoniae* isolates.

Plasmid replicon	***qnrS1* positive (n=157)**	***qnrS1* negative (n=328)**	***p*–value**
Col440II (n=50)	23 (14.6%)	27 (8.2%)	0.030
IncFIA(HI1) (n=91)	46 (29.3%)	45 (13.7%)	<0.001
IncFII(pKP91) (n=205)	87 (55.4%)	118 (36.0%)	<0.001
IncR (n=148)	64 (40.8%)	84 (25.6%)	0.001
IncN (n=20)	17 (10.8%)	3 (0.9%)	<0.001
IncX1_1 (n=10)	8 (5.1%)	2 (0.6%)	0.001
IncX1_4 (n=11)	10 (6.4%)	1 (0.3%)	<0.001

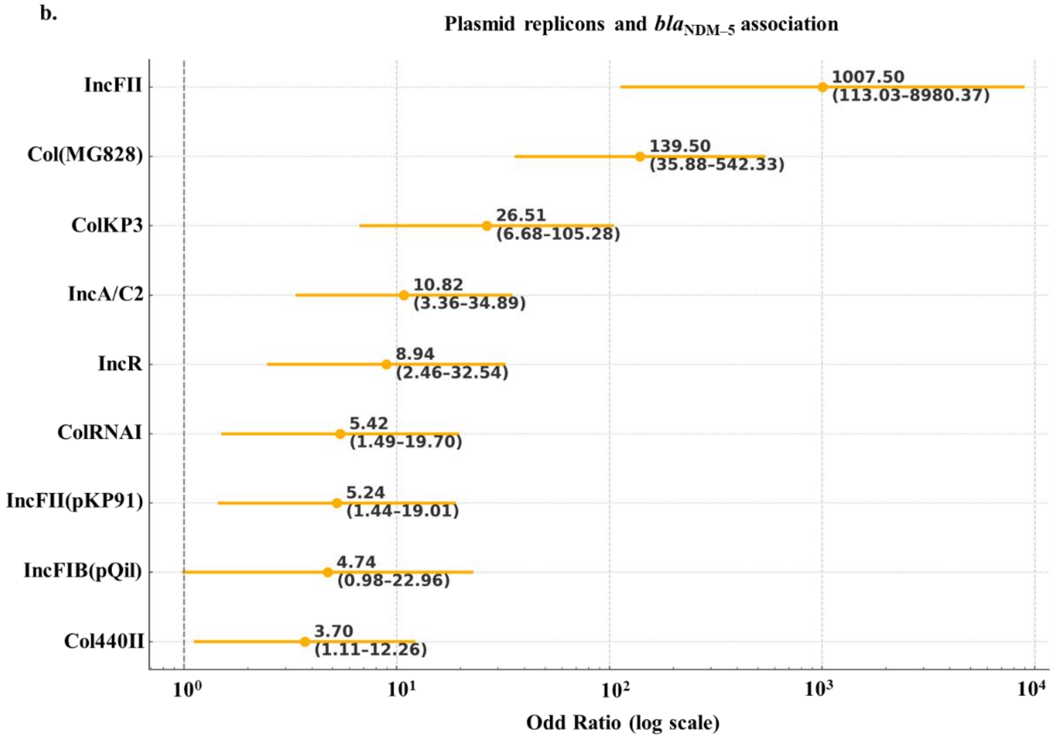
Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$.

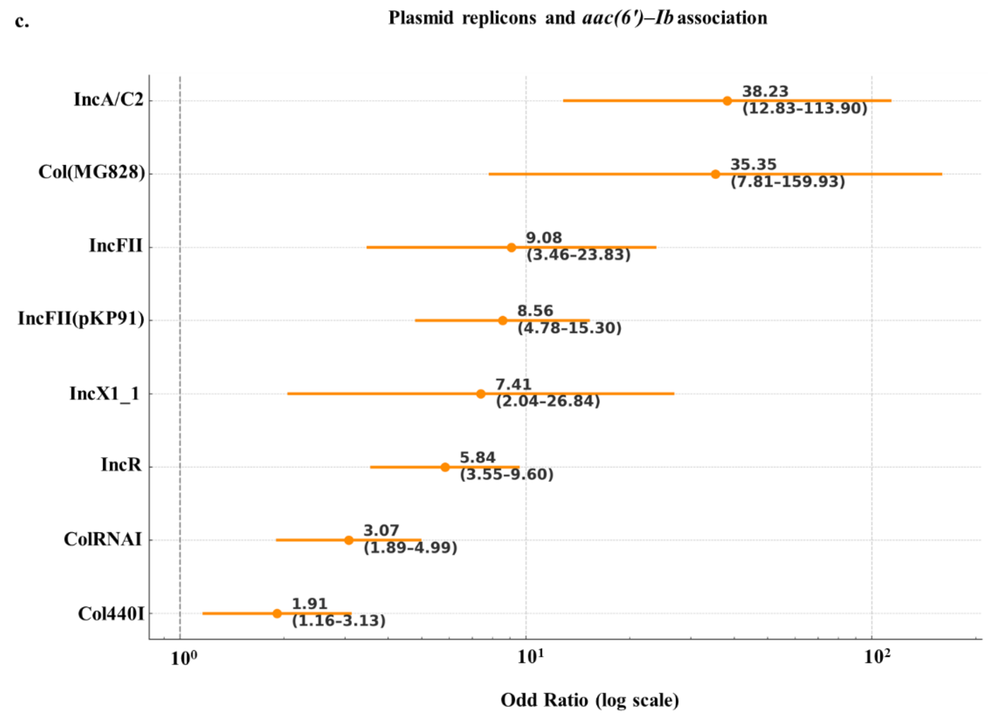
To assess the strength of association, this study reported for each plasmid replicon, not only the number and percentage of carriers in both positive and negative cohorts of the respective ARGs, along with the *p*-value, but also the odds ratio (OR) with its 95% confidence interval (CI). Replicons with ORs significantly greater than 1 indicate a positive association between the respective ARGs and their plasmid backbones in *K. pneumoniae*, thereby highlighting the primary plasmid vehicles most likely driving dissemination of these resistance determinants (Figure 4.24).

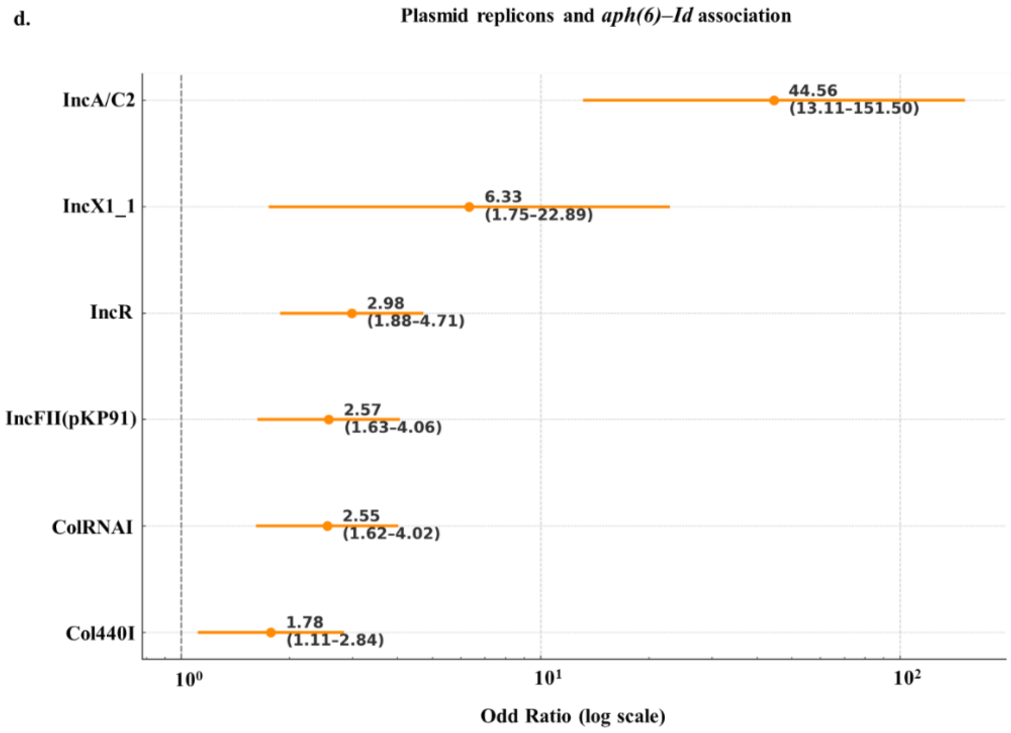
a.


Plasmid replicons and *bla*$_{\text{CTX-M-15}}$ association

b.


Plasmid replicons and *bla*$_{\text{NDM-5}}$ association


c.
Plasmid replicons and aac(6')–Ib association
IncA/C2
Col(MG828)
IncFII
IncFII(pKP91)
IncX1_1
IncR
ColRNAI
Col440I
38.23
(12.83–113.90)
35.35
(7.81–159.93)
9.08
(3.46–23.83)
8.56
(4.78–15.30)
7.41
(2.04–26.84)
5.84
(3.55–9.60)
3.07
(1.89–4.99)
1.91
(1.16–3.13)
10^0
10^1
10^2
Odd Ratio (log scale)


d.
Plasmid replicons and aph(6)–Id association
IncA/C2
IncX1_1
IncR
IncFII(pKP91)
ColRNAI
Col440I
44.56
(13.11–151.50)
6.33
(1.75–22.89)
2.98
(1.88–4.71)
2.57
(1.63–4.06)
2.55
(1.62–4.02)
1.78
(1.11–2.84)
10^0
10^1
10^2
Odd Ratio (log scale)

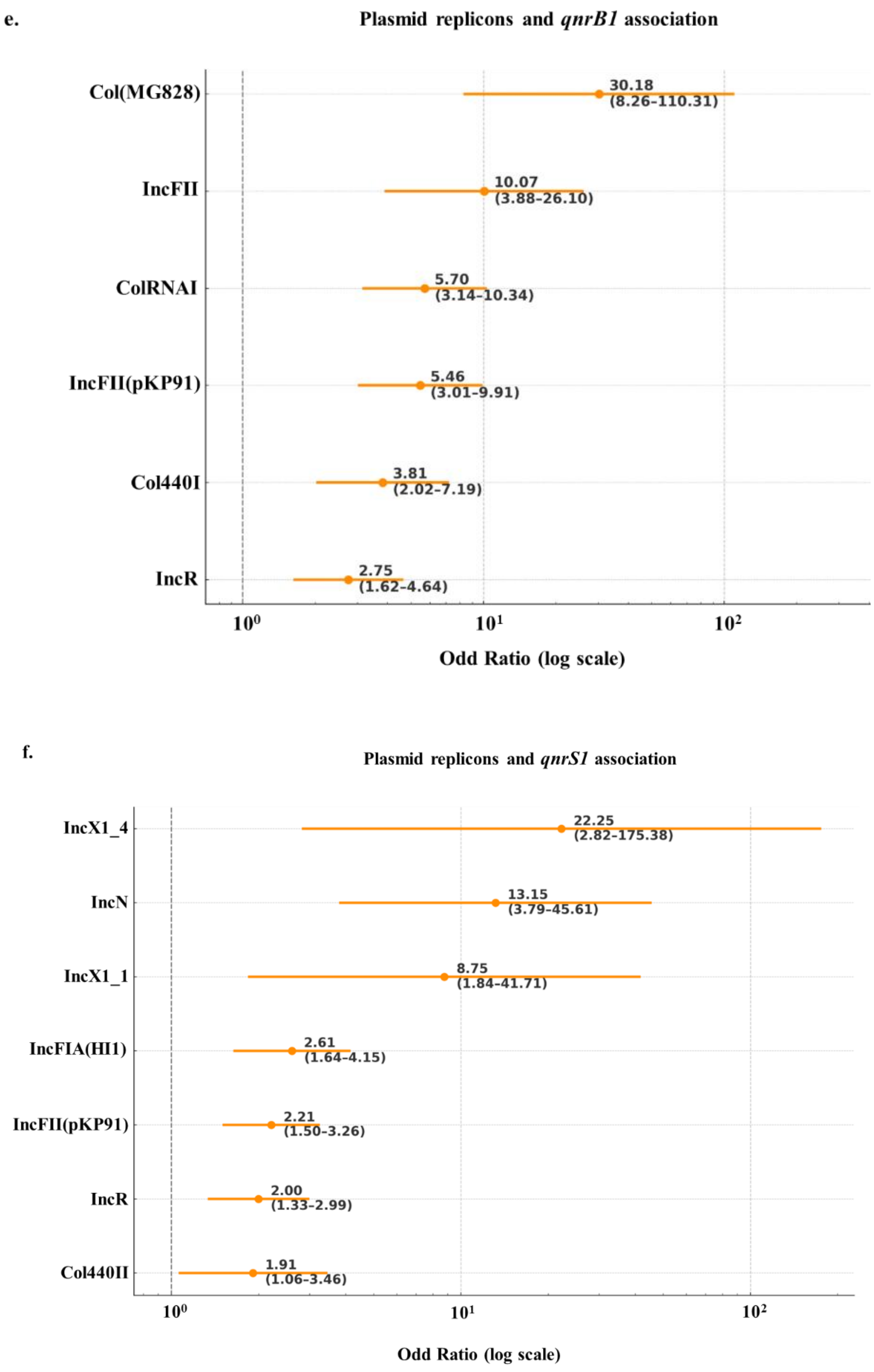


Figure 4.24 Forest plots showing the estimated ORs and 95% CIs for the association between plasmid replicon types and carriage of the ARGs among *K. pneumoniae* isolates. **a.** association between plasmid

replicons and *bla*$_{\text{CTX-M-15}}$ carriage, **b.** association between plasmid replicons and *bla*$_{\text{NDM–5}}$ carriage, **c.** association between plasmid replicons and *aac(6')–Ib* carriage, **d.** association between plasmid replicons and *aph(6)–Id* carriage, **e.** association between plasmid replicons and *qnrB1* carriage, and **f.** association between plasmid replicons and *qnrS1* carriage. Replicons are listed on the y-axis and ordered by increasing ORs, and points represent the OR estimates on a logarithmic x-axis, with horizontal bars denoting their 95% CIs. The dashed vertical line marks an OR of 1 (no association).

4.3 Discussion

The increasing level of AMR observed in *K. pneumoniae*, particularly in clinical settings, poses a serious threat to public health (Calland *et al.,* 2023). As *K. pneumoniae* are ecological generalists and capable of thriving in various environments, they have the potential to spread AMR genes across different settings (Thorpe *et al.,* 2022). Recent One Health studies from different countries have characterised *Klebsiella* from various settings, revealing that MDR *K. pneumoniae* is predominantly prevalent within hospital environments (Ludden *et al.,* 2020; Dereeper *et al.,* 2022; Thorpe *et al.,* 2022; Calland *et al.,* 2023; Yasmeen *et al.,* 2023; Jacob *et al.,* 2024; Hinthong *et al*., 2025; Mondol *et al*., 2025). My study, conducted in an LMIC setting, contributes to the existing evidence by providing a substantial sample of contemporaneous *K. pneumoniae* isolates from Mymensingh, Bangladesh. This study aimed to investigate the distribution and overlap of AMR among *K. pneumoniae* isolates across human, animal, and environmental reservoirs within the designated study area in Bangladesh.

The results of AST revealed that the overall susceptibility of *K. pneumoniae* from various sources showed high resistance to fosfomycin (62.8%), ciprofloxacin (47.5%), levofloxacin (46%), amoxicillin–clavulanic acid (36.2%), and high sensitivity to colistin (3.1%), ceftazidime–avibactam (3.5%), meropenem (4.6%) and imipenem (5.0%) (Table 4.1). Higher resistance to ciprofloxacin, levofloxacin, and amoxicillin–clavulanic acid may be attributed to the extensive use of these antibiotics in clinical and veterinary practices in Bangladesh (Chouduri *et al.,* 2018; Islam *et al.,* 2021; Mahmud *et al.,* 2022, Chowdhury *et al*., 2025). In contrast, the resistance to fosfomycin in Bangladesh, despite its rare application, may stem from the horizontal transfer of resistance genes, which can occur even without direct selective pressure from its use (Munny *et al.,* 2023). Higher sensitivity to colistin, ceftazidime–avibactam, meropenem, and imipenem, as supported by previous findings in Bangladesh, may be attributed to their limited availability and usage in Bangladesh (Ahmed *et al.,* 2019; Aminul *et al.,* 2021; Hussain *et al.,* 2023).

Beyond individual resistance frequencies, isolate-level co-resistance analysis in this study demonstrated clear clustering of resistance among commonly used antibiotic classes. The analysis revealed strong co-resistance between ciprofloxacin and levofloxacin and their co-occurrence with fosfomycin resistance (Figure 4.1). Similar fluoroquinolone-linked co-resistance profiles have been documented in *K. pneumoniae* isolates originating from diverse One Health reservoirs, including clinical and environmental sources (Ludden *et al*., 2020; Thorpe *et al*., 2022; Calland *et al*., 2023). Co-resistance among ESBLs, including third- and fourth-generation cephalosporins and aztreonam, is consistent with the widespread dissemination of ESBL-producing *K. pneumoniae*, in which β-lactamase genes are often co-located with resistance determinants for other antibiotic classes on conjugative plasmids (Rozwandowicz *et al*., 2018; Wyres & Holt, 2018). In contrast, the consistently low co-resistance involving carbapenems, colistin, and ceftazidime–avibactam in the dataset indicates that resistance to last-line agents has not yet become strongly embedded within broader multidrug resistance profiles.

Sample-wise susceptibility showed that *K. pneumoniae* from DARS, BF, and HRS exhibited high susceptibility to all classes of antibiotics except fosfomycin. *K. pneumoniae* from SSIs exhibited significantly higher resistance to ciprofloxacin, levofloxacin, fosfomycin, cefotaxime, ceftazidime, aztreonam and cefepime (Table 4.2). Although carbapenem resistance was higher only in SSIs, all of the clinical isolates (SSIs and UTIs) were sensitive to colistin. Previous studies reported similar antibiotic resistance patterns in various clinical isolates of *K. pneumoniae* in Bangladesh (Aminul *et al.,* 2021; Tanni *et al.,* 2021). Resistance to colistin remains low, mainly because it is infrequently prescribed in hospitals in Bangladesh, typically reserved for ICU patients. Unlike previous findings, I found no carbapenem resistance in UTIs, which may be due to the sampling criteria used in this study (only outdoor patients from the community were included, and children, pregnant women, and patients with co-morbidities were excluded). A recent study also reported higher resistance in *K. pneumoniae* for HAI than CAI in Bangladesh, which is consistent with the findings of the present study (Kawser *et al.,* 2025). Particularly alarming was the widespread resistance to 3GC, aminoglycosides, and fluoroquinolones found in water, flies, CCS, UTIs, and SSIs. These patterns mirror previous studies from Bangladesh that have documented resistance levels in *K. pneumoniae* in clinical, veterinary and environmental settings (Aminul *et al.,* 2021; Tanni *et al.,* 2021; Saha *et al.,* 2023; Kar *et al.,* 2024; Kawser *et al.,* 2025; Tanni *et al.,* 2025). The widespread use of 3GC, aminoglycosides, and fluoroquinolones for clinical management in hospitals, as well as in veterinary practices in Bangladesh, has led to resistance against these antibiotics (Hosain *et al.,* 2022; Rashid *et al.,* 2022). Across all samples, 22.6% (122/541) of *K. pneumoniae* in this study were MDR, and 3% (16/541) were XDR (Figure 4.2). MDR and XDR *K. pneumoniae* were most common in SSIs from the hospital, whereas no MDR or XDR *K. pneumoniae* were detected in DARS and UTIs in this study. None of the *K. pneumoniae* was pan–drug resistant (resistant to all classes of antibiotics). Data related to the frequency of MDR and XDR *K. pneumoniae* with respect to One Health sampling are very limited; however, a high incidence of MDR and XDR *K. pneumoniae* has been previously observed in clinical settings in Bangladesh (Kawser & Shamsuzzaman, 2022; Mahmud *et al.,* 2022; Kawser *et al.,* 2025).

Phenotypic resistance grouping (five groups based on susceptibility to major antibiotic classes) of *K. pneumoniae* was applied in this study to summarise antimicrobial susceptibility data into clinically and epidemiologically meaningful categories. Group A represents isolates susceptible to both 3GCs and carbapenems, while Group B comprises 3GC-resistant but carbapenem-susceptible isolates, a phenotype commonly used as a proxy for ESBL production (Wyres and Holt, 2018). Groups C–E capture increasing severity of carbapenem-associated resistance, with Group C representing intermediate carbapenem-resistant phenotypes that retain susceptibility to at least one major non-β-lactam class, and Groups D and E corresponding to multidrug-resistant and near-pan-resistant phenotypes, respectively. This class-based stratification reflects progressive loss of therapeutic options and is consistent with established antimicrobial resistance surveillance frameworks and MDR/XDR definitions proposed by Magiorakos *et al*. (2012) and adopted in global AMR surveillance initiatives, including WHO GLASS. Although these categories were defined phenotypically, they conceptually aligned with widely used genotypic classification approaches, such as Kleborate, which stratify *K. pneumoniae* according to ESBL production, carbapenemase presence, and resistance to last-line agents (Lam *et al*., 2021). Within the study area, 25.1% (136/541) of *K. pneumoniae* isolates across various sources were 3GC resistant but sensitive to carbapenems, and these isolates were recovered from different subdistricts. 3GC-resistant *K. pneumoniae* were more prevalent (36.9%) in Mymensingh Sadar than in other subdistricts. Only 4.8% (26/541) of the isolates were resistant to carbapenems, quinolones and aminoglycosides (Group D), and all of them were from Mymensingh Sadar (Figure 4.3 and Table 4.3). Location-wise multivariate logistic regression analyses also demonstrated that *K. pneumoniae* from MMCH in Mymensingh Sadar were significantly associated with resistance to multiple antibiotics ($p<0.05$) (Table 4.4). This finding indicates that the increased resistance in Mymensingh Sadar is attributed to the clinical isolates (SSIs) obtained from MMCH. Previous studies also reported high resistance in *K. pneumoniae* from tertiary hospitals (Tanni *et al.,* 2021).

This study also investigated the MIC values of different antibiotics for *K. pneumoniae* isolated from various samples. Values were interpreted using EUCAST (v.12.0) breakpoints for *K. pneumoniae*, with similar cut-offs applied across all isolates irrespective of sample source (EUCAST, 2022), enabling direct comparison across human, animal, and environmental compartments. Using these fixed cut-offs, *K. pneumoniae* isolates from SSIs demonstrated the highest reduced susceptibility, with MIC50 values exceeding EUCAST resistance thresholds for amoxicillin–clavulanic acid, piperacillin–tazobactam, cefotaxime, ceftazidime, cefepime, aztreonam, amikacin and gentamicin (Table 4.6). Similarly, CCS isolates showed elevated MIC50 values above resistance cut-offs for ciprofloxacin, levofloxacin, gentamicin and fosfomycin (Table 4.6). In the case of MIC90, SSI isolates exceeded EUCAST resistance breakpoints for almost all tested antibiotics, with the exception of tigecycline (2 mg/L) and colistin (0.5 mg/L) (Table 4.7). Conversely, the MIC90 of tigecycline (4 mg/L) and colistin (4 mg/L) was found to be higher in CCS, exceeding EUCAST epidemiological cut-off values (Table 4.7). The reduced MIC values of tigecycline and colistin may be due to their limited use in clinical practice (Aminul *et al.,* 2021), while the higher values in CCS may result from the widespread use of tetracycline and colistin in poultry farms (Hosain *et al.,* 2022; Islam *et al.,* 2020). Although MIC90 values for isolates from DARS, BF, and HRS largely remained below the breakpoint, fosfomycin MIC90 values frequently exceeded the cut-off across multiple non-clinical sources. Collectively, a high proportion of *K. pneumoniae* isolates with MIC50 and MIC90 values for different antibiotics above fixed EUCAST cut-offs in CCS, CMS, water, flies, SSIs and UTIs indicate that the bacterial population requires higher concentrations of antibiotics to inhibit growth, which is a strong signal of reduced susceptibility or emerging resistance to those antibiotics (Wang *et al.,* 2022).

The evading mechanisms of *K. pneumoniae* against 3GC, carbapenems, aminoglycosides and quinolones are crucial to investigate. In the present study, among the 131 distinct ARGs identified, $bla_{\text{TEM–1B}}$, $bla_{\text{CTX–M–15}}$, *aac(6')–Ib*, *aph(6)–Id*, *oqxA, oqxB, qnrS, qnrB, fosA, sul1, sul2 and tetA* showed higher prevalence in both clinical and non–clinical isolates, highlighting the pervasive distribution of ARGs in *K. pneumoniae* at human-animal-environment interfaces (Figure 4.6). Among ESBL genes, $bla_{\text{CTX–M–15}}$ was the most prevalent, comprising 23.5% (127/541) and detected in all sample types except DARS (Figure 4.7), which is consistent with a previous report from MMCH, Mymensingh, Bangladesh (Khan *et al.,* 2018). Khan *et al.* (2018) reported that 51.4% of *K. pneumoniae* clinical isolates carried the $bla_{\text{CTX–M–1}}$ group genes, with $bla_{\text{CTX–M–15}}$ being the dominant variant. Previous research has shown that the $bla_{\text{CTX–M–15}}$ gene is a highly prevalent ESBL gene, especially in South Asia, including Bangladesh, and is frequently identified in *E. coli* and *K. pneumoniae* isolates from both clinical and community settings (Sahoo *et al.,* 2022; Gondal *et al.,* 2023; Kawsar *et al.,* 2025). Safain *et al.* (2021) reported that $bla_{\text{CTX–M–1}}$ was the most common (63%) ESBL–resistant gene in Enterobacteriaceae from hospitalised patients in Bangladesh, where they found 28% in *K. pneumoniae*. Another study conducted in Dhaka found that 50% of *K. pneumoniae* harboured the $bla_{\text{CTX–M–15}}$ gene (Hussain *et al.,* 2023). The overall prevalence differences could be attributed to the sample types; previous reports were linked to clinical isolates, whereas my study included samples from diverse sources.

Among the carbapenem resistance alleles, $bla_{\text{NDM–5}}$ was the most commonly found in this study, followed by $bla_{\text{OXA–232}}$, $bla_{\text{OXA–181}}$, $bla_{\text{NDM–1}}$ and $bla_{\text{NDM–4}}$ (Figure 4.6). All identified carbapenem–resistant genes in *K. pneumoniae* originated from MMCH, with a single $bla_{\text{NDM–5}}$ positive *K. pneumoniae* found in hospital wastewater. This suggests potential transmission to the hospital environment. I also found that *K. pneumoniae* from SSIs co-harboured NDM–4/5 and OXA–181/232 carbapenemase. A previous study conducted in MMCH also reported $bla_{\text{NDM–1}}$, $bla_{\text{NDM–5}}$, $bla_{\text{NDM–7}}$ in *E. coli* and $bla_{\text{NDM–1}}$ in *K. pneumoniae* from clinical isolates (Khan *et al.,* 2018). A study from the largest tertiary care hospital in Bangladesh reported that $bla_{\text{NDM–1}}$ was the most prevalent among the CR-KP isolates, followed by $bla_{\text{NDM–5}}$, $bla_{\text{OXA–181}}$, and $bla_{\text{OXA–232}}$ (Okanda *et al.,* 2020). Another recent study from Dhaka Medical College Hospital, Bangladesh found $bla_{\text{NDM–5}}$ and $bla_{\text{OXA–181}}$ were the most frequently found carbapenemase genes from clinical *K. pneumoniae* (Kawasr *et al.,* 2025). These findings indicate changes in resistance patterns that may be due to the spread of carbapenemase among Enterobacterales (Alvarez *et al.,* 2024). Unlike previous reports from Bangladesh, India and Pakistan, all *K. pneumoniae* isolates from this study were negative for *bla*$_{\text{VIM}}$, *bla*$_{\text{KPC}}$, and *bla*$_{\text{IMP}}$ (Sahoo *et al.,* 2023; Farzanan *et al.,* 2023; Gondal *et al.,* 2023). For colistin resistance, *mcr-8.1* was the most frequently found *mcr* gene in *K. pneumoniae* isolates. I found both *mcr-1.1* and *mcr-8.1* circulating in the environment, on farms and among healthy humans living near the farms where *mcr-8.1* was the dominant mechanism. Although no *mcr* genes were found in clinical isolates in this study, Farzana *et al.* reported *mcr-8.1* in *K. pneumoniae* from clinical infections in a tertiary care hospital in Bangladesh (Farzana *et al.,* 2020). These findings, along with the previous reports, highlight the emergence of colistin resistance in *K. pneumoniae* in Bangladesh (Farzana *et al.,* 2020; Mondol *et al.,* 2025). For aminoglycoside resistance, the most prevalent resistant genes were *aph(6)–Id* (18.1%), *aph(3")–Ib* (17.2%), *aac(6')–Ib* (15.9%), *aadA2* (15.5%) and *aac(3)–IIa* (10.2%). The *oqxA* (97.6%) and *oqxB* 97.1%) were the most frequently identified quinolone–resistant genes, followed by *qnrS1*(29.0%) and *qnrB1* (12.4%), contributing to plasmid–mediated quinolone resistance. These findings align with the previous report from Dhaka, Bangladesh where they identified *aph(3")–Ib* (37.5%)*, aph(6)–Id* (35%)*, aac(6')–Ib* (31.3%)*, aadA2* (18.8%)*, aac(3)–IIa* (15.6%) are the frequent aminoglycoside-resistant genes along with AMR genes encoding for fluoroquinolone resistance such as *oqxA/oqxB* (100%), *qnrB1* (3.5%) and *qnrS* (3.5%) in *K. pneumoniae* isolates (Hussain *et al.,* 2023). While investigating the genotypic resistance in *K. pneumoniae*, I found that AMR-associated genes were distributed unevenly across different sources, where ESBL, carbapenemase, aminoglycoside and quinolone-resistant genes were predominantly found in clinical isolates, particularly in SSIs (Figure 4.7), which correspond to the findings of previous studies from South Asia, including India and Pakistan (Calland *et al.,* 2023; Jacob *et al.,* 2024; Thorpe *et al.,* 2022). ESBL gene *bla*$_{\text{CTX–M–15}}$ was found to be significantly associated with 3GC resistance, followed by *bla*$_{\text{NDM–5}}$ with carbapenem resistance, *aac(6')–Ib* and *aph(6)–Id* with aminoglycoside resistance, and *qnrS* and *qnrB1* with quinolone resistance in *K. pneumoniae* (p<0.05) (Table 4.8). I also found that β-lactamase, aminoglycoside, fluoroquinolone, sulfonamide and trimethoprim resistance genes were significantly correlated with

3GC and carbapenem resistance in *K. pneumoniae* (Table 4.9 and 4.10), indicating co-harbouring of multiple resistance genes in single isolates, limiting therapeutic options (Altayb *et al.,* 2022).

In this study, discordance between phenotypic resistance and detected ARGs was observed (Table 4.8), which can be attributed to several methodological and biological factors. This study focused on screening horizontally acquired resistance genes and did not include analysis of chromosomal point mutations. Consequently, resistance mechanisms primarily mediated by target-site mutations, most notably fluoroquinolone resistance associated with mutations in the quinolone resistance–determining regions of *gyrA* and *parC* were not captured (Hooper & Jacoby, 2015; Redgrave *et al*., 2014). Although plasmid-mediated quinolone resistance genes such as *qnrS1* and *qnrB1* were significantly associated with phenotypic resistance, these determinants typically confer low-level resistance and facilitate the selection of chromosomal mutations rather than acting as sole resistance mechanisms (Strahilevitz *et al*., 2009; Jacoby *et al*., 2014). Thus, the absence of *qnr* genes in some resistant isolates is more likely attributable to uncharacterized chromosomal mutations than to true genotypic–phenotypic discordance. Additionally, discordance may also include variability in gene expression, incomplete sequencing coverage, and inherent limitations of phenotypic antimicrobial susceptibility testing (Ellington *et al*., 2017; CLSI, 2023). Moreover, ABRicate relies on curated resistance gene databases and does not detect resistance arising from novel mutations or regulatory changes (Boolchandani *et al*., 2019). Collectively, these findings indicate that ARG-based genomic screening alone may underestimate resistance to antibiotics in which chromosomal mutations constitute the dominant resistance mechanism.

This study compared the clonal relatedness of *K. pneumoniae* across different niches to understand the distribution of STs with respect to the One Health approach. Results revealed the high genetic diversity of *K. pneumoniae* clones isolated in this study from human, farm and environmental samples. Among 260 identified STs in *K. pneumoniae*, ST37, ST490, ST15, ST147, ST48 and ST45 were the dominant STs (Figure 4.8). The evolution and diversity of *K. pneumoniae* have been reported in many parts of the world, including South Asia (Wyres *et al.,* 2019; Wyres *et al.,* 2020; Byarugaba *et al.,* 2023). Global problem clones (MDR STs) of *K. pneumoniae* such as ST11, ST15, ST29, ST37, ST147, ST307, ST101 were found in this study, which is consistent with the previous reports from clinical isolates in

Bangladesh (Farzana *et al.,* 2020; Hussain *et al.,* 2023; Kawser *et al.,* 2025). The findings of these high–risk clones in Bangladesh are not surprising, considering the global dissemination of these clones and the ease with which they spread and transmit not only within healthcare facilities but also in communities. Among the prevalent STs recovered from various sources, ST490 and ST48 were significantly associated with SSIs, whereas ST45 was linked to HRS (Table 4.11). ST490 and ST48 isolates were restricted to MMCH, indicating localised transmission within and around the hospital facilities (Figure 4.10). ST490 and ST48 associates with SSIs were MDR, whereas ST45 from HRS exhibited a low level of resistance according to Kleborate resistant score which is consistent with phenotypic resistance results of this study (Figure 4.13). Comparative analysis revealed a significant association of ST37, ST490, ST147 and ST48 with 3GC resistance in *K. pneumoniae*, followed by ST15 and ST48 with carbapenem resistance, and ST490, ST15, ST147 and ST48 with aminoglycoside resistance and quinolone resistance (Figure 4.15 – 4.18). Previous data suggested a strong association of particular resistance patterns with the clonal backgrounds, and ST11, ST14, ST15, ST23, and ST147 of *K. pneumoniae* have been reported episodically as the frequent host of ESBLs and carbapenemase (Farzana *et al.,* 2020; Wyres *et al.,* 2020; Byarugaba *et al.,* 2023).

K. pneumoniae was reported previously as an extremely transmissible organism and more prone to cause outbreaks than several other Enterobacterales (Gurieva *et al.,* 2018; Ludden *et al.,* 2020). In the present study, several genomic clusters were identified, comprising isolates with diverse STs, sample sources, sampling periods, and locations, which differed by ≤100 SNPs (Figure 4.19). Although MLST is commonly used to describe the population structure of *K. pneumoniae*, it is based on only seven housekeeping loci and therefore provides substantially lower resolution than core-genome SNP analysis (Maiden *et al*., 1998; Wyres & Holt, 2018). Consequently, isolates assigned to different STs may nonetheless cluster within relatively small core-genome SNP distances, particularly when ST divergence is driven by variation at one or a few loci rather than genome-wide evolutionary separation. Such discordance between MLST classification and core-genome relatedness has been widely reported in *K. pneumoniae* and reflects the limited discriminatory power of MLST for fine-scale phylogenetic inference (Wyres *et al*., 2019; Ludden *et al*., 2020). In addition, localised recombination affecting

MLST loci can result in ST reassignment despite high overall genomic similarity (Didelot & Wilson, 2015; Wyres *et al*., 2020). As recombination was identified and removed prior to SNP distance estimation using Gubbins, the observed ≤100 SNP clustering across distinct STs is unlikely to be an artefact of horizontal gene transfer affecting large genomic regions.

Importantly, the ≤100 SNP threshold applied in Figure 4.19 was used as an exploratory approach to examine broader phylogenetic structure and genomic relatedness across isolates from human, animal and environmental sources, rather than to infer transmission. This population-level clustering provided contextual insight into the genetic neighbourhoods within which more closely related isolates occurred, thereby informing subsequent, more stringent analyses focused on putative transmission events. Since there is no universally applicable SNP cut-off for defining an outbreak across all settings, SNP thresholds are best treated as pragmatic screening tools interpreted alongside epidemiological evidence and analytical context. Although outbreak SNP thresholds are not predefined, they can be inferred empirically by calibrating genomic distances against epidemiologically linked cases (Marsh *et al.,* 2019; Stimson *et al*., 2019). Consistent with this principle, I used the published working ranges (0 - 30 SNPs) to screen for plausible transmission (Stimson *et al*., 2019; Azarian & Black, 2024; Hinthong *et al*., 2025), followed by integration of ward-level epidemiology (admission ward and overlapping hospital stays) with genomic data. This approach identified two putative clonal outbreak clusters belonging to MDR ST490 and ST48 at the surgery, orthopaedics, obstetrics and gynaecology wards of MMCH (Figure 4.20). The combined epidemiological and genomic insights inferred the following probable transmission events: 1) Within the same ward, overlapping of patients' hospital stay, and isolates with the SNPs difference of ≤30 were suggestive of sequential patient–to–patient transmission. 2) Isolates differed by very few SNPs (0 to 2), suggesting the sequential transmission or concurrent acquisition of an identical clone from a common source. 3) In case of different wards, the probable transmission might be through health care workers or the usage of a common device by the patients of different wards. However, this interpretation is limited by the lack of colonisation screening. This study did not investigate the role of the carrier state during the putative clinical outbreak. The carrier might be patients infected with outbreak isolates, other patients, caregivers or health care workers (Hawkey, 2015;

Cimmino *et al.,* 2016; Shahida *et al.,* 2016). Perhaps the involvement of pre-existing exogenous sources along with sequential patient-to-patient transmission can be responsible for the occurrences (Roisin *et al.,* 2016). In addition, the lack of in-ward environmental sampling in the hospital limits the ability to directly link putative outbreaks to hospital reservoirs. Hospital outbreaks can increase the clinical burden of successful MDR clones, which can spread between/within hospitals, the community via faecal carriage, and in environmental settings through poor drainage systems (Hassing *et al.,* 2015; Hawkey, 2015; Cimmino *et al.,* 2016).

Plasmids serve as major vehicles for the horizontal gene transfer of critical AMR determinants in *K. pneumoniae*, enhancing its capacity to develop and disseminate multidrug resistance (Ramirez *et al*., 2014; Li *et al*., 2025). Therefore, this study focused on examining the plasmid–driven spread of AMR in *K. pneumoniae* within the study area with regard to One Health. Plasmid typing identified a wide variety of plasmid replicons in *K. pneumoniae* from various sources (Figure 4.21 and Table 4.12). Hierarchical clustering based on plasmid replicon presence and absence using Jaccard distance revealed that some isolates shared highly similar plasmid profiles, whereas others displayed diverse and heterogeneous plasmid content, indicating substantial variability in plasmid carriage across the population (Figure 4.22). The observed heterogeneity indicates that AMR in *K. pneumoniae* is not driven solely by clonal expansion but also by horizontal plasmid-mediated dissemination (Rozwandowicz *et al*., 2018). Notably, IncFIB(K)Kpn3 was the most prevalent replicon, aligning with the previous report (Wyres *et al.,* 2020). I found that several replicons showed source-specific enrichment with IncFIB(K)Kpn3 and IncHI1B(pNDM–MAR) significantly associated with *K. pneumoniae* from HRS, IncN and IncX1 linked to CCS, CMS, and water-derived isolates (p<0.05) (Figure 4.23), indicating ecological structuring of plasmid distribution (Jacob *et al.,* 2024). Col440I, Col440II, Col(MG828), ColKP3, ColRNAI, IncA/C2, IncFIB(pQil), IncFII, IncFII(pKP91), and IncR were found to be significantly associated with the ESBL gene *bla*$_{\text{CTX–M–15}}$ and the carbapenem–resistant gene *bla*$_{\text{NDM–5}}$ (p<0.05) (Table 4.13 and Table 4.14). Previous studies have also reported a significant association between the *bla*$_{\text{CTX–M–15}}$ gene and IncF–type plasmids, which are known for their broad host range and ability to carry multiple resistance genes (Carattoli, 2009; Negeri *et al.,* 2023; Ikhimiukor *et* *al.,* 2024). Refath *et al.* (2023) documented that the plasmid–mediated horizontal dissemination of bla_{NDM} among various species of Enterobacterales primarily occurred via IncFII and IncX3 in Bangladesh. Another study from India also reported that bla_{NDM} was associated with IncFII–like plasmids in *K. pneumoniae* (Shankar *et al.,* 2021). The findings from this study indicate various mechanisms of HGT for the spread of $bla_{\text{CTX–M–15}}$ and $bla_{\text{NDM–5}}$, especially when compared to earlier reports. Furthermore, plasmid replicons associated with $bla_{\text{CTX–M–15}}$ and $bla_{\text{NDM–5}}$ were also significantly enriched in SSIs rather than to other sample sources (Figure 4.23). This finding indicates that healthcare environments may play a role in the spread of plasmid–mediated ESBL and carbapenemase resistance, likely due to lapses in aseptic practices, contaminated instruments, or colonisation of surgical staff and/or patients.

In the present study, IncFII(pKP91) and IncR replicons were frequently associated with genes that confer resistance to ESBL, carbapenemase, aminoglycosides, and quinolones ($bla_{\text{CTX–M–15}}$, $bla_{\text{NDM–5}}$, *aac(6')–Ib* and *aph(6)–Id*, *qnrB1* and *qnrS1*), aligning with earlier findings in India (Shankar *et al.,* 2021). Interpretation of odds ratios (ORs) further supports this, as replicons such as IncFII(pKP91) and IncR exhibited consistently elevated ORs across multiple resistance classes, indicating high gene penetrance and stable maintenance of diverse ARGs within widely disseminated plasmid backbones, a recognised feature of IncF- and IncR-type plasmids (Carattoli, 2009; Wyres *et al*., 2020). In contrast, very high ORs observed for less prevalent replicons likely reflect strong lineage-specific enrichment rather than widespread dissemination. Importantly, ORs describe genetic co-carriage and dissemination potential rather than phenotypic resistance magnitude, which is more accurately reflected by MIC data (San Millan, 2018). The frequent co-occurrence of ESBL, carbapenemase, aminoglycoside, and quinolone resistance genes on the same plasmid replicons provides a clear genetic basis for co-resistance. Therefore, under antimicrobial pressure, selection for a single resistance determinant can co-select for multiple unrelated ARGs located on shared mobile genetic platforms, facilitating the persistence and spread of MDR *K. pneumoniae* across the human–animal–environment interface (Partridge *et al*., 2018).

The major findings of this chapter strengthened the understanding of the vital resistance determinants of *K. pneumoniae* in Bangladesh within the One Health framework. The shared *K. pneumoniae* clones, AMR genes, and plasmids across various environments revealed several critical aspects of AMR distribution, clonal diversity, and phylogenetic relationships. These insights highlighted the prevalent resistance patterns and mobile genetic elements of *K. pneumoniae* that transcend traditional sectoral boundaries. Notably, the spread of MDR *K. pneumoniae* was more prominent among hospitalised patients and chickens in poultry farms. My results indicated that hospitals acted as hotspots for the dissemination of *K. pneumoniae* AMR, while isolates from healthy human volunteers, domestic animals near farms, free–flying birds, and environmental samples were mostly susceptible to the current antimicrobials used to treat human disease. Furthermore, the potential outbreak of MDR *K. pneumoniae* ST490 and ST48 is likely to be originated in hospital environments rather than from animals or natural conditions. The genetic diversity among local *K. pneumoniae* isolates from different sources allowed me to identify various STs from humans, animals, and their environment, which may prove crucial in managing the public and animal health threats posed by MDR *K. pneumoniae* in the future.

Chapter 5

Investigating the Epidemiology of Hypervirulent *Klebsiella pneumoniae* in Bangladesh: Insights from One Health Genomic Surveillance

5.1 Introduction

K. pneumoniae stands among the most formidable bacterial pathogens worldwide with remarkable phenotypic, genotypic and pathogenic diversity (Stanton & Wyres, 2024). This bacterium is best known for its two distinct pathotypes, referred to as the classical *K. pneumoniae* (cKP) and the hypervirulent *K. pneumoniae* (hvKP). The cKP strains exhibit high genetic diversity and are frequently MDR but typically of lower virulence. They encompass globally disseminated antibiotic-resistant clones, including ST11, ST15, ST48, ST147, ST231, ST258, ST307 and ST512, which frequently harbour ESBLs ($bla_{\text{CTX-M-15}}$) and carbapenemases (bla_{KPC}, bla_{NDM}, $bla_{\text{OXA-48-like}}$). cKP are primarily associated with HAIs such as pneumonia, bloodstream infections, urinary tract infections and wound infections, particularly in neonates and immunocompromised patients. In contrast to cKP, hvKP are generally susceptible to most antibiotics yet capable of causing severe, rapidly progressing CAIs, posing a unique challenge (Wyres *et al*., 2020; Kochan *et al*., 2023; Russo *et al*., 2024).

Over the past two decades, hvKP has evolved as a significant public health concern due to its exceptional virulence and ability to cause severe, life-threatening invasive infections such as pyogenic liver abscesses and meningitis in otherwise healthy individuals, as well as in immunocompromised people (Paczosa & Mecsas, 2016). ST23, ST65, ST66, ST86, and ST380 are the dominant lineages causing hypervirulent infections globally (Wyres *et al*., 2020). These strains possess enhanced virulence attributes associated with pathogenicity loci responsible for siderophore overproduction (aerobactin, encoded by *iuc*; salmochelin, *iro*; yersiniabactin, *ybt*), hypermucoviscosity (hypermucoidy operons: *rmpADC*, *rmpA2*), genotoxin (colibactin, *clb*) and virulence plasmids (pK2044Kp, pLVPK). The gene clusters encoding these features are usually carried on ICEs (for *ybt*, *clb*), or on large plasmids (for *iuc, iro, rmpADC* and its ortholog *rmpA2*) (Lee *et al*., 2017; Spadar *et al*., 2022; Stanton & Wyres, 2024). Previous studies have identified *iucA, iroB*, *peg-344, rmpA*, *rmpA2* and virulence plasmids as key molecular markers for defining hvKP (Russo *et al*., 2018; Russo *et al*., 2024). Hypervirulence-associated factors also include the capsular polysaccharide, which defines the capsular serotype (K-antigen type) and the lipopolysaccharide (LPS, O-antigen type), with K1 and K2 serotypes reported to be significantly associated with hvKP (Lei *et al*., 2024).

Although the hvKP and cKP strains have traditionally been considered non-overlapping, recent global genomic studies have revealed the emergence of convergent strains, which possess features of both the hvKP and cKP strains. Convergence can occur through several mechanisms, including the acquisition of resistance determinants by hvKP strains, the acquisition of virulence factors by MDR cKP lineages, or through the acquisition of hybrid plasmids carrying both AMR and virulence determinants (Wyres *et al*., 2020; Lan *et al*., 2021; Pu *et al*., 2023; Lei *et al*., 2024). The hvKP (ST23) acquiring multiple AMR plasmids, cKP (ST11) acquiring the pLVPK-like virulence plasmid, and *K. pneumoniae* ST2096 acquiring hybrid plasmids carrying both AMR and virulence genes are some examples of convergence (Gu *et al*., 2018; Shankar *et al*., 2020, 2022). These convergent strains, particularly carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP), not only cause severe infections but also limit the treatment options, further increasing the threat to global health (Lee *et al*., 2017; Hetta *et al*., 2025). The spread of such strains is particularly worrisome in LMICs like Bangladesh, where treatment options are limited and diagnostics are suboptimal (Hussain *et al*., 2023).

Global dissemination of key hvKP lineages (ST23, ST65, ST86) and convergent strains (ST11, ST231, ST2096), including CR-hvKP strains, has been documented in previous studies, particularly with higher prevalence in East and Southeast Asia (Karlsson *et al*., 2019; Beyrouthy *et al*., 2020; Shankar *et al*., 2020; Wyres *et al*., 2020; Hernández *et al*., 2021; Imtiaz *et al*., 2021; Lan *et al*., 2021; Zhu *et al*., 2022; Hussain *et al*., 2023; Dingiswayo *et al*., 2024; ECDC, 2024; Merla *et al*., 2024; Nguyen *et al*., 2024). A recent study reported LMICs in Asia, specifically India, Pakistan and Vietnam, as hotspots for these convergent strains (Silvester *et al*., 2022). Despite these findings, limited data exist from Bangladesh on the prevalence, reservoirs, and genetic structure of hvKP and its convergent forms (Kawser & Shamsuzzaman, 2022; Hussain *et al*., 2023).

Considering the growing global concern on the convergence of hypervirulence and AMR and the existing knowledge gap in the epidemiological mapping of hvKP in Bangladesh, this study aims to comprehensively investigate the molecular epidemiology of hvKP across community, farm, and healthcare settings.

This chapter specifically aims to:

1. Characterise the virulence profiles of *K. pneumoniae* circulating at the human-animal-environment interface.
2. Determine the prevalence and clonal relatedness of hvKP.
3. Investigate the convergence of AMR and virulence determinants in *K. pneumoniae*.
4. Assess the role of plasmids in the dissemination of virulence and AMR determinants among convergent *K. pneumoniae*.

5.2 Results

5.2.1 Virulence profile of *K. pneumoniae*

A total of 541 *K. pneumoniae* isolates were sequenced using the Illumina NovaSeq 6000 platform. Virulence genes and ARGs were identified using the latest VFDB and ABRicate databases. Kleborate (v.3.0) was used to determine virulence and AMR profiles, while Kaptive was used to identify surface antigens (K and O antigens) (Wyres *et al.,* 2016; Lam *et al.,* 2021).

Based on the VFDB screening and Kleborate analysis, 37 virulence genes were identified among the 541 *K. pneumoniae* isolates. Among the identified virulence loci, core virulence genes such as *entA*, *entB*, *fepC*, *ompA*, the ECP pilus loci *yagV/W/X/Y/Z* and the regulator *ykgK* were present in more than 95% of *K. pneumoniae* (Table 5.1). Accessory virulence genes (*ybt*, *iro*, *clb*, *iuc*, *rmp*), which are typically found on mobile elements such as genomic islands, ICEs or plasmids, showed variable prevalence. These accessory genes are also considered virulence markers in *K. pneumoniae*. Yersiniabactin cluster (*ybtA*, *ybtE*, *ybtP/Q/S/T/U/X)* and associated genes (*irp1*, *irp2*) required for yersiniabactin biosynthesis were found in 20.9% (113/541) *K. pneumoniae*. Salmochelin siderophore operon (*iroB/C/N*) was present in 3.3% (18/541) isolates, with *iroD* and *iroE* detected in 3.1% (17/541) and 0.4% (2/541), respectively. Aerobactin biosynthesis genes (*iucA*, *iucB*, *iucC*, *iucD*), along with their regulator *iutA* were found in 3.7% (20/541) of the isolates. Colibactin (*clb*) was present in only 0.9% (5/541) of *K. pneumoniae*. The hypercapsule-regulator genes, *rmpA*, *rmpA2*, *rmpC*, and *rmpD*, were present in 3.7%, 3.5%, 3.0%, and 3.0% of the isolates, respectively.

The prevalence of accessory virulence genes varied depending on the source of isolation (Table 5.2). The prevalence of *ybt* was highest at 65.6% in SSIs, followed by 21.4% in UTIs, 18.4% in HRS, 13.3% in DARS, 8.0% in water, 7.5% in flies, 4.8% in CCS, and 3.7% in CMS strains. Salmochelin (*iro*) was absent from water, CMS, DARS, and BF, and found at 7.5% in SSIs, 7.1% in UTIs, 4.3% in HRS, 1.6% in CCS and 1.3% in flies. Aerobactin (*iuc*) was found only in 12.9% of SSIs, 3.8% of HRS and 1.3% of fly isolates. Colibactin (*clb*) was exclusive to strains from human samples, at 4.3% in SSIs and 0.5%

in HRS. Capsule-regulator genes (*rmp*) occurred in 11.8% of SSIs, 4.3% of HRS and 1.3% of flies, but were absent from all other sources (Table 5.2).

Table 5.1 Prevalence of virulence loci in 541 *K. pneumoniae* isolates.

Category	**Gene(s)**	**Function/System**	**Prevalence (n, %)**
Core	*entA*, *entB*, *fepC*	Enterobactin synthesis and uptake	*entA*: 522 (96.5%)
			entB: 540 (99.8%)
			fepC: 541 (100%)
	ompA, *yagV/W/X/Y/Z*, *ykgK (ECP)*	Adhesion	*ompA*: 541 (100%)
			yagV/X/Y/Z: 540 (99.8%)
			yagW: 539 (99.6%)
			ykgK (*ECP*): 535 (98.9%)
Accessory	*ybtA*, *ybtE*, *ybtP/Q/S/T/U/X*, *irp1*, *irp2*	Yersiniabactin siderophore (*ybt*, *irp*)	*ybtA/E/P/Q/S/T/U/X*: 113 (20.9%)
			irp1/2: 113 (20.9%)
	iroB/*C*/*D*/*E*/*N*	Salmochelin siderophore (*iro*)	*iroB/C/N*: 18 (3.3%)
			iroD: 17 (3.1%)
			iroE: 2 (0.4%)
	iucA/*B*/*C*/*D*, *iutA*	Aerobactin siderophore (*iuc*, *iut*)	*iucA/B/C/D*: 20 (3.7%)
			iutA: 20 (3.7%)
	clb (*clbA-clbQ* cluster)	Colibactin genotoxin (*clb*)	*clb*: 5 (0.9%)
	rmpA, *rmpA2*, *rmpC*, *rmpD*	Capsule regulators for hypercapsule production and hypermucoidy	*rmpA*: 20 (3.7%)
			rmpA2: 19 (3.5%)
			rmpC: 16 (3.0%)
			rmpD: 16 (3.0%)

n, number.

Table 5.2 Prevalence of key virulence markers identified in *K. pneumoniae* across various sample sources.

Source (n)	**Siderophore systems**			**Colibactin**	**Regulator of mucoid phenotype**
	***ybt* cluster + *irp* (n=113)**	***iro* (n=18)**	***iuc* + *iutA* (n=20)**	***clb* (n=5)**	***rmp* (n=20)**
Water (25)	2 (8.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Fly (80)	6 (7.5%)	1 (1.3%)	1 (1.3%)	0 (0%)	1 (1.3%)
CCS (62)	3 (4.8%)	1 (1.6%)	0 (0%)	0 (0%)	0 (0%)
CMS (54)	2 (3.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
DARS (15)	2 (13.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
BF (13)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
UTIs (14)	3 (21.4%)	1 (7.1%)	0 (0%)	0 (0%)	0 (0%)
SSIs (93)	61 (65.6%)	7 (7.5%)	12 (12.9%)	4 (4.3%)	11 (11.8%)
HRS (185)	34 (18.4%)	8 (4.3%)	7 (3.8%)	1 (0.5%)	8 (4.3%)

n, number.; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs.

Of the 541 *K. pneumoniae* isolates analysed by Kaptive, 56 different K antigen types (capsular polysaccharide) were assigned to 56.2% (n=304) isolates and 43.8% (n=237) could not be assigned a known K-locus. Among the typable strains, K3 was the most commonly found (3.7%, n=20), followed by K12, K30, K64 and K8 (each, 2.2%, n=12), and K15 (2.0%, n=11). Each of the remaining K types comprised ≤1.7% of the collection (Figure 5.1).

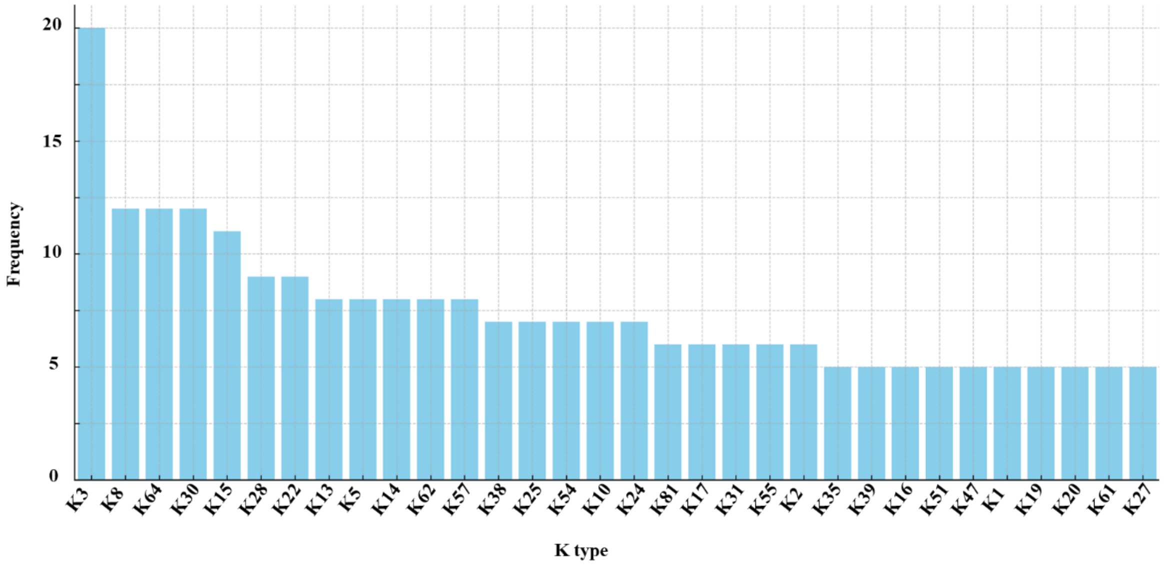


Figure 5.1 Frequency of K antigen types (n≥5) in *K. pneumoniae* isolates.

In case of O antigens (lipopolysaccharide), 10 different O types were assigned to 92.6% (n=501) of the isolates, with 7.4% (n=40) remaining untypeable (Figure 5.2). O1ab (29.4%, 159/541), O3b (19.6%, 106/541) and O2afg (15.0%, 81/541) were the dominant O serotypes.

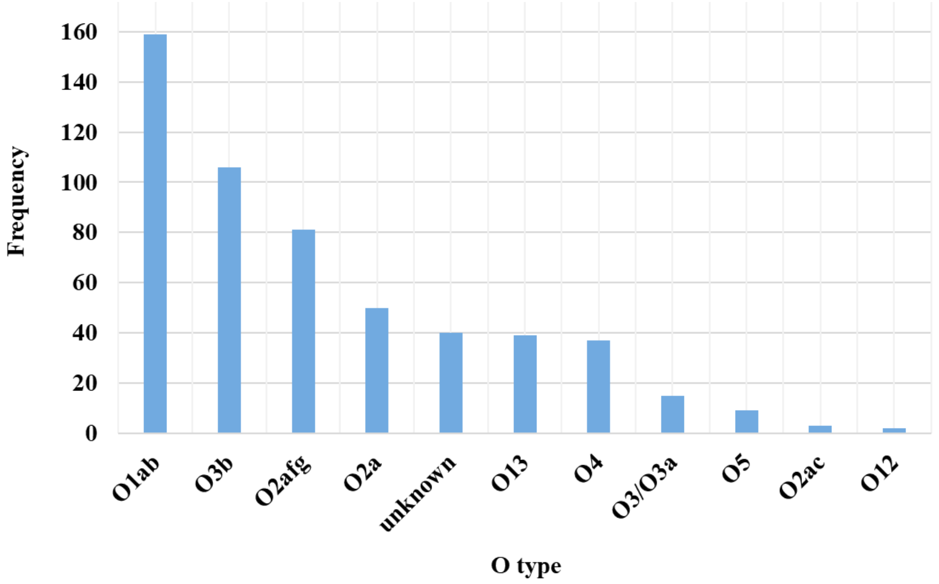


Figure 5.2 Frequency of O antigen types (n≥5) in *K. pneumoniae* isolates.

When combining K and O antigens, 94 distinct K-O type pairings were found. The most frequent combination was K3-O2afg (n=19), followed by K30-O1ab (n=12), K15-O4 (n=11), K28-O2a, K64-O1ab and K14-O3b (each, n=8), K13-O3b and K54-O1ab (each, n=7), K81-O13, K8-O2afg, K5-O3b, K62-O2a, K31-O3b and K2-O1b (each, n=6), K19-O1ab, K22-O1ab, K1-O1ab and K61-O2a (each, n=5) (Figure 5.3).

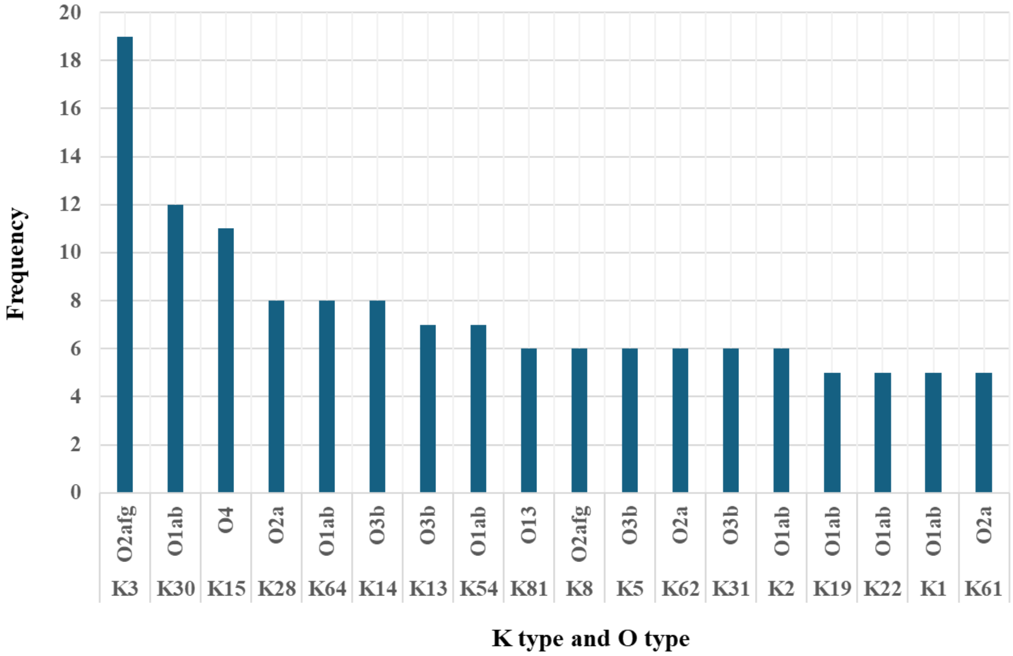


Figure 5.3 Frequency of K-O antigen pairings (n≥5) in *K. pneumoniae* isolates.

Kleborate assigned six scores (0 to 5) to the *K. pneumoniae* isolates based on the presence of genes encoding the virulence factors *ybt*, *iuc*, and *clb* (Table 5.3). Salmochelin (*iro*) and the hypermucoidy regulator locus (*rmp*) were *not* directly scored, since they almost always co-occurred with *iuc* (Kleborate v.3.0). Isolates scoring 3 or more were classified as hypervirulent. Out of 541 isolates, 78.4% (424) lacked all three loci (score 0) and 17.9% (97) carried only *ybt* (score 1). None of the isolates had *clb* without *iuc* (score 2). Four isolates (0.7 %) scored 3 (*iuc* alone), 11 (2.0 %) scored 4 (*iuc* + *ybt*), and five (0.9 %) scored 5 with the full complement of loci.

Table 5.3 Kleborate virulence score distribution and prevalence in *K. pneumoniae*.

Kleborate score	**Loci present**	**Virulence category**	**Prevalence, n (%)**
0	None of yersiniabactin (*ybt*), colibactin (*clb*) or aerobactin (*iuc*)	Classical/Non-virulent	424 (78.4%)
1	*ybt* only	Low	97 (17.9%)
2	*clb* without *iuc* (regardless of *ybt*)	Moderate	0
3	*iuc* only (without *ybt* or *clb*)	High	4 (0.7%)
4	*iuc* with *ybt* (without *clb*)	High	11 (2.0%)
5	*iuc*, *ybt* and *clb*	Very High	5 (0.9%)

n, number.

According to the Kleborate criteria, out of 541 *K. pneumoniae* isolates, 20 (3.7 %, scored ≥ 3) were classified as genotypically hypervirulent. As shown in Figure 5.4, 19 of these 20 hvKP were recovered from human-derived samples (SSIs and HRS), and one from flies. Breaking this down by the score category:

- **Score 3 (n = 4):** two isolates from SSI patients, one from a healthy volunteer, and one from flies.
- **Score 4 (n = 11):** six isolates from SSI patients and five from healthy volunteers.
- **Score 5 (n = 5):** four isolates from SSI patients and one from a healthy volunteer.

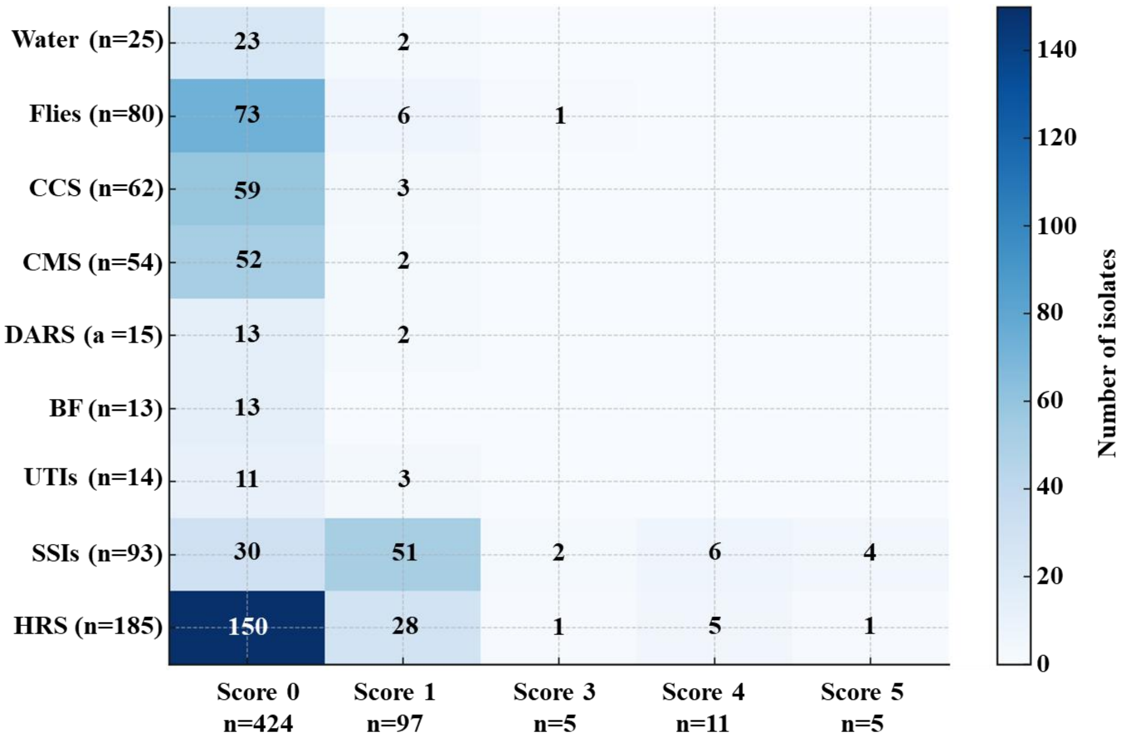


Figure 5.4 Heatmap showing the Kleborate virulence score distribution in *K. pneumoniae* across different sample sources. n, number. The heatmap was created in Python using Matplotlib (v.3.7).

5.2.2 Clonality of hvKP in this study

MLST profiling of genotypically determined 20 hvKP isolates revealed nine diverse sequence types (Figure 5.5). ST23 and ST86 were the most frequently found, each occurring in five isolates, followed by ST420 (n=4) and single isolates from ST11, ST29, ST231, ST160, ST660 and ST2096.

All five hvKP with a virulence score of 5 (carrying *ybt*, *iro*, *iuc*, *clb*, and *rmp*) belonged to ST23. Four of these hypervirulent isolates were recovered from SSIs at MMCH, and one from HRS near the Tarakanda layer farm. These ST23 isolates uniformly exhibited the K1 (capsular) and O1ab (lipopolysaccharide) antigen types (Figure 5.5).

The ST86 (n=5) isolates were recovered exclusively from HRS across five different sampling locations (Mymensingh layer, Muktagachha broiler, Muktagachha layer, Trishal broiler and Tarakanda layer farms). All ST86 strains had a virulence score of 4, with the presence of *ybt*, *iro*, *iuc*, and *rmp*, but lacked *clb*. Their antigenic profile was consistent with K2 and O1ab antigen types. Of the four ST420 isolates, 3 (virulence score 4) were isolated from SSIs (MMCH) and one (virulence score 3) from HRS (Muktagachha layer farm). They also had a similar antigenic presentation with K20 and O1ab. A single fly-derived isolate from the Gauripur layer farm was identified as ST660 (K16/O1ab), and the remaining isolates (ST11, ST29, ST160, ST231 and ST2096), each of which exhibited different virulence scores (score 3 or 4) and diverse K–O antigen combinations, were all obtained from SSIs.

Comparative analysis revealed a significant association between K1, K2, and K20 serotypes and hvKP strains compared to low-virulent or cKP (*p*<0.05) (Table 5.5).

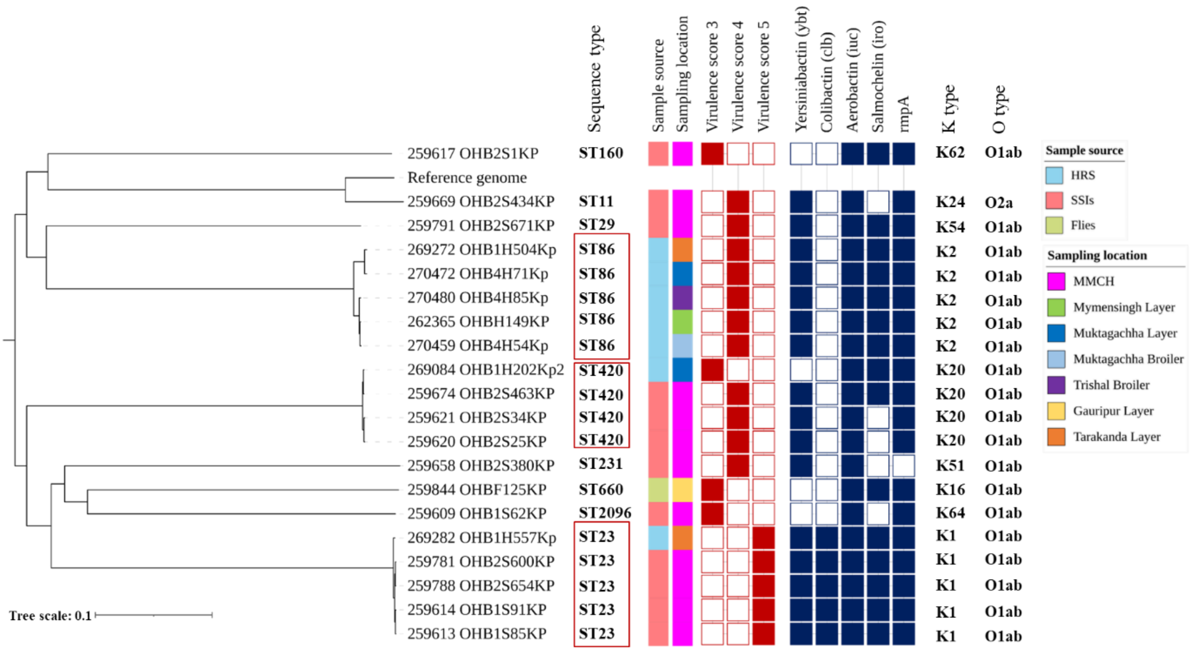


Figure 5.5 Clonal distribution of hvKP across various sources. The ML tree was generated using VeryFastTree (v.4.0) and visualised in iTOL (v.6). Core-genome alignment was performed using the Bactmap pipeline (v.1.0.0). The PubMLST database was used for 7 loci MLST profiling. The latest VFDB database, Kleborate analytical pipeline and Kaptive serotyping tool were used to screen for virulence genes, virulence scores and K and O antigen types, respectively.

Table 5.4 Comparative analysis of K antigen types with genotypically determined hvKP versus low-virulent/cKP.

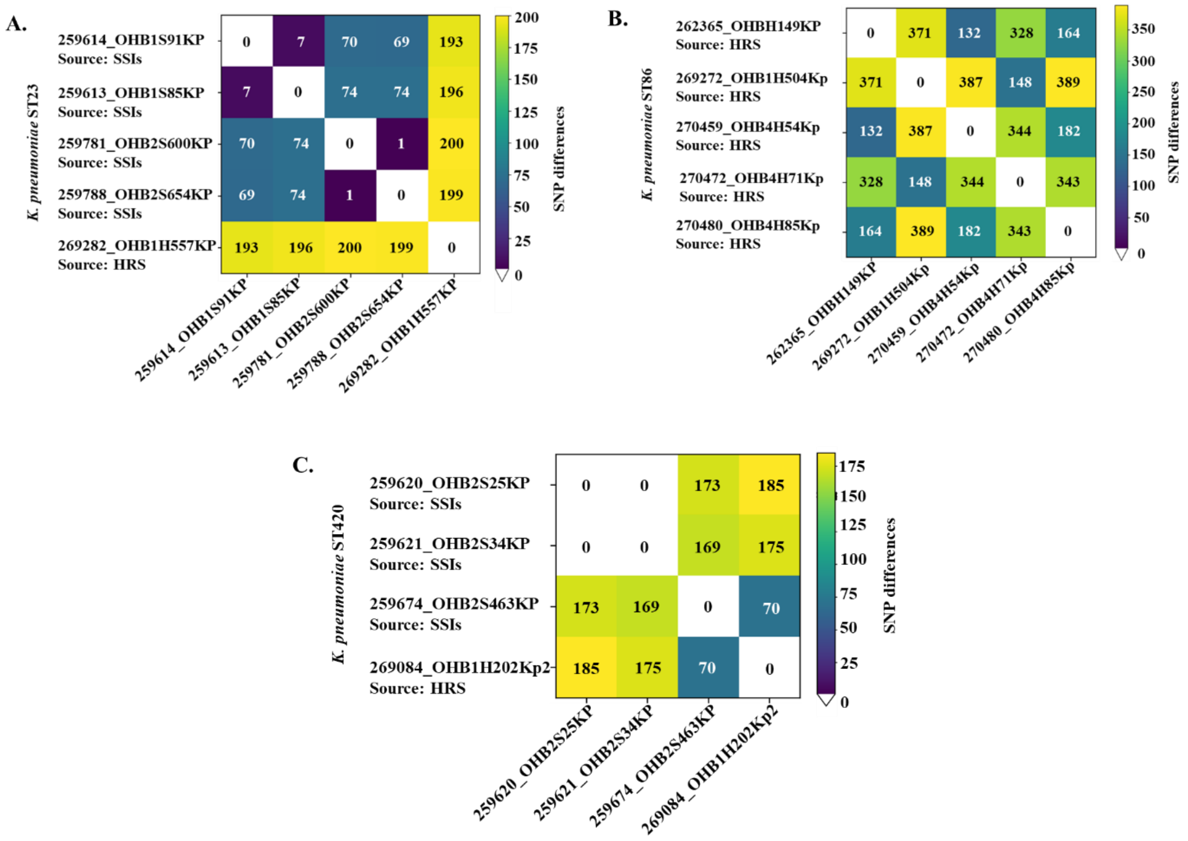
K type	**hvKP (n=20)**	**low-virulent/cKP (n=521)**	***p*-value**	**OR**	**95% CI**
K1 (n=5)	5 (25.0%)	0 (0)	<0.001	NA	NA
K2 (n=6)	5 (25.0%)	1 (0.2%)	<0.001	173.333	19.062 - 1576.176
K16 (n=5)	1 (5.0%)	4 (0.8%)	0.172	6.803	0.725 - 63.811
K20 (n=5)	4 (20.0%)	1 (0.2%)	<0.001	130.000	13.742 - 1229.791
K24 (n=7)	1 (5.0%)	6 (1.2%)	0.233	4.518	0.518 - 39.406
K51 (n=5)	1 (5.0%)	4 (0.8%)	0.172	6.803	0.725 - 63.811
K54 (n=7)	1 (5.0%)	6 (1.2%)	0.233	4.518	0.518 - 39.406
K61 (n=8)	1 (5.0%)	7 (1.3%)	0.262	3.865	0.453 - 33.003
K64 (n=12)	1 (5.0%)	11 (2.1%)	0.367	2.440	0.300 - 19.881

Values in parentheses indicate column percentage. Statistical significance was set at $p<0.05$. NA, not applicable. K antigen types that had a significantly higher association with the hvKP were highlighted.

Core-genome SNP analysis of *K. pneumoniae* ST23 isolates (n=5) revealed two tight clusters (Figure 5.6A). Isolates 259781_OHB2S600KP and 259788_OHB2S654KP differed by only 1 SNP, while isolates 259614_OHB1S91KP and 259613_OHB1S85KP differed by 7 SNPs. All other pairwise distances ranged from 69 to 200 SNPs, where isolate 269282_OHB1H557KP was 193–200 SNPs away from all other strains.

Pairwise SNP distances among the five *K. pneumoniae* ST86 isolates ranged from 0 to 389 SNPs (Figure 5.6B). The closest pair, 262365_OHBH149KP and 270459_OHB4H54Kp, differed by 132 SNPs.

Pairwise SNP distances among the four *K. pneumoniae* ST420 isolates ranged from 0 to 185 SNPs (Figure 5.6C). Isolates 259620_OHB2S25KP and 259621_OHB2S34KP were identical at the core-genome level (0 SNPs). Isolates 259674_OHB2S463KP and 269084_OHB1H202Kp2 differed by 70 SNPs, forming a separate cluster. Between these two clusters, SNP distances were 169–185 SNPs.



	259614_OHB1S91KP	259613_OHB1S85KP	259781_OHB2S600KP	259788_OHB2S654KP	269282_OHB1H557KP
259614_OHB1S91KP Source: SSIs	0	7	70	69	193
259613_OHB1S85KP Source: SSIs	7	0	74	74	196
259781_OHB2S600KP Source: SSIs	70	74	0	1	200
259788_OHB2S654KP Source: SSIs	69	74	1	0	199
269282_OHB1H557KP Source: HRS	193	196	200	199	0

	262365_OHBH149KP	269272_OHB1H504Kp	270459_OHB4H54Kp	270472_OHB4H71Kp	270480_OHB4H85Kp
262365_OHBH149KP Source: HRS	0	371	132	328	164
269272_OHB1H504Kp Source: HRS	371	0	387	148	389
270459_OHB4H54Kp Source: HRS	132	387	0	344	182
270472_OHB4H71Kp Source: HRS	328	148	344	0	343
270480_OHB4H85Kp Source: HRS	164	389	182	343	0

	259620_OHB2S25KP	259621_OHB2S34KP	259674_OHB2S463KP	269084_OHB1H202Kp2
259620_OHB2S25KP Source: SSIs	0	0	173	185
259621_OHB2S34KP Source: SSIs	0	0	169	175
259674_OHB2S463KP Source: SSIs	173	169	0	70
269084_OHB1H202Kp2 Source: HRS	185	175	70	0

Figure 5.6 Pairwise SNP distance heatmap of hvKP lineages. **A.** SNP distances among ST23 isolates, **B.** SNP distances among ST86 isolates and **C.** SNP distances among ST420 isolates. In the heatmap, all diagonal cells are white and labelled "0", which reflects the self-comparisons resulting in no SNP differences.

5.2.3 Convergence of AMR and virulence determinants in *K. pneumoniae*

Convergent *K. pneumoniae* strains are defined as isolates that concurrently harbour genetic determinants of MDR and hypervirulence. In this study, convergence was operationally defined as the co-occurrence of a resistance score > 0 and a virulence score ≥ 3 within a single isolate.

Figure 5.7 illustrates the distribution of MDR (resistance score > 0), hypervirulent (virulence score ≥ 3), and convergent (MDR + hypervirulence) *K. pneumoniae* sequence types identified in this study. The predominant STs identified, such as ST15, ST37, ST48, ST147, and ST490, were primarily MDR with low virulence potential, whereas ST86 and ST660 exhibited high virulence but limited AMR potential. ST29 was detected in both MDR and hypervirulent isolates; however, these traits occurred in distinct isolates. No ST29 isolate concurrently harboured both MDR and hypervirulence determinants. Notably, ST11, ST23, ST160, ST231, ST420, and ST2096 harboured genetic markers associated with both MDR and hypervirulence, indicating convergent phenotypes.

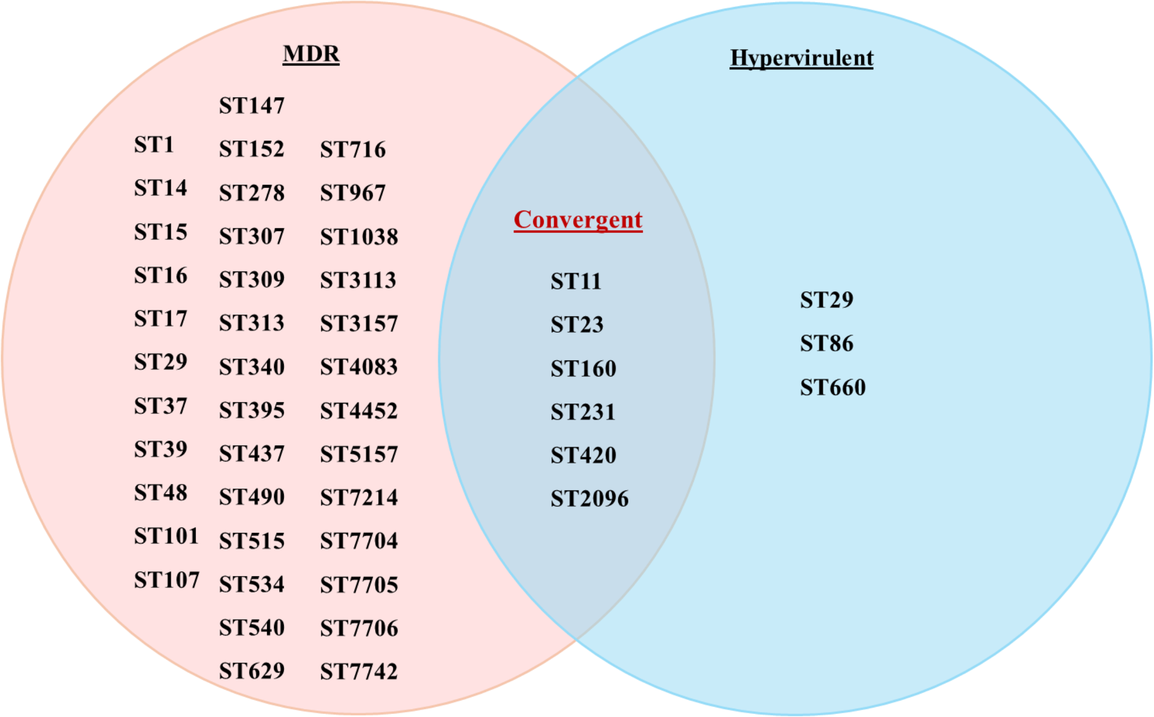


Figure 5.7 Venn diagram illustrating the distribution of MDR, hypervirulent and convergent *K. pneumoniae* sequence types identified in this study.

Among 541 *K. pneumoniae* isolates, only 2.0% (n=11) exhibited convergence of hypervirulence (virulence score ≥3) and MDR (resistance score >0) (Figure 5.8). Convergent isolates were exclusively recovered from patients with SSIs at MMCH, comprising ST23 (n=4), ST420 (n=3), and one each of ST11, ST231, ST160, and ST2096. All convergent isolates harboured the ESBL gene *bla*$_{\text{CTX-M-15}}$. Furthermore, three isolates were both carbapenem-resistant and hypervirulent: ST231 and ST2096 carried the carbapenemase gene *bla*$_{\text{OXA-232}}$, while ST11 carried *bla*$_{\text{OXA-181}}$. Among the hvKP, all strains recovered from HRS and flies exhibited a low resistance score (resistance score 0).

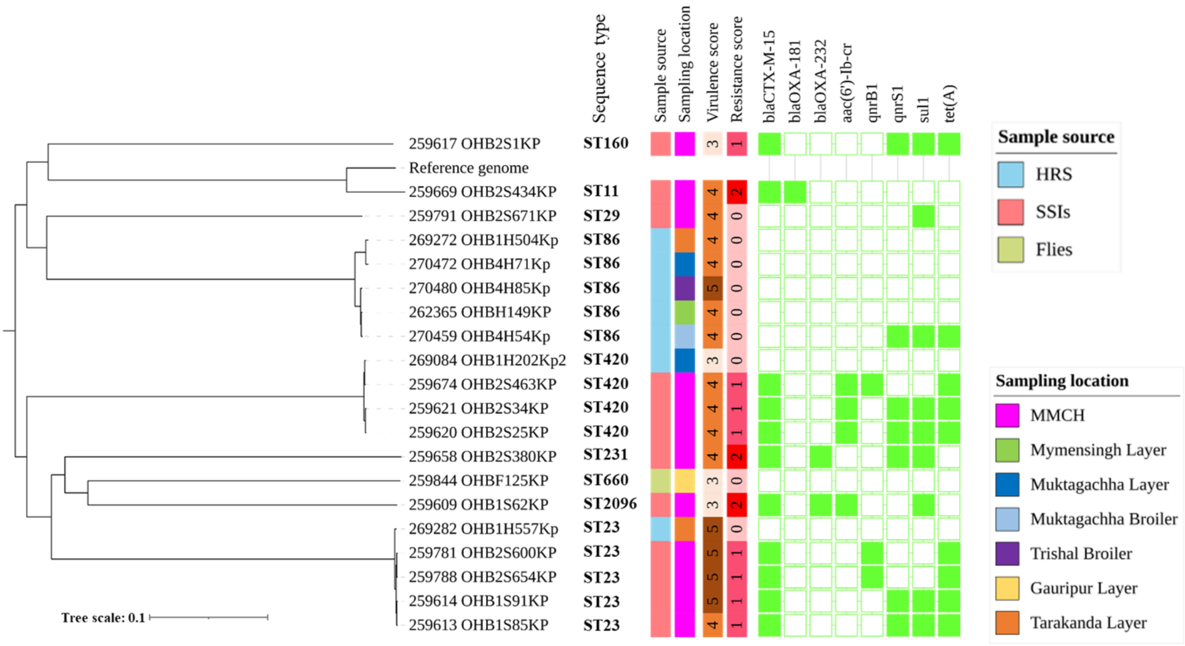


Figure 5.8 Clonal distribution of hvKP and convergent *K. pneumoniae* across various sources. The ML tree was generated using VeryFastTree (v.4.0) and visualised in iTOL (v.6). Core-genome alignment was performed using the Bactmap pipeline (v.1.0.0). The PubMLST database was used for 7 loci MLST profiling. The Kleborate analytical pipeline was used to screen for virulence and resistance scores, and the latest ABRicate database was used to screen for ARGs.

5.2.4 Plasmid-mediated dissemination of virulence and AMR determinants in convergent *K. pneumoniae*

To evaluate the role of plasmids in the dissemination of virulence determinants among *K. pneumoniae*, the convergent CR-hvKP isolates (n=3) were subjected to both short-read (Illumina NovaSeq 6000) and long-read (PromethION) sequencing. Plasmid replicon types were identified using BLASTn against the PlasmidFinder database, and genomic characterisation was performed through a hybrid assembly combining both sequencing data.

Isolate OHB1S62KP (*K. pneumoniae* ST2096) harboured both AMR (*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{OXA-232}}$) and virulence-associated gene loci (*iuc* and *rmp*). Plasmid profiling revealed that virulence genes were carried on a complete circular hybrid plasmid (IncFIB_pNDM-Mar + IncFIB(K)Kpn3; 304,102 bp), which also carried the ESBL gene (*bla*$_{\text{CTX-M-15}}$) on the same plasmid (Figure 5.9A). Genetic context analysis identified a conserved ~2800 bp segment arranged as "insertion sequence–**–mfs transporter–*iucA*–*iucB*–*iucC*–*iucD*–*iutA*–**–*rmpD*–*rmpA2*–**–insertion sequence", flanked downstream by IS*630* and upstream by IS*110*. The carbapenemase gene *bla*$_{\text{OXA-232}}$ was carried separately on a ColKP3 (6,141 bp) plasmid (Figure 5.9B).

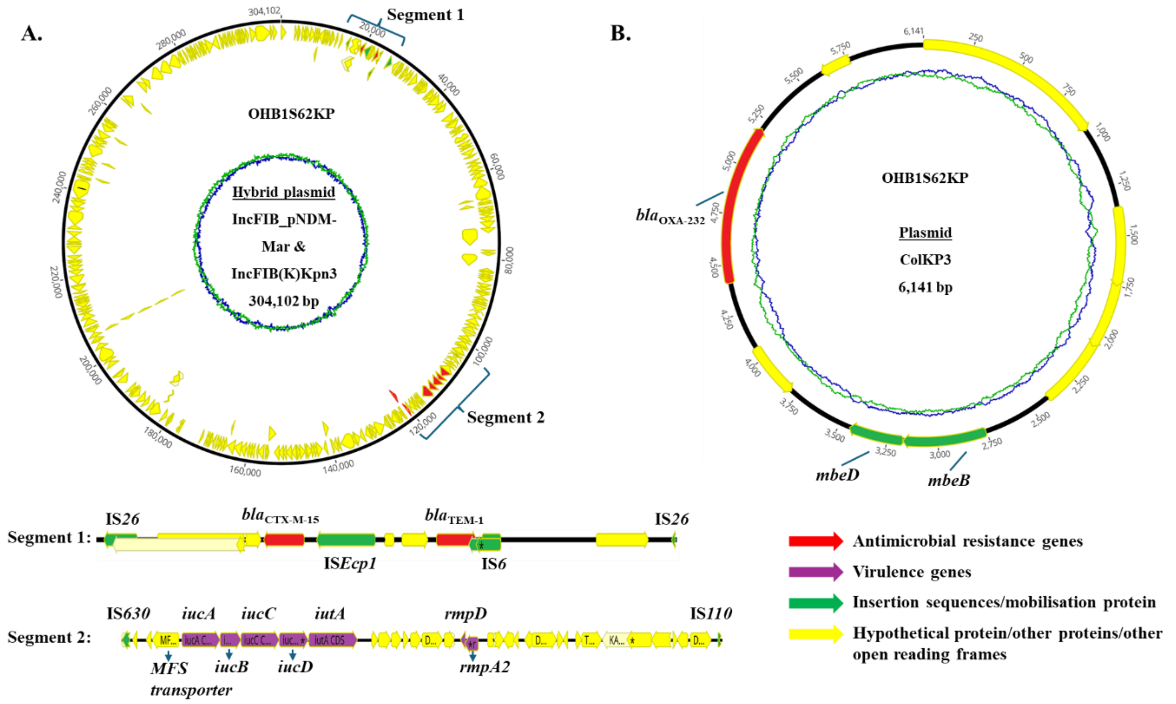


Figure 5.9 Schematic layout of plasmid (IncFIB_pNDM-Mar + IncFIB(K)Kpn3) carrying virulence loci (A) and ColKP3 plasmid (B) carrying *bla*$_{\text{OXA-232}}$ in OHB1S62KP isolate. Arrows represent the position and transcriptional direction of the open reading frames. The figures were produced using Geneious Prime (v.2025.1).

Isolate OHB2S380KP (*K. pneumoniae* ST231) carried the hypervirulence-associated genes on a complete circular hybrid plasmid (IncFII_(pAMA1167-NDM-5) + IncFIA; 74,402 bp) that also encoded sulphonamide resistance gene *sul1* and macrolide resistance gene *mphA* (Figure 5.10A). The aerobactin (*iuc*) gene cluster "mfs transporter–*iucA*–*iucB*–*iucC*–*iucD*–*iutA*–insertion sequence" was flanked upstream by IS*1A*. Similar to isolate OHB1S62KP, *bla*$_{\text{OXA-232}}$ was found on a separate ColKP3 (6,141 bp) plasmid with a conserved arrangement of mobilisation proteins (*bla*$_{\text{OXA-232}}$–hypothetical protein–*mbeB*–*MbeD*) and additional plasmid-associated proteins (Figure 5.10B).

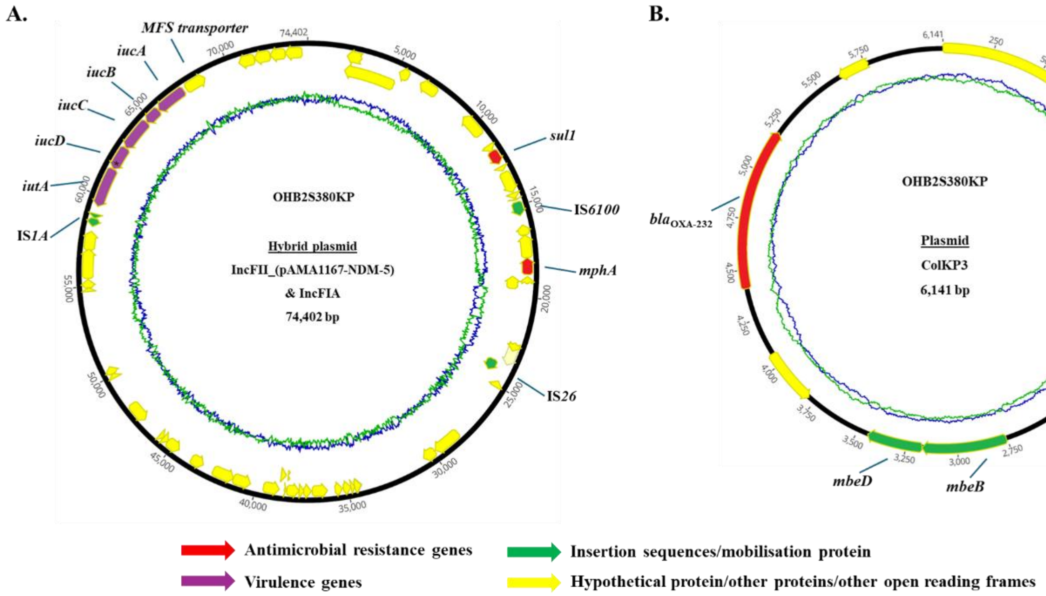


Figure 5.10 Schematic layout of plasmid (IncFII_(pAMA1167-NDM-5) + IncFIA) carrying virulence loci (A) and ColKP3 plasmid (B) carrying *bla*$_{\text{OXA-232}}$ in OHB2S380KP isolate. Arrows represent the position and transcriptional direction of the open reading frames. The figures were produced using Geneious Prime (v.2025.1).

Isolate OHB2S434KP (*K. pneumoniae* ST11) possessed virulence genes on an incomplete plasmid (83,044 bp) that contained the same conserved virulence segment "IS*630*–**–mfs transporter–*iucA*–*iucB*–*iucC*–*iucD*–*iutA*–**–*rmpD*–*rmpA2*–**–IS*110*", as observed in OHB1S62KP isolate (Figure 5.9A and 5.11A). In this isolate, the carbapenem resistance gene *bla*$_{\text{OXA-181}}$ was located on a complete circular hybrid plasmid (IncFIB(K) + IncFIB_pNDM-Mar; 99,277 bp), with *bla*$_{\text{OXA-181}}$ flanked downstream by IS*Ecp1* and upstream by IS*Kox1* (Figure 5.11B).

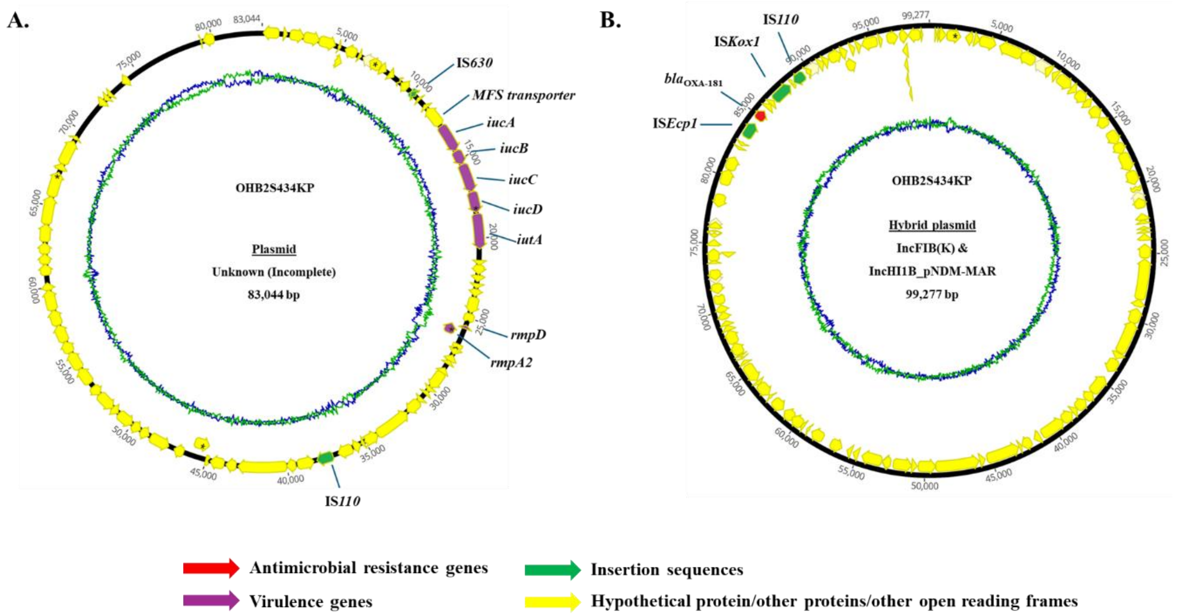


Figure 5.11 Schematic layout of plasmid carrying virulence loci (A) and plasmid IncFIB(K) + IncFIB_pNDM-Mar) (B) carrying *bla*$_{\text{OXA-181}}$ in OHB2S434KP isolate. Arrows represent the position and transcriptional direction of the open reading frames. The figures were produced using Geneious Prime (v.2025.1).

5.3 Discussion

Although hvKP is well known for causing fatal CAIs, it was historically susceptible to many antibiotics. However, reports from multiple regions have documented the emergence of 'convergent' hvKP and cKP lineages that couple MDR with enhanced virulence. Moreover, an increasing number of CR-hvKP are being detected, raising the prospect of a new clinical crisis. Despite the clinical importance of these developments, genomic epidemiological data of such strains from Bangladesh are very sparse. To expand our understanding of the genomics of hvKP and convergent strains in Mymensingh, this study performed WGS-based characterisation of 541 *K. pneumoniae* isolates collected within a One Health sampling framework.

The findings of this study revealed a clear separation between ubiquitous, chromosome-encoded core virulence loci and less frequent, mobile-element-encoded accessory virulence loci. Core determinants such as *entA*, *entB, fepC*, *ompA*, the ECP pilus locus (*yagV/W/X/Y/Z*, *ykgK*) were present in >95% of isolates (Table 5.1), consistent with a conserved pathogenic backbone that contributes to baseline pathogenicity (Holt *et al*., 2015; Paczosa & Mecsas, 2016; Lam *et al*., 2018; Hussain *et al*., 2023). In contrast, accessory virulent loci, especially siderophore systems (*ybt*, *iro*, *iuc*), genotoxin (*clb*) and genes related to hypercapsule production and hypermucoviscosity (*rmpA*, *rmpA2*) were infrequent and source dependent. The *ybt* was the dominant accessory siderophore (20.9%, 113/541) and was highly enriched in SSIs (65.6%), followed by UTIs (21.4%) and HRS (18.4%) strains and was less common in animals and environmental strains (Table 5.1 and 5.2). The prevalence of *ybt* was lower than the global reports (around 30 - 40%) but consistent with the inclusion of non-clinical sources (community, farm and environment) in my sampling frame (Lam *et al*., 2018). When stratified by source, the findings aligned with previous studies, which reported that >60% of clinical isolates from invasive infections carried *ybt*, consistent with its role in iron acquisition during tissue invasion (Palmieri *et al*., 2021; Jati *et al*., 2023). Plasmid-associated virulence genes, which are considered the hallmark of the hypervirulent pathotype, aerobactin operon (*iuc/iutA*), salmochelin (*iroB*) and *rmp* capsule regulators, were present at a lower prevalence (3.3 - 3.7%) (Shankar *et al*., 2021; Spadar *et al*., 2022; Russo *et al*., 2024). Colibactin was rarely found, only in 0.9% (5/541) of *K. pneumoniae,* and all were human-derived strains. The broader, multi-source sampling (including environmental and non-invasive sources) used in this study is likely to lessen the prevalence of accessory virulence compared with hospital-only cohorts (Palmieri *et al*., 2021; Hussain *et al*., 2023). Together, these findings suggest limited circulation of hvKP in Mymensingh, with accessory hypervirulence markers concentrated in invasive human disease (SSIs).

Based on Kaptive serotyping, a heterogeneous distribution of 56 K types (assigned to 56.2% isolates) and 10 O types (assigned to 92.6% isolates) was identified in *K. pneumoniae*. K3, K12, K30, K64 and K8 were commonly (>10 isolates) found K types, whereas O1ab (29.4%, 159/541), O3b (19.6%, 106/541) and O2afg (15.0%, 81/541) were the dominant O serotypes across the isolates from various sources (Figure 5.1 and 5.2). These findings are consistent with recent regional and global reports, where large Indian and multinational datasets described broad K serotype diversity and a handful of prevalent O serotypes, with O1/O2/O3/O5 accounting for around 80% of clinical infections, similar to O1/O2/O3 predominance identified in this study (Miller *et al*., 2024; Shamanna *et al*., 2024). A relatively high proportion of isolates (43.8%) had K-untyped genomes, which might be due to the ongoing expansion of the KL catalogue and technical constraints (e.g., fragmented assemblies), as emphasised in the latest Kaptive version (v.3.0) (Stanton *et al*., 2025). Data showed many different K-O pairings (overall 94 combinations) among *K. pneumoniae* isolates, suggesting frequent recombination and rearrangement that characterise the *K. pneumoniae* population (Figure 5.3) (Miller *et al*., 2024). A recent study from Bangladesh also reported O1 predominance with heterogeneous K profiles (K1, K2, K24, K30, K62, K64), providing further regional concordance with identified K and O type distribution (Kawser *et al*., 2025).

In the present study, 96.3% (521/541) of *K. pneumoniae*, including all isolates from water, CCS, CMS, DARS, BF, and UTIs, showed low Kleborate virulence potential (scored 0/1) (Table 5.3 and Figure 5.4). Out of 541 isolates, 20 (3.7 %) were hypervirulent (scored ≥ 3) and contained hypervirulent genetic determinants. This finding is consistent with the VFDB database findings of this study, where 3.7% isolates contained hypervirulence markers (*iuc/iutA* and *rmpA/rmpA2*). The low burden of hvKP aligns with recent hospital surveillance showing low hvKP proportions (3.7% in an Italian one-year screening, around 1.8% in a study from the USA) (Kochan *et al*., 2023; Merla *et al*., 2024). However, among the 20 highly virulent *K. pneumoniae* isolates, 12 (60%) were from SSIs, 7 (35%) were from HRS, and one was from flies (5%). This data suggests that hypervirulence-associated loci (*iuc/rmp*) concentrate in invasive clinical infections, including SSIs, rather than in non-invasive sources and environmental reservoirs (Zhao *et al*., 2020; Araújo *et al*., 2024). Notably, although flies have been shown to harbour AMR *K. pneumoniae*, to the best of my knowledge, there are no such prior reports confirming the presence of genomically defined hypervirulent genes in *K. pneumoniae* from flies (Poudel *et al*., 2019; Cook *et al*., 2025).

MLST profiling of the genotypically determined 20 hvKP strains identified nine STs (ST23, ST86, ST420, ST29, ST660, ST11, ST231, ST160 and ST2096), encompassing both hvKP and cKP lineages. All five hvKP belonged to the canonical hvKP lineage ST23:K1:O1ab scored 5 and harboured *ybt*, *iro*, *iuc, clb* and *rmp*. Other frequently found hvKP included ST86:K2:O1ab (n=5) and ST420:K20:O1ab (n=4) (Figure 5.5). Comparative analysis revealed a significant enrichment of K1, K2, and K20 capsule types among highly virulent isolates compared to low-virulent or cKP ($p<0.05$) (Table 5.4). O1ab was the predominant O type, occurring in 19 out of 20 highly virulent isolates (Figure 5.5). These findings accord with the global studies, linking K1, K2, K20 and O1 serotypes with hypervirulent lineages such as ST23, ST65, ST86 and ST420 (Zhang *et al*., 2016; Shankar *et al*., 2020; Wyres *et al*., 2020; Eger *et al*., 2021; Merla *et al*., 2024; Shamanna *et al*., 2024; Stanton & Wyres, 2024). Studies from Bangladesh also reported overlapping clones (ST11, ST23, ST29, ST231, ST420) and a predominance of K1, K2, and K20 with O1 among circulating high-risk/hvKP, corroborating the findings of this study (Hussain *et al*., 2023; Kawser *et al*., 2025). The only hvKP isolated from flies belonged to ST660:K16:O1ab, in line with the reports from China indicating that ST660:16 clinical isolates are strongly associated with hvKP (Zhang *et al*., 2016; Lu *et al*., 2018).

Core-genome SNP analysis of hvKP ST23 isolates identified two SSI-derived tight clusters with pairwise distances of 1 and 7 SNPs, well below commonly used transmission cut-offs for *K. pneumoniae* (≤10–25 SNPs), suggesting recent transmission or recent divergence. The remaining ST23 comparisons ranged from 69 to 200 SNPs, including one isolate (recovered from HRS) with 193–200 SNPs, which differed from all others, indicating separate introductions (Figure 5.5 and 5.6A). Comparable SNP spans have been documented for ST23-K1 clusters in recent surveillance (e.g., 0–38 SNPs in EU/EEA datasets) (ECDC, 2024; Luterbach *et al.*, 2025). *K. pneumoniae* ST86 recovered from HRS at various sampling sites, exhibited broad diversity, with the closest pair differing by 132 SNPs and the range extending up to 389 SNPs, indicating polyclonal circulation (Figure 5.5 and 5.6B). For ST420 (n=4), two independent clusters were evident, where one pair (recovered from SSIs) was core-genome identical (0 SNPs), indicating probable direct or near-direct transmission, while a second pair differed by 70 SNPs, showing that they might be distantly related. The differences of 169–185 SNPs between the clusters indicate separate lineages (Figure 5.5 and 5.6C). Previous reports of micro-clusters with two clonal ST420 isolates from patient samples also support the findings of this study (Eger *et al.*, 2021).

Out of 541 *K. pneumoniae*, the dominant STs (ST15, ST37, ST48, ST147 and ST490) identified in this study were MDR with low virulence, while ST86 and ST660 were highly virulent but low AMR lineages (Figure 5.7). Notably, ST29 was observed among both MDR and hypervirulent isolate groups. However, since no ST29 isolate concurrently harboured MDR and hypervirulence determinants, ST29 was not classified as a convergent lineage, consistent with definitions of convergence requiring co-localisation of AMR and virulence genes within the same isolate. Although no genomic evidence of convergence was observed in the current dataset, ST29 could represent a potential lineage at risk of future convergence, given its presence in both populations (Lam *et al.*, 2018; Wyres *et al.*, 2020). Only 2.0% (11/541) of isolates showed true convergence (virulence score ≥ 3 plus resistance score > 0), confined to *K. pneumoniae* from SSIs at MMCH and spanning ST23 (n = 4), ST420 (n = 3), and singletons ST11, ST160, ST231 and ST2096 (Figure 5.8). These findings are consistent with a recent large-scale genomic analysis of invasive *K. pneumoniae* from South and Southeast Asia, which reported that AMR-hypervirulence convergence remains overall infrequent but is disproportionately enriched among bloodstream and other invasive hospital isolates, often involving ST23 and high-risk MDR lineages acquiring virulence or resistance plasmids (Hinthong *et al.*, 2025). In this study, 55% (11/20) of the hvKP were convergent. All convergent strains carried *bla*$_{\text{CTX-M-15}}$, while three were CR-hvKP

(ST231/ST2096 with *bla*$_{\text{OXA-232}}$, ST11 with *bla*$_{\text{OXA-181}}$). These findings align with the well-described dichotomy between MDR cKP and hvKP lineages and their convergence, noting cKP/hvKP typically has low virulence/resistance unless it acquires AMR/virulence plasmids or other hybrid genetic elements (Wyres *et al*., 2020; Lan *et al*., 2021; Lei *et al*., 2024). Regionally, these results are consistent with recent studies from Bangladesh, reporting high ESBL burdens dominated by *bla*$_{\text{CTX-M-15}}$ and frequent OXA-48–like carbapenemases (*bla*$_{\text{OXA-181}}$ and *bla*$_{\text{OXA-232}}$) (Hussain *et al*., 2023; Kawser *et al*., 2025). In India and China, the expansion of OXA-232–associated ST231 and the emergence of ST2096 carrying hybrid virulence-resistance plasmids provide clear precedents for the convergent ST231/ST2096 findings of this study, underscoring the regional mobility of OXA-48-like determinants in *K. pneumoniae* convergent strains (Nagaraj *et al*., 2021; Shankar *et al*., 2022; Li *et al*., 2025). All the highly virulent isolates from HRS and flies showed lower resistance potential (resistance score 0), consistent with the broad observation that hvKP in non-hospital reservoirs tends to retain susceptibility and only sporadically acquires major AMR determinants. Contemporary cohort analyses confirm that hvKP (especially ST23/KL1) is generally less likely to acquire AMR than hospital lineages (Wyres *et al*., 2020; Tang *et al*., 2025). This strengthens the inference that our hypervirulent isolates from HRS or flies represent community-like hvKP rather than circulating hospital clones.

Considering the clinical importance of CR-hvKP*,* this study performed targeted genomic analysis of the three convergent isolates. All three CR-hvKP convergent strains belonged to MDR high-risk lineages (ST11, ST231 and ST2096), acquiring hypervirulence loci and β-lactamase genes via plasmid-mediated gene exchange. In OHB1S62KP (*K. pneumoniae* ST2096), a large IncFIB-hybrid (IncFIB_pNDM-Mar + IncFIB(K)Kpn3; ~304 kb) plasmid co-carried both virulence loci (*iucA/B/C/D/iutA* and *rmpA2*) and resistance loci (*bla*$_{\text{CTX-M-15}}$), while carbapenem resistance gene (*bla*$_{\text{OXA-232}}$) was encoded on a separate 6,141-bp ColKP3 plasmid (Figure 5.9). This finding is similar to the Indian *K. pneumoniae* ST2096 report, where aerobactin/*rmpA2* reside on IncFIB/IncHI1B (pNDM-MAR)-type “hybrid” virulence plasmids and OXA-232 sits on a small mobilisable ColKP3 backbone (Shankar *et al*., 2022). In OHB2S434KP (*K. pneumoniae* ST11), the hypervirulence determinant (*iucA/B/C/D/iutA*) module was present on an incomplete plasmid (~83 kb), while *bla*$_{\text{OXA-181}}$ localised to an IncFIB-hybrid replicon (~99 kb) with the hallmark IS*Ecp1*-linked genetic context, echoing the predominant OXA-181 mobilisation route (Figure 5.11) (Potron *et al*., 2011). Across OHB1S62KP (ST2096) and OHB2S434KP (ST11), the virulence region followed a conserved segment (mfs transporter–*iucA*–*iucB*–*iucC*–*iucD*–*iutA*–**–*rmpD*–*rmpA2*) flanked by IS*630* and IS*110*, which is consistent with recent descriptions of IS-bracketed aerobactin/*rmpA2* cassettes that facilitate capture and remodelling of hypervirulence loci on hybrid or mosaic plasmids (Kuzina *et al*., 2023; Shapovalova *et al*., 2024). OHB2S380KP (*K. pneumoniae* ST231) carried *iuc* inserted by IS*1A* (upstream) on a circular IncFII/IncFIA hybrid plasmid (~74 kb) that also encoded *sul1* with IS*6100* and a macrolide resistance determinant *mphA* with IS*26* (Figure 5.10). Again, *bla*$_{\text{OXA-232}}$ was on a ColKP3 plasmid as widely documented for ST231 outbreaks in India, China and elsewhere (Shankar *et al*., 2021; Chen *et al*., 2023; Spadar *et al*., 2023). These findings indicate that convergence seems to be driven primarily by plasmids exchanging genetic regions such as emerging "hybrid" hypervirulence–AMR plasmids, where IS-bracketed virulence cassettes and AMR genes integrate onto F-type backbones, rather than clonal expansion of a single lineage.

Although the analysis includes a limited number of convergent isolates (n=3), hybrid long- and short-read sequencing was employed to resolve plasmid structure and gene context rather than to infer sequence variation. While Oxford Nanopore reads have a higher per-base error rate, this is largely random and can be effectively corrected by Illumina-based polishing (Wick *et al*., 2017; De Maio *et al*., 2019). Long reads were used primarily to resolve plasmid circularity, repetitive insertion sequences and the physical linkage of virulence and resistance loci. Illumina-only assemblies would have been sufficient to identify virulence and resistance genes and assign plasmid replicons, but would likely have fragmented plasmid assemblies, limiting confidence in the localisation of virulence and resistance determinants to the same or distinct plasmids (Arredondo-Alonso *et al*., 2017). This limitation is particularly relevant for F-type hybrid plasmids enriched in mobile genetic elements. Hybrid assemblies, therefore, enabled robust structural inference at the isolate level.

This One Health WGS survey found a low prevalence of genotypically determined hvKP with high concentration in SSIs. Convergence was detected exclusively among SSI isolates, where all carried *bla*$_{\text{CTX-M-15}}$ and three were CR-hvKP. Core genome SNP analysis revealed small, closely related clusters, highlighting the risks of outbreaks and therapeutic resistance, particularly in hospital settings. However, canonical hvKP lineages were also recovered from healthy humans and flies, indicating potential community and environmental reservoirs. These findings emphasise the importance of SSI-focused infection prevention in hospitals, routine genomic surveillance with timely screening for hypervirulence markers and plasmid tracking to mitigate further dissemination.

Chapter 6

Investigating Genomic Epidemiology of Non-pneumoniae *Klebsiella* spp. Across the One Health Sectors in Bangladesh

6.1 Introduction

The *Klebsiella* genus comprises several ecologically diverse species found across humans, animals, plants, food chains, insects and environmental reservoirs such as soil and water. Taxonomically, the *K. pneumoniae* species complex (KpSC) includes *K. pneumoniae*, *K. quasipneumoniae*, and *K. variicola*, while the *K. oxytoca* species complex (KoSC) encompasses *K. oxytoca*, *K. michiganensis*, *K. pasteurii*, *K. grimontii*, and related taxa. Formerly classified as *Enterobacter aerogenes*, *K. aerogenes* is also placed within the genus *Klebsiella*. These *Klebsiella* spp. circulate at the human-animal-environment interface, with recent cross-sectoral genomic surveys underscoring their ubiquity and public health importance (Wyres *et al*., 2020; Yang *et al*., 2021; Thorpe *et al*., 2022; Prinzi & Moore, 2023; Rodríguez-Medina *et al*., 2024).

Within the *Klebsiella* genus, *K. pneumoniae* has received the most attention due to its significant global burden on healthcare settings and the spread of ESBL and carbapenemase resistance genes. It is recognised as a priority AMR threat by WHO (Abbas *et al*., 2024; WHO, 2024). However, increasing resolution from genome-based identification shows that the non-pneumoniae *Klebsiella* spp. are substantial contributors to disease and AMR dissemination. The non-pneumoniae *Klebsiella* are frequently misidentified by routine phenotypic or conventional methods, biasing epidemiology and masking species-specific risk profiles. They can carry mobile resistomes, such as ESBLs and globally important carbapenemases, including NDM, KPC, and OXA-48 (Farzana *et al*., 2020; Boyd *et al*., 2022; Campos-Madueno *et al*., 2022; Li *et al*., 2024; Tian *et al*., 2025). Within KpSC, *K. quasipneumoniae* and *K. variicola* are regularly recovered from clinical samples but are under-recognised at the species level (Rodrigues *et al*., 2018; Rodríguez-Medina *et al*., 2024; McAndrew *et al*., 2025; Ohno *et al*., 2025). The KoSC members, including *K. oxytoca*, *K. michiganensis*, *K. pasteurii* and *K. grimontii,* can carry the kleboxymycin gene cluster, which produces enterotoxins (tilimycin/tilivalline), believed to be involved in antibiotic-associated haemorrhagic colitis (Yang *et al*., 2021; Campos-Madueno *et al*., 2022; Maghembe *et al*., 2024; Osbelt *et al*., 2024). Besides KpSC and KoSC, *K. aerogenes* acts as a hospital-adapted opportunist that can cause bloodstream and other invasive infections (Wesevich *et al*., 2020; Guedes *et al*., 2024).

The *Klebsiella* spp. also cause diseases in animals. A large, multi-species genomic survey of isolates from diseased animals identified diverse *Klebsiella* spp. *K. variicola* has been associated with bovine mastitis, and the members of both KpSC and KoSC are also reported across food-animal production systems and food products, highlighting potential food-chain connections (Klaper *et al*., 2021; Giannattasio-Ferraz *et al*., 2022; Martin *et al*., 2025). Environmental reservoirs relevant to the transmission of non-pneumoniae *Klebsiella* spp. have been repeatedly documented. Surface water and hospital water systems (sinks, drains, toilets, etc.) have served as persistent sources for KoSC (e.g., *K. oxytoca*, *K. michiganensis*) outbreaks, and contaminated consumer products such as detergent dispensers have seeded neonatal colonisation events (Lowe *et al*., 2012; Chapman *et al*., 2020; Thorpe *et al*., 2022; Larsen *et al*., 2025).

In Bangladesh, most studies have focused on *K. pneumoniae* and HAIs (Farzana *et al*., 2020; Mahmud *et al*., 2022; Tanni *et al*., 2022; Hussain *et al*., 2023; Kawser *et al*., 2025). Although *K. quasipneumoniae*, *K. variicola*, and *K. aerogenes* have been reported in clinical infections in Bangladesh, the available data remain limited, particularly concerning cases beyond *K. pneumoniae* and the frequency of genomic characterisations (Farzana *et al*., 2019; Mazumder *et al*., 2023; Kawser *et al*., 2025). In addition to clinical settings, *K. oxytoca* has also been reported in poultry meats, while *K. quasipneumoniae* has been identified in the wastewater of a tertiary care hospital in Bangladesh (Ferdous *et al*., 2022; Tanni *et al*., 2025).

Considering existing scientific evidence regarding the public health importance of non-pneumoniae *Klebsiella* spp., this study aims to investigate the genomic epidemiology of these organisms across human, animal, and environmental sectors in Bangladesh. The specific objectives for this chapter are as follows:

1. To estimate the prevalence of non-pneumoniae *Klebsiella* spp. across human, animal and environmental samples in Mymensingh, Bangladesh.
2. To determine the AMR and virulence profiles of non-pneumoniae *Klebsiella* spp. at the human-animal-environment interface.
3. To determine the clonality of *K. quasipneumoniae* and *K. variicola* in the study area.

6.2 Results

6.2.1 Prevalence of non-pneumoniae *Klebsiella* spp. in the study area

A total of 3759 samples were collected from nine ecological sources (water, flies, CCS, CMS, DARS, BF, SSIs, UTIs and HRS). Culture-based isolation, followed by WGS, identified eight distinct *Klebsiella* spp. among the positives: *K. pneumoniae*, *K. quasipneumoniae*, *K. variicola*, *K. michiganensis*, *K. aerogenes*, *K. pasteurii*, *K. oxytoca* and *K. grimontii* (Figure 6.1).

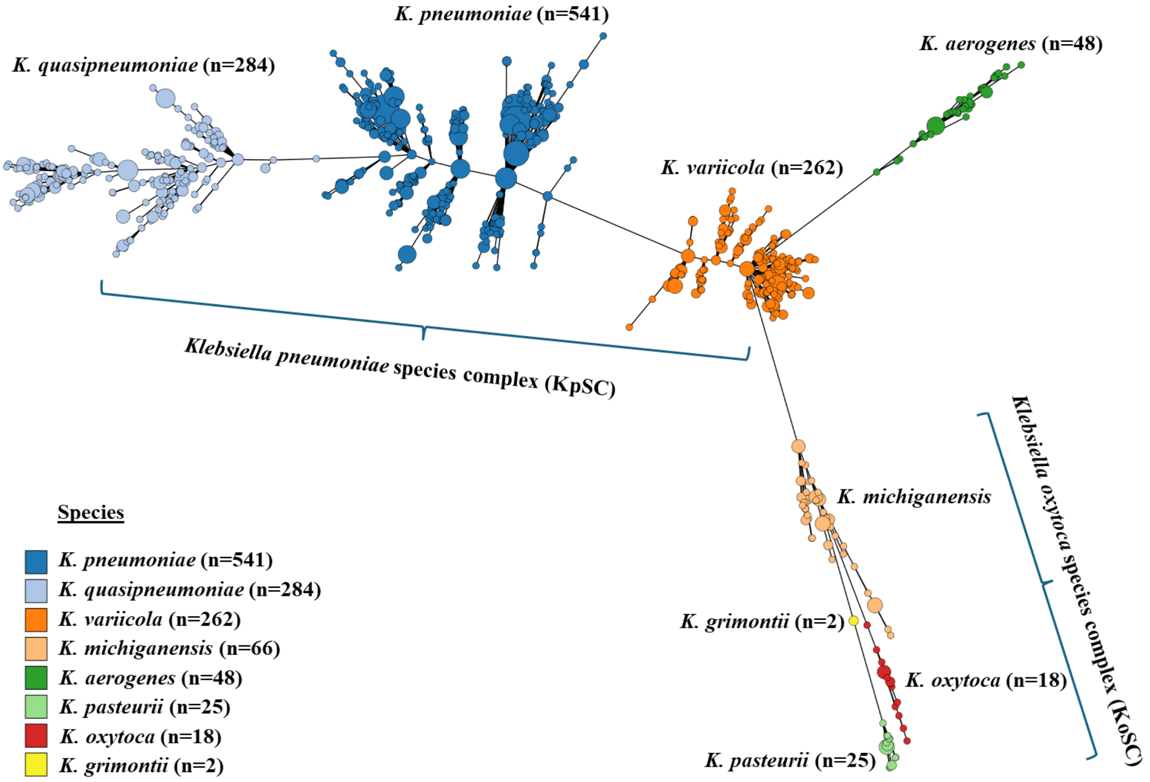
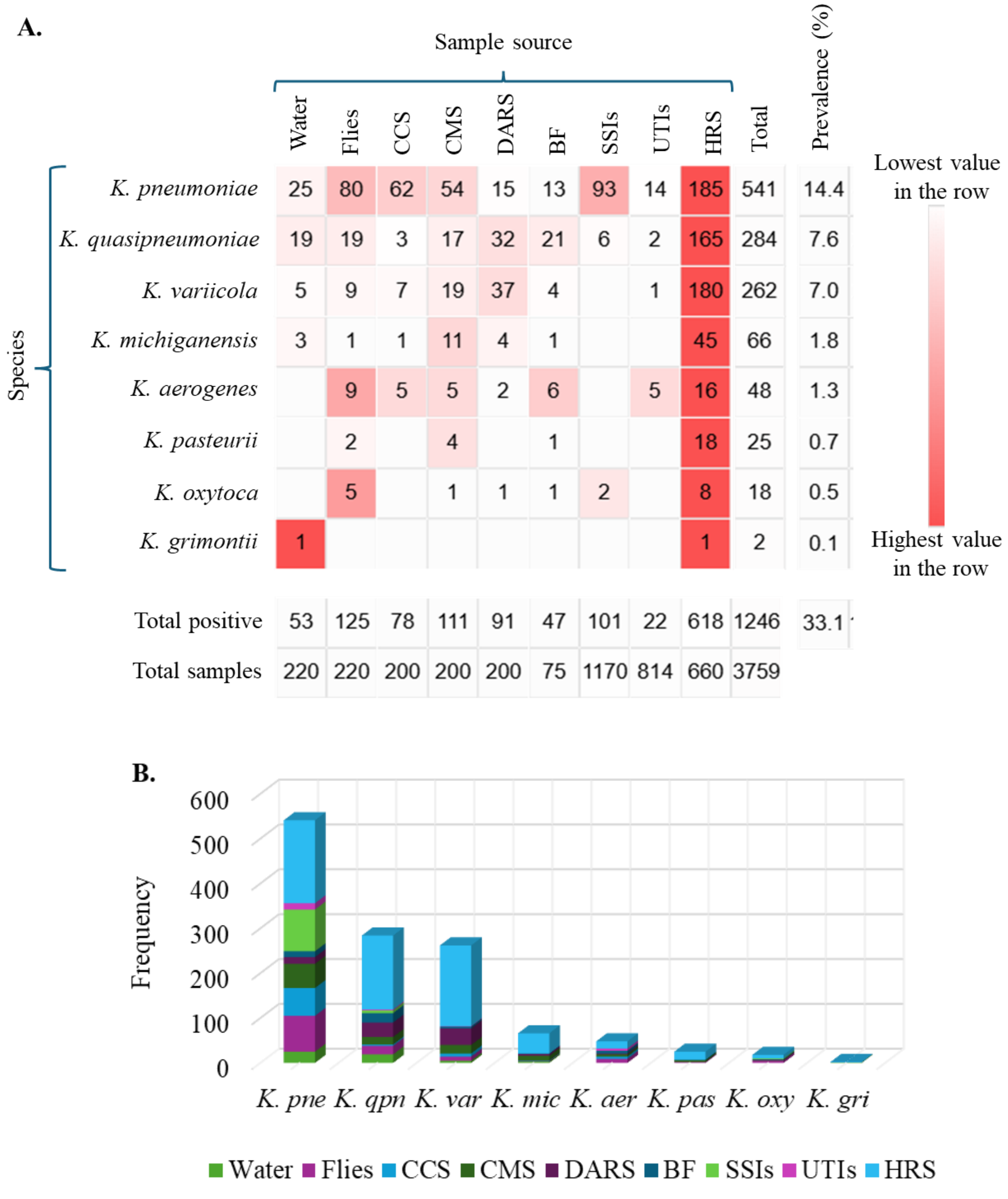


Figure 6.1 Minimum spanning tree showing the distribution of *Klebsiella* spp. identified in this study. This minimum spanning tree was constructed from core genes and annotated using the GrapeTree (Zhou *et al.*, 2018). Node colours correspond to individual species.

The overall prevalence of *Klebsiella* spp. from different samples was 33.1% (1246/3759), as detailed in Figure 6.2. Among the *Klebsiella* spp., the most frequently isolated species was *K. pneumoniae* (14.4%, 541/3759) and constituted 43.4% (541/1246) of all *Klebsiella* isolates (a detailed analysis of *K. pneumoniae* has been discussed in Chapters 3, 4 and 5). In addition to *K. pneumoniae* within the KpSC, *K. quasipneumoniae* and *K. variicola* showed prevalences of 7.6% (284/3759) and 7.0% (262/3759), respectively. The members of KoSC were less commonly found, with the prevalence of *K. michiganensis* at 1.8% (66/3759), followed by *K. pasteurii* at 0.7% (25/3759), *K. oxytoca* at 0.5% (18/3759), and *K. grimontii* at 0.1% (2/3759). The overall prevalence of *K. aerogenes* was 1.3% (48/3759). Non-pneumoniae *Klebsiella* spp. together comprised 56.6% (705/1246) of all *Klebsiella* isolates.

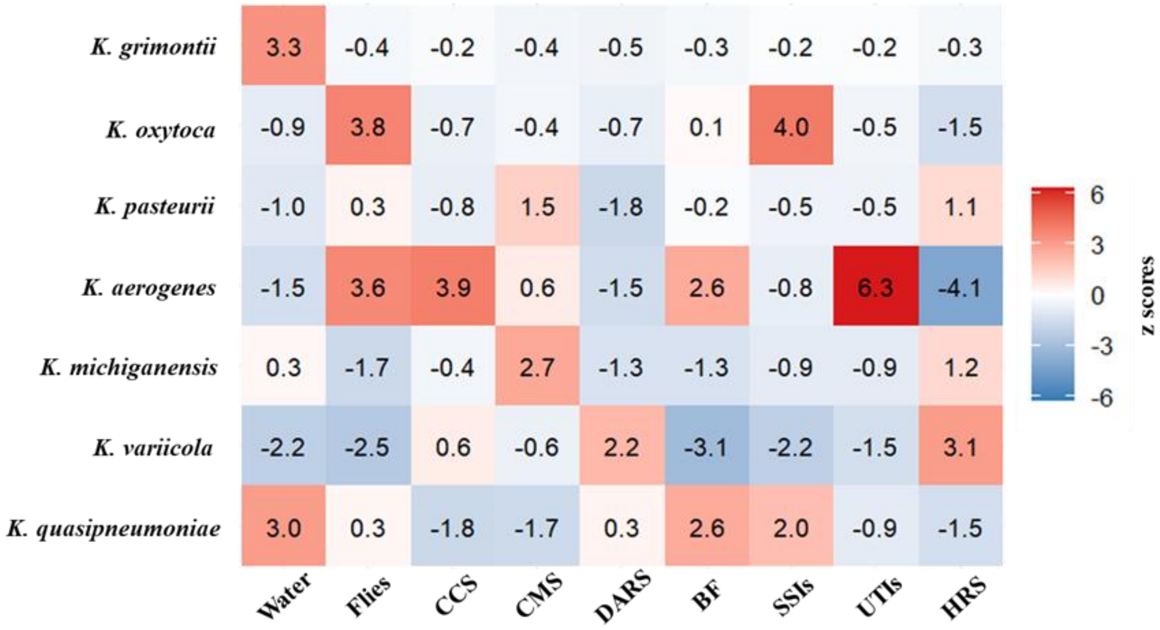


Species	Water	Flies	CCS	CMS	DARS	BF	SSIs	UTIs	HRS	Total	Prevalence (%)
K. pneumoniae	25	80	62	54	15	13	93	14	185	541	14.4
K. quasipneumoniae	19	19	3	17	32	21	6	2	165	284	7.6
K. variicola	5	9	7	19	37	4		1	180	262	7.0
K. michiganensis	3	1	1	11	4	1			45	66	1.8
K. aerogenes		9	5	5	2	6		5	16	48	1.3
K. pasteurii		2		4		1			18	25	0.7
K. oxytoca		5		1	1	1	2		8	18	0.5
K. grimontii	1								1	2	0.1
Total positive	53	125	78	111	91	47	101	22	618	1246	33.1
Total samples	220	220	200	200	200	75	1170	814	660	3759	

Figure 6.2 Prevalence and distribution of *Klebsiella* spp. across various sample sources. **A.** Heatmap shows the distribution of *Klebsiella* spp. isolates by species and sources. Rows represent species, and

columns represent sample sources. The colour gradient from white to red indicates the lowest to the highest values in each row. Blank cell indicates zero positive isolates. The rows at the bottom show the total number of positive isolates and the total number of samples of that source. Species-specific prevalence (%) was calculated as the total number of specific *Klebsiella* species divided by the total number of samples collected, then multiplied by 100. **B.** The stacked bar chart shows the frequency of *Klebsiella* spp. within each sample type. *K. pne*, *K. pneumoniae*; *K. qpn*, *K. quasipneumoniae*; *K. var*, *K. variicola*; *K. mic*, *K. michiganensis*; *K. aer*, K. aerogenes; *K. pas*, *K. pasteurii*; *K. oxy*, *K. oxytoca*; *K. gri*, *K. grimontii*; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs.

A chi-square test demonstrated a significant association between several non-pneumoniae *Klebsiella* spp. and sample sources (χ^2 (48) = 180.5, $p = 3.1 \times 10^{-17}$; Cramér's V = 0.21) (Figure 6.3). The heatmap shows that *K. quasipneumoniae* (z = 3.0) and *K. grimontii* (z = 3.3) were enriched in water. *K. variicola* was over-represented in HRS (z = 3.1) and under-represented in BF (z = −3.1). *K. aerogenes* was significantly over-represented in UTIs (z = 6.3) and also enriched in CCS (z = 3.9) and Flies (z = 3.6) but under-represented in HRS (z = −4.1). *K. oxytoca* was enriched in SSIs (z = 4.0) and Flies (z = 3.8). Other cells were close to expectation. The remaining source species combinations exhibited either slight enrichment (positive values) or depletion (negative values), but none reached the threshold value (z-score of ≥ 3).



	Water	Flies	CCS	CMS	DARS	BF	SSIs	UTIs	HRS
K. grimontii	3.3	-0.4	-0.2	-0.4	-0.5	-0.3	-0.2	-0.2	-0.3
K. oxytoca	-0.9	3.8	-0.7	-0.4	-0.7	0.1	4.0	-0.5	-1.5
K. pasteurii	-1.0	0.3	-0.8	1.5	-1.8	-0.2	-0.5	-0.5	1.1
K. aerogenes	-1.5	3.6	3.9	0.6	-1.5	2.6	-0.8	6.3	-4.1
K. michiganensis	0.3	-1.7	-0.4	2.7	-1.3	-1.3	-0.9	-0.9	1.2
K. variicola	-2.2	-2.5	0.6	-0.6	2.2	-3.1	-2.2	-1.5	3.1
K. quasipneumoniae	3.0	0.3	-1.8	-1.7	0.3	2.6	2.0	-0.9	-1.5

Figure 6.3 Association between non-pneumoniae *Klebsiella* spp. and sample sources (standardised residuals). Heatmap of standardised Pearson residuals from a chi-square test of independence for the 7×9 contingency table of non-pneumoniae *Klebsiella* spp. by sample sources. Numbers in the cells represent residual z-scores, and the colour bar encodes magnitude and sign (red indicates more than expected, blue indicates less than expected, and white indicates approximately expected). Overall association: χ^2 (48) = 180.5, $p = 3.1\times10^{-17}$; Cramér's V = 0.21 (small–moderate effect). Cells with a z-score of ≥ 3 indicate a strong association. CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs.

6.2.2 AMR and virulence profiles of non-pneumoniae *Klebsiella* spp.

6.2.2.1 Antibiogram of non-pneumoniae *Klebsiella* spp.

All non-pneumoniae *Klebsiella* spp. (n=705) isolated from various sources were subjected to AST against 16 clinically relevant antibiotics using the agar dilution method. The antibiogram study revealed that out of 284 *K. quasipneumoniae*, 140 (49.3%) showed resistance to fosfomycin, followed by levofloxacin (20.8%, 59/284), ciprofloxacin (15.1%, 43/284) and amoxicillin–clavulanic acid (14.8%, 42/284). Low resistance was observed to cephalosporins (cefotaxime 7.0%, 20/284; ceftazidime 10.2%, 29/284; cefepime 5.6%, 16/284), aztreonam (6.3%, 18/284), piperacillin–tazobactam (1.1%, 3/284), tigecycline (4.6%, 13/284), colistin (3.5%, 10/284) and aminoglycosides (amikacin 0.7%, 2/284; gentamicin 0.7%, 2/284). All *K. quasipneumoniae* from different sources were susceptible to ceftazidime–avibactam, imipenem and meropenem (Table 6.1). The resistance burden varied by source, where 50% or more of *K. quasipneumoniae* recovered from SSIs (n=6) and UTIs (n=2) showed resistance to amoxicillin–clavulanic acid, cefotaxime, ceftazidime, cefepime, ciprofloxacin, levofloxacin and fosfomycin. A small subset (n=3) of *K. quasipneumoniae* from CCS showed 100% resistance to ciprofloxacin, levofloxacin, fosfomycin and tigecycline. *K. quasipneumoniae* recovered from water (n=19) and flies (n=19) showed high resistance to fosfomycin at 68.4% and 42.1%, respectively (Table 6.1).

Table 6.1 Antibiotic susceptibility patterns of *K. quasipneumoniae* (n=284) isolated from various samples.

Attributes	**Resistance to respective antibiotics, n (%)**															
Sample sources	**AMC**	**TZP**	**CTX**	**CAZ**	**FEP**	**CZA**	**ATM**	**IPM**	**MEM**	**CIP**	**LVX**	**AMK**	**GEN**	**FOS**	**TGC**	**CST**
Water (n=19)	4 (21.1)	1 (5.3)	2 (10.5)	3 (15.8)	2 (10.5)	0 (0)	2 (10.5)	0 (0)	0 (0)	7 (36.8)	7 (36.8)	1 (5.3)	0 (0)	13 (68.4)	2 (10.5)	1 (5.3)
Flies (n=19)	2 (10.5)	0 (0)	1 (5.3)	1 (5.3)	1 (5.3)	0 (0)	1 (5.3)	0 (0)	0 (0)	5 (26.3)	4 (21.1)	0 (0)	0 (0)	8 (42.1)	0 (0)	2 (10.5)
CCS (n=3)	1 (33.3)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (100)	3 (100)	1 (33.3)	1 (33.3)	3 (100)	3 (100)	1 (33.3)
CMS (n=17)	4 (23.5)	1 (5.9)	1 (5.9)	3 (17.6)	1 (5.9)	0 (0)	1 (5.9)	0 (0)	0 (0)	6 (35.3)	6 (35.3)	0 (0)	0 (0)	13 (76.5)	3 (17.6)	1 (5.9)
DARS (n=32)	1 (3.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6.3)	2 (6.3)	0 (0)	0 (0)	18 (56.3)	0 (0)	2 (6.3)
BF (n=21)	3 (14.3)	0 (0)	0 (0)	2 (9.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.8)	1 (4.8)	0 (0)	0 (0)	3 (14.3)	0 (0)	1 (4.8)
SSIs (n=6)	3 (50.0)	0 (0)	4 (66.7)	4 (66.7)	5 (83.3)	0 (0)	4 (66.7)	0 (0)	0 (0)	3 (50.0)	3 (50.0)	0 (0)	1 (16.7)	6 (100)	1 (16.7)	0 (0)
UTIs (n=2)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50.0)	1 (50.0)	0 (0)	0 (0)	1 (50.0)	1 (50.0)	0 (0)
HRS (n=165)	23 (13.9)	0 (0)	11 (6.7)	14 (8.5)	6 (3.6)	0 (0)	10 (6.1)	0 (0)	0 (0)	15 (9.1)	32 (19.4)	0 (0)	0 (0)	75 (45.5)	3 (1.8)	2 (1.2)
Overall (n=284)	**42 (14.8)**	**3 (1.1)**	**20 (7.0)**	**29 (10.2)**	**16 (5.6)**	**0 (0)**	**18 (6.3)**	**0 (0)**	**0 (0)**	**43 (15.1)**	**59 (20.8)**	**2 (0.7)**	**2 (0.7)**	**140 (49.3)**	**13 (4.6)**	**10 (3.5)**

Values in parentheses indicate row percentage. The heatmap was generated according to the resistance percentage. N, number; AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime–avibactam; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin; AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline; CST, Colistin. The colour scale from white to red indicates the lowest to the highest resistance percentage in a row.

Among 262 *K. variicola* isolates, the overall resistance was the highest to fosfomycin (46.2%, 121/262), whereas overall resistance to other antibiotics remained low: amoxicillin–clavulanic acid (7.3%, 19/262), levofloxacin (6.9%, 18/262), ciprofloxacin (1.9%, 5/262), cefotaxime (1.1%, 3/262), ceftazidime (0.8%, 2/262), cefepime (0.8%, 2/262), aztreonam (0.8%, 2/262), piperacillin–tazobactam (0.4%, 1/262), gentamicin (0.8%, 2/262), tigecycline (1.9%, 5/262) and colistin (4.6%, 12/262). No resistance was detected to ceftazidime–avibactam, imipenem, meropenem and amikacin. Source-specific analysis revealed higher resistance to fosfomycin in isolates from water (80.0%, 4/5), CMS (68.4%, 13/19), BF (50.0%, 2/4), DARS (43.2%, 16/37), HRS (45.0%, 81/180) and flies (33.3%, 3/9). Colistin resistance was found in *K. variicola* from flies (44.4%, 4/9), CCS (28.6%, 2/7), BF (25%, 1/4), water (20%, 1/5), CMS (10.5%, 2/19) and DARS (5.4%, 2/37), while absent in HRS and UTI isolates. Resistance to ciprofloxacin and levofloxacin was concentrated in CCS (42.9% for each), whereas isolates from other sources showed little to no resistance (Table 6.2).

Given the lower number of isolates across different sample types from KoSC members (*K. michiganensis*, *K. pasteurii*, *K. oxytoca*, and *K. grimontii*) and *K. aerogenes*, Table 6.3 presents only the overall resistance patterns. Across the five species, fosfomycin showed an overall high resistance burden, particularly in *K. pasteurii* (52.0%, 13/25), *K. aerogenes* (41.7%, 20/48), and *K. michiganensis* (40.9%, 27/66). However, a low resistance rate was found in *K. oxytoca* (16.7%, 3/18), with no instances of resistance in *K. grimontii*. Fluoroquinolone resistance was observed in *K. aerogenes* (ciprofloxacin 16.7%, 8/48; levofloxacin 14.6%, 7/48), followed by *K. pasteurii* (12.0% for both, 3/25), *K. oxytoca* (11.1% for both, 2/18) and *K. michiganensis* (ciprofloxacin 7.6%, 5/66; levofloxacin 6.1%, 4/66). *K. aerogenes* demonstrated a striking amoxicillin–clavulanic acid resistance (83.3%, 40/48), while β-lactam resistance in KoSC members was low (≤4.0%) for amoxicillin–clavulanic acid; cefotaxime/ceftazidime/cefepime all 0%). Resistance to aztreonam was detected only in *K. oxytoca* (5.6%, 1/18). Gentamicin resistance was observed in *K. aerogenes* (10.4%, 5/48), *K. oxytoca* (11.1%, 2/18), and *K. michiganensis* (2/66, 3.0%), but absent in *K. pasteurii* and *K. grimontii*. The overall resistance to tigecycline was low, with the highest rate observed in *K. aerogenes* (12.5%, 6/48).

Table 6.2 Antibiotic susceptibility patterns of *K. variicola* (n=262) isolated from various samples.

Attributes	**Resistance to respective antibiotics, n (%)**															
Sample sources	**AMC**	**TZP**	**CTX**	**CAZ**	**FEP**	**CZA**	**ATM**	**IPM**	**MEM**	**CIP**	**LVX**	**AMK**	**GEN**	**FOS**	**TGC**	**CST**
Water (n=5)	1 (20.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	4 (80.0)	0 (0)	1 (20.0)
Flies (n=9)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	3 (33.3)	1 (11.1)	4 (44.4)
CCS (n=7)	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (42.9)	3 (42.9)	0 (0)	2 (28.6)	2 (28.6)	2 (28.6)	2 (28.6)
CMS (n=19)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (68.4)	0 (0)	2 (10.5)
DARS (n=37)	2 (5.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16 (43.2)	0 (0)	2 (5.4)
BF (n=4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50.0)	0 (0)	1 (25.0)
UTIs (n=1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
HRS (n=180)	14 (7.8)	1 (0.6)	3 (1.7)	2 (1.1)	2 (1.1)	0 (0)	2 (1.1)	0 (0)	0 (0)	2 (1.1)	14 (7.8)	0 (0)	0 (0)	81 (45.0)	2 (1.1)	0 (0)
Overall (n=262)	**19 (7.3)**	**1 (0.4)**	**3 (1.1)**	**2 (0.8)**	**2 (0.8)**	**0 (0)**	**2 (0.8)**	**0 (0)**	**0 (0)**	**5 (1.9)**	**18 (6.9)**	**0 (0)**	**2 (0.8)**	**121 (46.2)**	**5 (1.9)**	**12 (4.6)**

Values in parentheses indicate row percentage. The heatmap was generated according to the resistance percentage. N, number; AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime–avibactam; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin; AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline; CST, Colistin. The colour scale from white to red indicates the lowest to the highest resistance percentage in a row.

Table 6.3 Antibiotic susceptibility patterns of members of KoSC (*K. michiganensis*, *K. pasteurii*, *K. oxytoca* and *K. grimontii*) and *K. aerogenes*.

Attributes	**Resistance to respective antibiotics, n (%)**															
Species	**AMC**	**TZP**	**CTX**	**CAZ**	**FEP**	**CZA**	**ATM**	**IPM**	**MEM**	**CIP**	**LVX**	**AMK**	**GEN**	**FOS**	**TGC**	**CST**
K. michiganensis (n=66)	2 (3.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (7.6)	4 (6.1)	0 (0)	2 (3.0)	27 (40.9)	1 (1.5)	0 (0)
K. pasteurii (n=25)	1 (4.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (12.0)	3 (12.0)	0 (0)	0 (0)	13 (52.0)	1 (4.0)	0 (0)
K. oxytoca (n=18)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.6)	0 (0)	0 (0)	2 (11.1)	2 (11.1)	0 (0)	2 (11.1)	3 (16.7)	0 (0)	0 (0)
K. grimontii (n=2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
K. aerogenes (n=48)	40 (83.3)	0 (0)	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (16.7)	7 (14.6)	0 (0)	5 (10.4)	20 (41.7)	6 (12.5)	0 (0)

Values in parentheses indicate row percentage. The heatmap was generated according to the resistance percentage. N, number; AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime–avibactam; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin; AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline; CST, Colistin. The colour scale from white to red indicates the lowest to the highest resistance percentage in a row.

6.2.2.2 Genomic AMR determinants and Kleborate-derived resistance scores of non-pneumoniae *Klebsiella* spp.

All non-pneumoniae *Klebsiella* spp. isolates were sequenced using the Illumina (NovaSeq 6000) platform. Based on sequencing results, ARGs and virulence genes were identified using the latest ABRicate and VFDB databases, respectively. Kleborate (v.3.0) was also used to determine virulence and resistance profiles (Wyres *et al.,* 2016; Lam *et al.,* 2021).

Across nine sample sources, *K. quasipneumoniae* (n=284) carried a broad panel of ARGs (Figure 6.4). Among β-lactamases, *bla*$_{\text{OKP-B-16}}$ (n=34), *bla*$_{\text{OKP-A-5}}$ (n=30) and *bla*$_{\text{TEM-1B}}$ (n=16) were commonly found across sources, whereas *bla*$_{\text{CTX–M–15}}$ (n=17) was the most predominant ESBL gene. The ESBL *bla*$_{\text{CTX–M–15}}$ was more frequently present in HRS (n=9) with additional detections in water, flies, CMS and UTIs. Another ESBL *bla*$_{\text{CTX–M–55}}$ was rare (1 in SSIs), and a single carbapenemase *bla*$_{\text{OXA–181}}$ was identified in an isolate recovered from water. Regarding aminoglycoside resistance, *aph(6)–Id* (n=12), *aph(3")–Ib* (n=12), *aadA2* (n=7) were the commonly identified genes. The fluoroquinolone resistance genes *oqxA/oqxB* were the most common and detected in all sample types and especially enriched in HRS (158 each). They also showed high counts in isolates from DARS (30/29), BF (21/21), water (17/17) and flies (15/15). The acquired quinolone resistance gene was mainly *qnrS1* (n=28; HRS=8) while *qnrB1* was rare (n=4). Fosfomycin resistance gene *fosA* was also common (n=188), predominantly found in HRS (n=111) and present in flies (n=16), water (n=10), DARS (n=22), and BF (n=14). Trimethoprim/sulfonamide resistance markers (*dfrA1*, n=12; *sul1*, n=22 and *sul2*, n=21) were also found in some isolates. The *tet(A)* (n=29) was the most frequently identified tetracycline resistance gene. For colistin resistance, *mcr–8* (n=4) was identified in *K. quasipneumoniae* recovered from water, CCS, CMS and HRS.

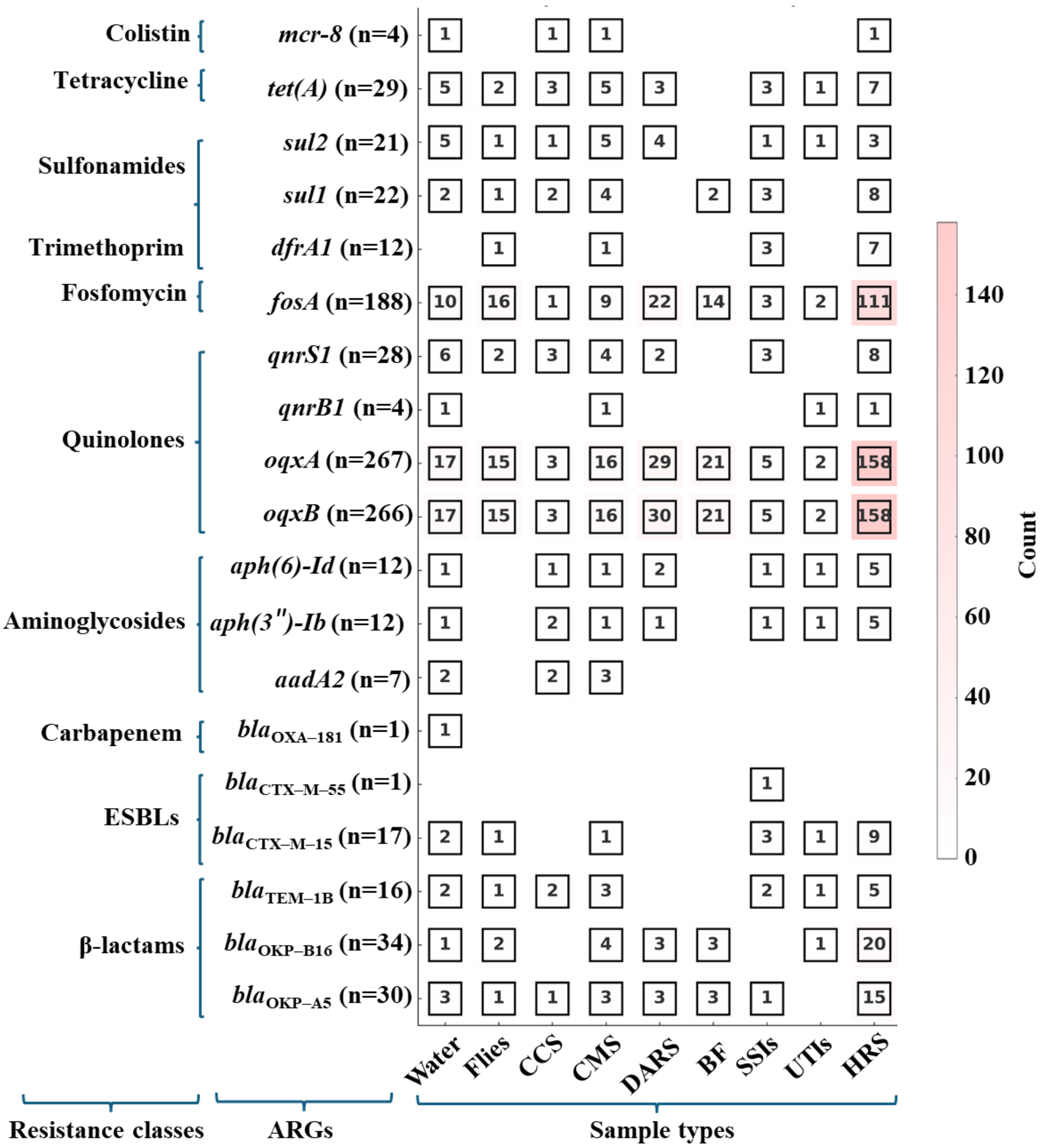


Figure 6.4 Distribution of ARGs in *K. quasipneumoniae* across various sources. The colour scale from white to red indicates the lowest to the highest resistance frequency. n, number; ARGs, antibiotic resistance genes; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs.

Resistome analysis of *K. variicola* revealed a predominance of intrinsic determinants, mainly *fosA* (n=261), *oqxA/oqxB* (n=259/n=260), and the species-specific *bla*$_{\mathrm{LEN}}$ β-lactamases (*bla*$_{\mathrm{LEN16}}$, n=99; *bla*$_{\mathrm{LEN24}}$, n=84; *bla*$_{\mathrm{LEN2}}$, n=39; *bla*$_{\mathrm{LEN17}}$, n=16; other *bla*$_{\mathrm{LEN}}$ alleles ≤8) (Figure 6.5). Non-LEN β-lactamases were uncommon (*bla*$_{\mathrm{SHV\text{-}187}}$, n=2; *bla*$_{\mathrm{OKP\text{-}B\text{-}8}}$, *bla*$_{\mathrm{SED\text{-}1}}$, *bla*$_{\mathrm{ACT\text{-}6}}$, *bla*$_{\mathrm{ACT\text{-}12}}$, *bla*$_{\mathrm{MAL\text{-}1}}$ each with frequency of 1). ARGs including the ESBL *bla*$_{\mathrm{CTX\text{–}M\text{-}15}}$ (n=1), *qnrS1* (n=1), *tet(D)* (n=1), *sul2* (n=1), and *aph(3')-Ia* (n=1) were infrequently identified in *K. variicola*.

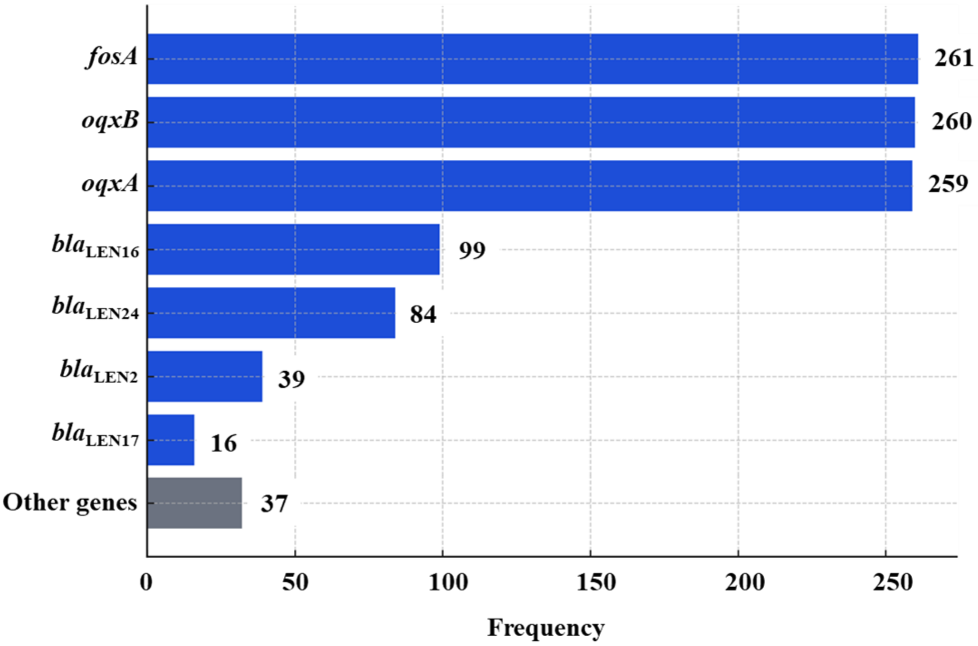


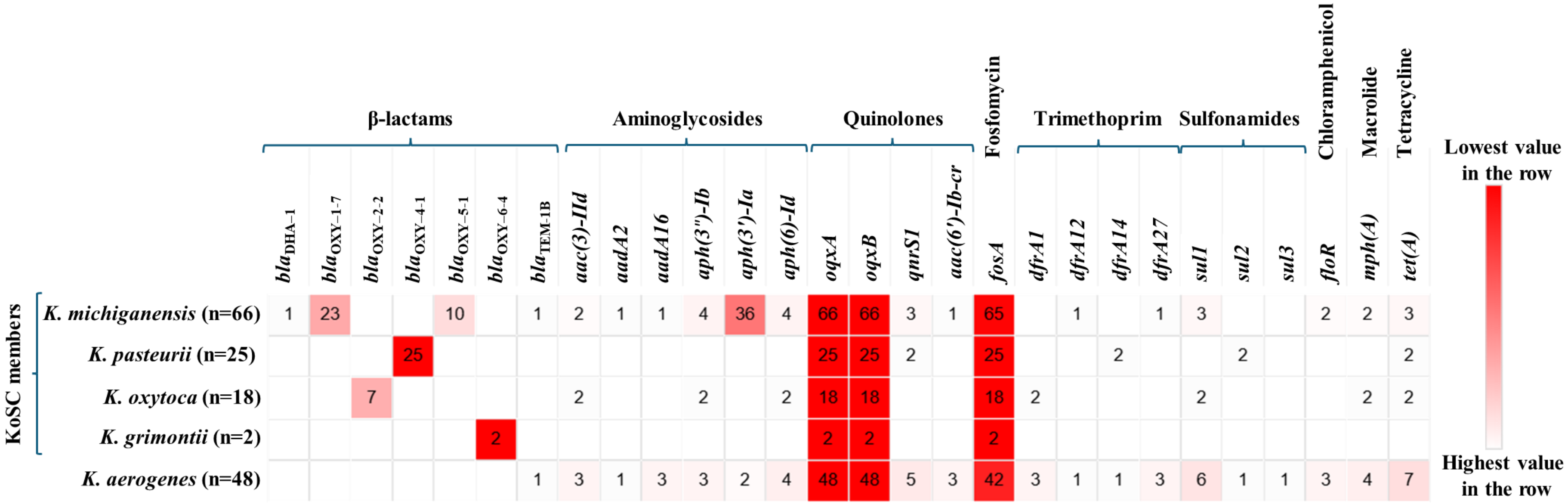
Figure 6.5 Distribution of ARGs in *K. variicola*. The top seven genes are plotted individually. The other genes bar pools the remaining alleles (*bla*$_{\mathrm{CTX\text{–}M\text{–}15}}$, *bla*$_{\mathrm{LEN18}}$, *bla*$_{\mathrm{LEN19}}$, *bla*$_{\mathrm{LEN20}}$, *bla*$_{\mathrm{LEN21}}$, *bla*$_{\mathrm{LEN22}}$, *bla*$_{\mathrm{LEN25}}$, *bla*$_{\mathrm{SHV\text{-}187}}$, *bla*$_{\mathrm{OKP\text{-}B\text{-}8}}$, *bla*$_{\mathrm{SED\text{-}1}}$, *bla*$_{\mathrm{ACT\text{-}6}}$, *bla*$_{\mathrm{ACT\text{-}12}}$, *bla*$_{\mathrm{MAL\text{-}1,}}$ *mdf(A)*, *qnrS1*, *tet(D)*, *sul2*, and *aph(3')-Ia*).

Among the members of KoSC (*K. michiganensis, K. oxytoca, K. pasteurii, K. grimontii*) and *K. aerogenes*, different ARG profiles were identified. Across species, *oqxAB* and *fosA* were commonly found, while β-lactam, aminoglycoside, fluoroquinolone, tetracycline, and phenicol resistance determinants varied in frequency (Figure 6.6).

In *K. michiganensis* (n=66), *oqxA/oqxB* were present in all isolates and *fosA* in 65 out of 66 (98.5%). Species-associated β-lactamases were dominated by *bla*$_{\text{OXY-1-7}}$ (23/66, 34.8%) and *bla*$_{\text{OXY-5-1}}$ (10/66, 15.2%), with infrequent *bla*$_{\text{DHA-1}}$ and *bla*$_{\text{TEM-1B}}$ (each 1/66, 1.5%). Aminoglycoside resistance determinants were led by *aph(3')-Ia* (36/66, 54.5%), with *aph(3")-Ib* and *aph(6)-Id* (each 4/66, 6.1%) and *aac(3)-IId/aadA2/aadA16* (≤3.0%) were less common. Quinolone resistance genes *qnrS1* (3/66, 4.5%) and *aac(6')-Ib-cr* (1/66, 1.5%) were detected infrequently. Other resistance determinants were detected at low levels: *tet(A)* (3/66, 4.5%), *mph(A)* (2/66, 3.0%), *floR* (2/66, 3.0%), *sul1* (3/66, 4.5%), and *dfrA12/dfrA27* (1/66 each, 1.5%).

In *K. pasteurii* (n=25), *bla*$_{\text{OXY-4-1}}$, *oqxA/oqxB*, and *fosA* were found in all isolates (25/25, 100%). Low-frequency genes (each 2/25, 8.0%) included *qnrS1*, *tet(A)*, *mph(A)*, *sul2*, and *dfrA14*. In *K. oxytoca* (n=18), *oqxA/oqxB* and *aac(6')-Ib-cr* were found in all isolates (18/18, 100%). β-lactamase *bla*$_{\text{OXY-2-2}}$, occurred in 38.9% (7/18). Several genes (*aac(3)-IId*, *fosA*, *tet(A)*, *mph(A)*, *sul1*, and *dfrA1*) were detected at 11.1% (2/18) each. In *K. grimontii* (n=2), both isolates carried only *bla*$_{\text{OXY-6-4}}$, *oqxA/oqxB*, and *fosA* (2/2, 100%).

In *K. aerogenes* (n=48), *oqxA/oqxB* (48/48) and *fosA* (42/48, 87.5%) were the most commonly identified ARGs. β-lactamase *bla*$_{\text{TEM-1B}}$ was rarely found (1/48, 2.1%). Aminoglycoside resistance genes were diverse and less common including *aph(6)-Id* (4/48, 8.3%), *aac(3)-IId/aph(3")-Ib/aadA16* (3/48 each, 6.2%), *aph(3')-Ia* (2/48, 4.2%), *aadA2* (1/48, 2.1%). Quinolone resistance determinants were *qnrS1* (5/48, 10.4%) and *aac(6')-Ib-cr* (3/48, 6.2%). Other resistance genes occurred sporadically: *tet(A)* (7/48, 14.6%), *mph(A)* (4/48, 8.3%), *floR* (3/48, 6.2%), *sul1* (6/48, 12.5%), *sul2/sul3* (1/48 each, 2.1%), *dfrA1/dfrA27* (3/48 each, 6.2%) and *dfrA12/dfrA14* (1/48 each, 2.1%).



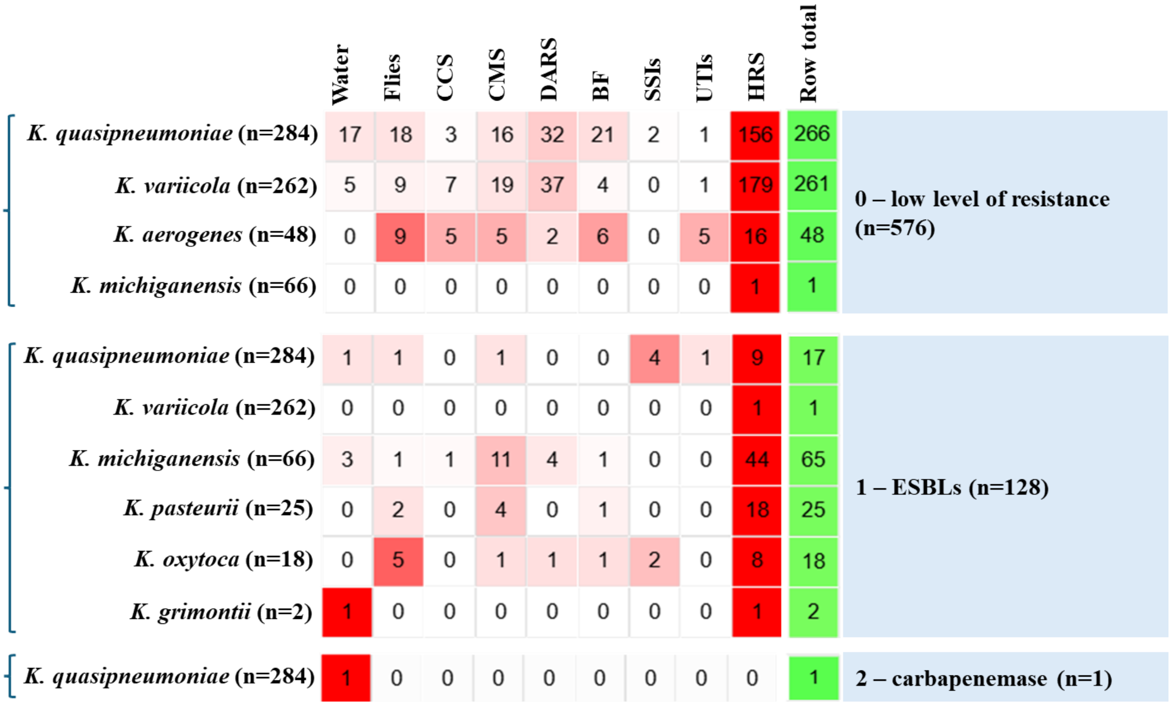
		β-lactams							Aminoglycosides						Quinolones				Fosfomycin	Trimethoprim				Sulfonamides			Chloramphenicol	Macrolide	Tetracycline
		$bla_{\text{DHA-1}}$	$bla_{\text{OXY-1-7}}$	$bla_{\text{OXY-2-2}}$	$bla_{\text{OXY-4-1}}$	$bla_{\text{OXY-5-1}}$	$bla_{\text{OXY-6-4}}$	$bla_{\text{TEM-1B}}$	*aac(3)-IId*	*aadA2*	*aadA16*	*aph(3'')-Ib*	*aph(3')-Ia*	*aph(6)-Id*	*oqxA*	*oqxB*	*qnrS1*	*aac(6')-Ib-cr*	*fosA*	*dfrA1*	*dfrA12*	*dfrA14*	*dfrA27*	*sul1*	*sul2*	*sul3*	*floR*	*mph(A)*	*tet(A)*
KoSC members	***K. michiganensis*** **(n=66)**	1	23			10		1	2	1	1	4	36	4	66	66	3	1	65		1		1	3			2	2	3
	K. pasteurii **(n=25)**				25										25	25	2		25			2			2				2
	K. oxytoca **(n=18)**			7					2			2		2	18	18			18	2				2				2	2
	K. grimontii **(n=2)**						2								2	2			2										
	K. aerogenes **(n=48)**							1	3	1	3	3	2	4	48	48	5	3	42	3	1	1	3	6	1	1	3	4	7

Figure 6.6 Distribution of ARGs across KoSC members and *K. aerogenes*. Each cell shows the count of isolates carrying the indicated gene. The colour gradient from white to red indicates the minimum to maximum values in each row.

Kleborate assigned four resistance scores (0 to 3): 0 indicating a low level of resistance; 1 = ESBL positive, 2 = carbapenemase positive; 3 = carbapenemase plus colistin positive (Discussed in Chapter 4) (Lam *et al.,* 2021). Among 705 isolates from seven *Klebsiella* spp. and nine sources, species-level differences were observed in Kleborate resistance scores (Figure 6.7).

K. quasipneumoniae isolates mostly scored 0 (266/284), with a few exceptions, where isolates scored 1 in HRS (n=9), SSIs (n=4), UTIs (n=1), CMS (n=1), and flies (n=1), along with a single isolate recovered from water (1/284) scored 2. Out of 262 *K. variicola* isolates, 261 scored 0, and only one isolate from HRS scored 1. All *K. aerogenes* (n=48) scored 0. Almost all isolates from KoSC members, including *K. michiganensis* (65/66), *K. pasteurii* (25/25), *K. oxytoca* (18/18) and *K. grimontii* (2/2), scored 1, with one isolate from *K. michiganensis* scoring 0.

Although most isolates from KoSC members scored 1 (indicating the presence of ESBLs), all were phenotypically susceptible to the 3GCs tested in this study (Table 6.3). Further analysis of the Kleborate findings revealed that *K. quasipneumoniae* and *K. variicola* isolates scoring 1 carried either $bla_{\text{CTX–M–15}}$ or $bla_{\text{CTX–M–55}}$ ESBLs. On the contrary, different species-specific OXY β-lactamase variants (OXY1-1, OXY1-2, OXY1-3, OXY1-4, OXY2-1, OXY2-2, OXY2-6, OXY2-7, OXY2-8, OXY4-1, OXY5-1 and OXY6-4) carried by the KoSC members were identified as ESBLs by Kleborate, hence providing a score of 1.



	Water	Flies	CCS	CMS	DARS	BF	SSIs	UTIs	HRS	Row total	
K. quasipneumoniae (n=284)	17	18	3	16	32	21	2	1	156	266	0 – low level of resistance (n=576)
K. variicola (n=262)	5	9	7	19	37	4	0	1	179	261	
K. aerogenes (n=48)	0	9	5	5	2	6	0	5	16	48	
K. michiganensis (n=66)	0	0	0	0	0	0	0	0	1	1	
K. quasipneumoniae (n=284)	1	1	0	1	0	0	4	1	9	17	1 – ESBLs (n=128)
K. variicola (n=262)	0	0	0	0	0	0	0	0	1	1	
K. michiganensis (n=66)	3	1	1	11	4	1	0	0	44	65	
K. pasteurii (n=25)	0	2	0	4	0	1	0	0	18	25	
K. oxytoca (n=18)	0	5	0	1	1	1	2	0	8	18	
K. grimontii (n=2)	1	0	0	0	0	0	0	0	1	2	
K. quasipneumoniae (n=284)	1	0	0	0	0	0	0	0	0	1	2 – carbapenemase (n=1)

Figure 6.7 Distribution of Kleborate resistance scores across non-pneumoniae *Klebsiella* spp. and sample sources. Heatmap shows the number of isolates for each combination of species (rows) and sample source (columns), stratified by Kleborate resistance score. The colour gradient from white to red indicates the minimum to maximum values in each row. The rightmost green column in each panel gives the total for the respective species within the score category. 'n' indicates the total number of isolates in each species. Cells with a value of "0" indicate no isolates for that combination. CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs.

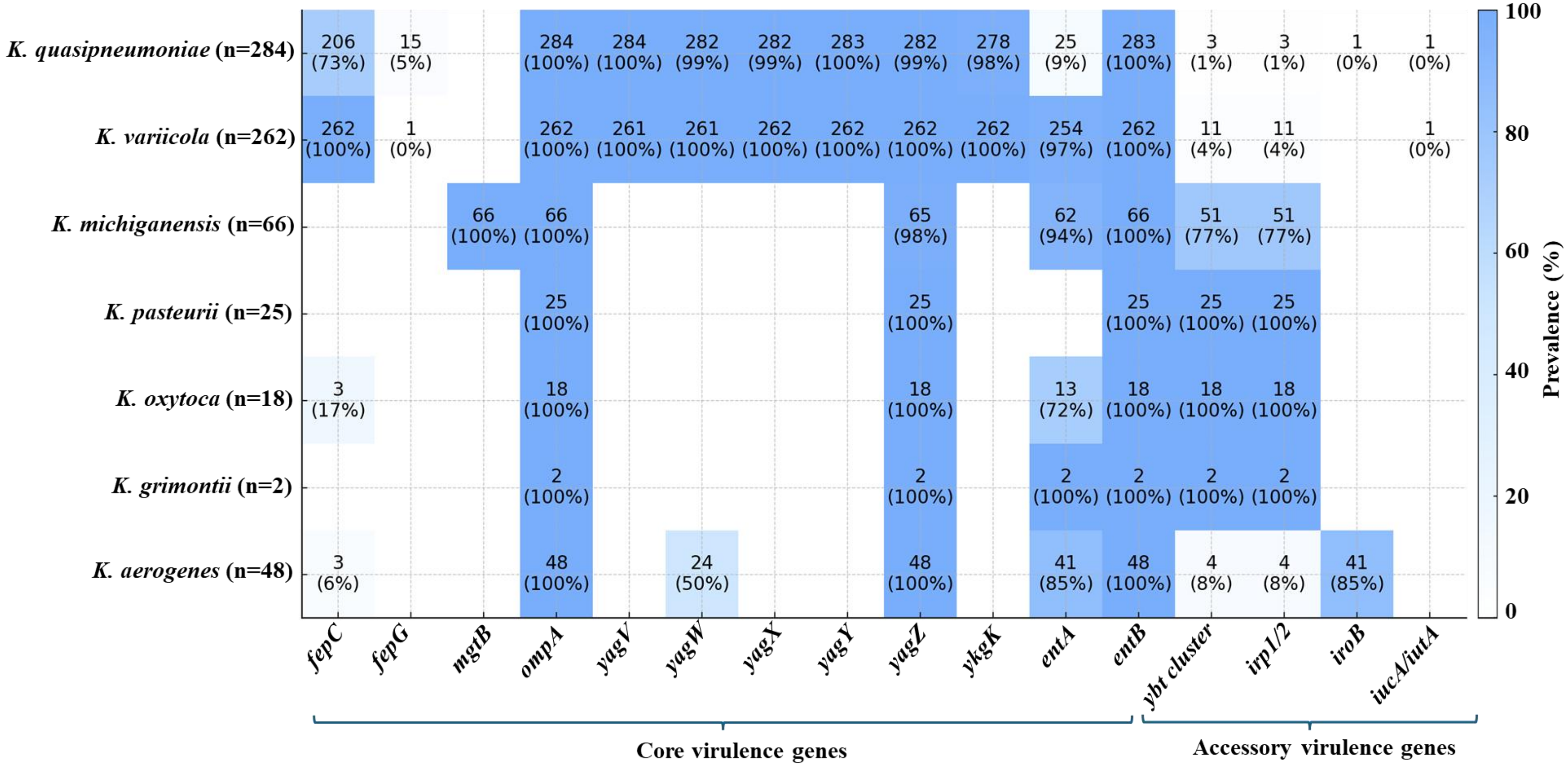
6.2.2.3 Genomic virulence determinants and Kleborate-derived virulence scores of non-pneumoniae *Klebsiella* spp.

Based on the VFDB screening and Kleborate analysis, different virulence genes were identified among the non-pneumoniae *Klebsiella* spp. (n=705) from diverse sources (Lam *et al*., 2021). Among the identified virulence loci, core virulence genes such as *entA*, *entB*, *fepC*, *fepG, ompA*, *mgtB*, *yagV/W/X/Y/Z*, *ykgK*, and accessory virulence genes (*ybt*, *iro*, *iuc*) were found variably across the species (Figure 6.8). The function of these genes was discussed in Chapter 5.

In *K. quasipneumoniae* (n=284), the core virulence gene *yagV/W/X/Y/Z*, *ykgK,* and *ompA* were present in more than 98% isolates. The enterobactin core *entB* was found in ~100% isolates (283/284), whereas *entA* was uncommon (9%, 25/284). The enterobactin transporter *fepC* was identified in 73% (206/284) isolates, followed by *fepG* (5%, 15/284). The yersiniabactin cluster (*ybtA/E/P/Q/S/T/U/X*) with *irp1/2* was identified in only 3 isolates, whereas salmochelin (*iroB*), and aerobactin (*iucABCD/iutA*) were each detected in a single isolate. In *K. variicola* (n=262), *fepC, yagV/W/X/Y/Z*, *ykgK, ompA, entA* and *entB* were found in 97% to 100% isolates. The *ybt* and *irp1/2* were infrequently identified (4%, 11/262). The *iucABCD/iutA* were detected in only one genome.

In the KoSC members of this study, *yagZ* was commonly found (98-100%), whereas other components of this locus were absent. *ompA* and *entB* were present in all isolates across the species. The *entA* was present in *K. michiganensis* (94%, 62/66), *K. oxytoca* (72%, 13/18), and *K. grimontii* (100%, 2/2) but was not detected in *K. pasteurii*. The *mgtB* was distinctive to *K. michiganensis* and present in all isolates (66/66). The *ybt* and *irp1/2* were present in all isolates of *K. pasteurii*, *K. oxytoca* and *K. grimontii*, whereas prevalence was 77% (51/66) in *K. michiganensis*. No *iucABCD/iutA* was detected in KoSC members.

In *K. aerogenes* (n=48), *ompA* and *entB* were present in 100% of the isolates, whereas *entA* and *fepC* were present in 85% (41/48) and 6% (3/48), respectively. The *yagZ* (48/48) and *yagW* (24/48) were identified while other components were absent. The *ybt* and *irp1/2* were less commonly found (8%, 4/48). The salmochelin (*iroB*) was highly enriched (41/48, 85%) in *K. aerogenes* than in other members of the non-pneumoniae *Klebsiella* spp., and no *iucABCD/iutA* was detected.



	fepC	*fepG*	*mgtB*	*ompA*	*yagV*	*yagW*	*yagX*	*yagY*	*yagZ*	*ykgK*	*entA*	*entB*	*ybt cluster*	*irp1/2*	*iroB*	*iucA/iutA*
K. quasipneumoniae **(n=284)**	206 (73%)	15 (5%)		284 (100%)	284 (100%)	282 (99%)	282 (99%)	283 (100%)	282 (99%)	278 (98%)	25 (9%)	283 (100%)	3 (1%)	3 (1%)	1 (0%)	1 (0%)
K. variicola **(n=262)**	262 (100%)	1 (0%)		262 (100%)	261 (100%)	261 (100%)	262 (100%)	262 (100%)	262 (100%)	262 (100%)	254 (97%)	262 (100%)	11 (4%)	11 (4%)		1 (0%)
K. michiganensis **(n=66)**			66 (100%)	66 (100%)					65 (98%)		62 (94%)	66 (100%)	51 (77%)	51 (77%)		
K. pasteurii **(n=25)**				25 (100%)					25 (100%)			25 (100%)	25 (100%)	25 (100%)		
K. oxytoca **(n=18)**	3 (17%)			18 (100%)					18 (100%)		13 (72%)	18 (100%)	18 (100%)	18 (100%)		
K. grimontii **(n=2)**				2 (100%)					2 (100%)		2 (100%)	2 (100%)	2 (100%)	2 (100%)		
K. aerogenes **(n=48)**	3 (6%)			48 (100%)		24 (50%)			48 (100%)		41 (85%)	48 (100%)	4 (8%)	4 (8%)	41 (85%)	

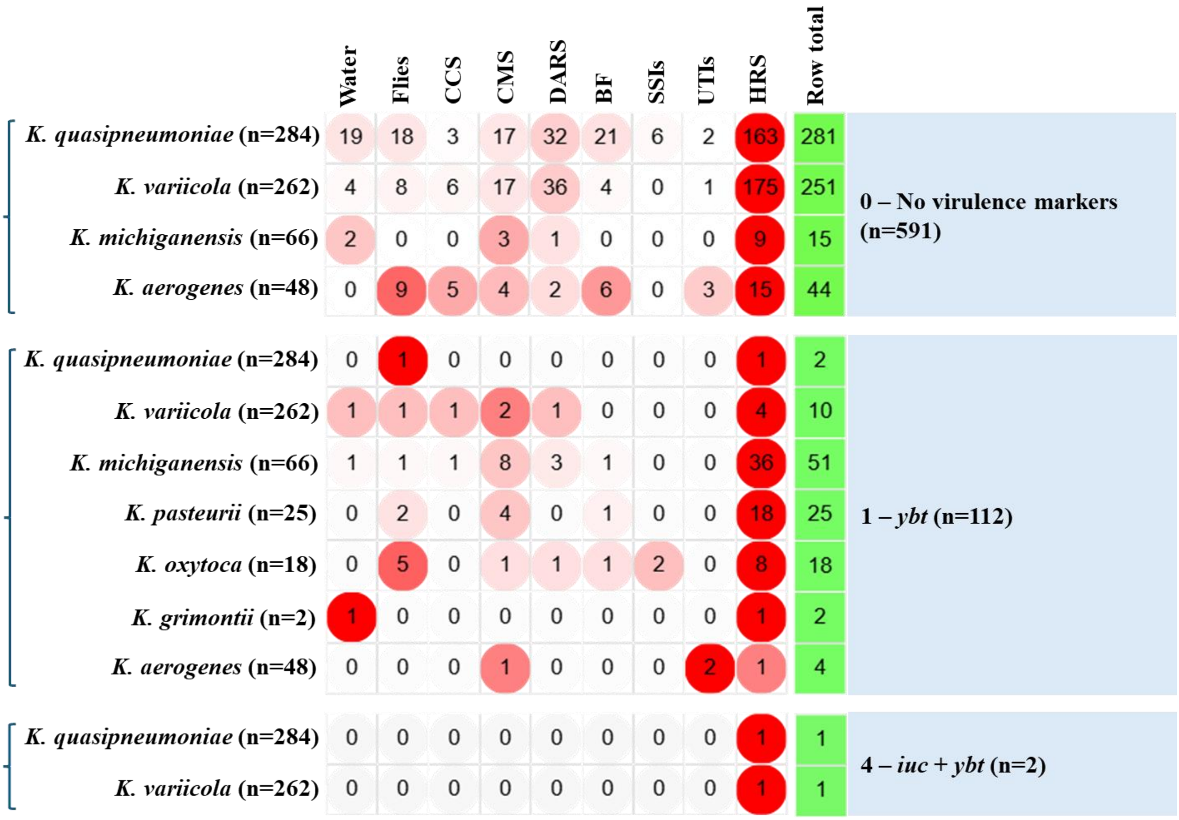
Figure 6.8 Distribution of virulence-associated genes in non-pneumoniae *Klebsiella* spp. identified in this study. The heatmap shows the frequency and prevalence (%) of isolates for each combination of species (rows) and gene markers (columns). The colour gradient from white to blue indicates the minimum to maximum prevalence (0 to 100%). Cells with zero counts are left blank.

Kleborate assigned six virulence scores (0 to 5) based on the presence of genes encoding the virulence factors *ybt*, *iuc* and *clb*: 0 = no *ybt*, *iuc* or *clb*; 1 = *ybt* only; 2 = *clb* without *iuc*; 3 = *iuc* only, 4 = *iuc* with *ybt*; and 5 = *iuc*, *ybt* and *clb* (Discussed in Chapter 5). Isolates scoring 3 or more were classified as hypervirulent.

Out of 705 non-pneumoniae *Klebsiella* isolates, 84% (591) scored 0 (lacked all three virulence loci), while 15.8% (112) scored 1 (*ybt* only). Only two isolates scored 4 (carried hypervirulent gene marker *iuc*, along with *ybt*) (Figure 6.9). The frequency of isolates corresponded to different categories, varied between species and sources. Isolates with scores of 1 and 4 were mostly from HRS (71/114), followed by CMS (16/114), flies (10/114), DARS (5/114), BF (3/114), water (3/114), and two each from CCS, SSIs, and UTIs.

By species, *K. quasipneumoniae* (n=284) mostly scored 0 (281/284), with a score of 1 in isolates from flies (n=1) and HRS (n=1). One *K. quasipneumoniae* recovered from HRS was found with a score of 4. In *K. variicola*, 251 out of 262 (95%) scored 0, while 4 isolates from HRS, 2 from CMS, 1 from CCS, DARS, flies, and water scored 1. A single isolate from HRS scored 4.

More than 75% (51/66) of *K. michiganensis* scored 1 across various sources (HRS 36, CMS 8, DARS 3, and single isolates from BF, CCS, flies, and water), while the remaining scored 0 (15/66). In *K. pasteurii* (n=25), *K. oxytoca* (n=18) and *K. grimontii* (n=2), all isolates from various sources scored 1 (Figure 6.9). *K. aerogenes* mostly scored 0 (44/48) with scores 1 in UTIs (n=2), CMS (n=1), and HRS (n=1).



	Water	Flies	CCS	CMS	DARS	BF	SSIs	UTIs	HRS	Row total	
***K. quasipneumoniae* (n=284)**	19	18	3	17	32	21	6	2	163	281	**0 – No virulence markers (n=591)**
***K. variicola* (n=262)**	4	8	6	17	36	4	0	1	175	251	
***K. michiganensis* (n=66)**	2	0	0	3	1	0	0	0	9	15	
***K. aerogenes* (n=48)**	0	9	5	4	2	6	0	3	15	44	
***K. quasipneumoniae* (n=284)**	0	1	0	0	0	0	0	0	1	2	**1 – *ybt* (n=112)**
***K. variicola* (n=262)**	1	1	1	2	1	0	0	0	4	10	
***K. michiganensis* (n=66)**	1	1	1	8	3	1	0	0	36	51	
***K. pasteurii* (n=25)**	0	2	0	4	0	1	0	0	18	25	
***K. oxytoca* (n=18)**	0	5	0	1	1	1	2	0	8	18	
***K. grimontii* (n=2)**	1	0	0	0	0	0	0	0	1	2	
***K. aerogenes* (n=48)**	0	0	0	1	0	0	0	2	1	4	
***K. quasipneumoniae* (n=284)**	0	0	0	0	0	0	0	0	1	1	**4 – *iuc* + *ybt* (n=2)**
***K. variicola* (n=262)**	0	0	0	0	0	0	0	0	1	1	

Figure 6.9 Distribution of Kleborate virulence scores across non-pneumoniae *Klebsiella* spp. and sample sources. Heatmap shows the number of isolates for each combination of species (rows) and sample source (columns), stratified by Kleborate virulence score. The colour gradient from white to red indicates the minimum to maximum values in each row. The rightmost green column in each panel gives the total for the respective species within the score category. 'n' indicates the total number of isolates in each species. Cells with a value of "0" indicate no isolates for that combination. CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs.

The AMR and virulence profiles of non-pneumoniae *Klebsiella* spp. (n=705) identified by Kleborate in this study, are summarised in Figure 6.10. Kleborate-derived resistance and virulence scores were predominantly low: resistance score 0 in 576 (82%), score 1 in 128 (18%), and score 2 in only one isolate, while virulence scores were 0 in 591 (84%), 1 in 112 (16%), and 4 in 2 (<1%).

To assess the convergence of MDR and hypervirulence, paired resistance–virulence score combinations were analysed. All isolates with resistance scores of 1 and 2 showed low virulence potential (either a virulence score of 0 or 1). The only two hypervirulent strains (virulence score 4) identified each exhibited low resistance potential with a score of 0.

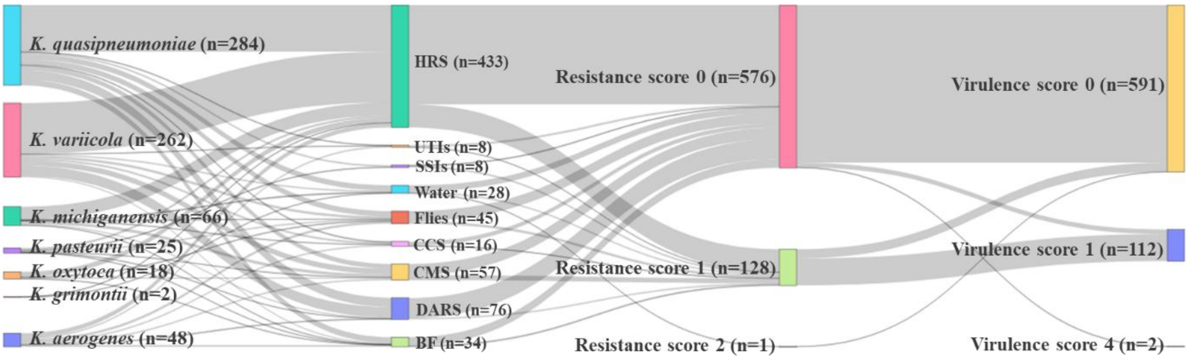


Figure 6.10 Sankey diagram showing the distribution of non-pneumoniae *Klebsiella* spp. isolates by species, source, resistance score and virulence scores. This Sankey diagram was generated in Python. CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; HRS, human rectal swabs; UTIs, urinary tract infections; SSIs, surgical site infections; and BF, bird faeces. Resistance score 0, no ESBL, no carbapenemase, indicating a low level of resistance; score 1, ESBL positive but no carbapenemase; and score 2 = carbapenemase positive. Virulence score 0, classical/non-virulent; score 1, low virulence with *ybt* only; score 4, high virulence with *iuc* plus *ybt*.

6.2.2.4 Sequence type diversity in *K. quasipneumoniae* and *K. variicola* with their resistance and virulence profiles

Multilocus sequence typing (MLST) revealed a diverse range of sequence types (STs) in both *K. quasipneumoniae* (n=284) and *K. variicola* (n=262). *K. quasipneumoniae* comprised 192 STs, with five dominant STs (each having ≥5 isolates): ST138 (n=11), ST526 (n=10), ST3387 (n=6), ST2676 (n=5), ST5682 (n=5). Additionally, 37 STs had 2–4 isolates, 150 were singletons, and 5 remained untyped. *K. variicola* comprised 187 STs (31 STs had 2–4 isolates, 152 singletons; 6 untyped) with dominant STs being ST355 (n=10), ST347 (n=8), ST1562 (n=6) and ST1899 (n=5).

All isolates of the major *K. quasipneumoniae* lineages, including ST138 (HRS 5, DARS 3, flies 2 and CMS 1), ST526 (HRS 5, CMS 2, water 2, DARS 1), ST3387 (all HRS), and ST2676 (HRS 4, water 1) exhibited resistance and virulence scores of 0. The ST5682 (all HRS) included four isolates with resistance score 1 (carrying *bla*$_{\text{CTX-M-15}}$ or *bla*$_{\text{CTX-M-55}}$), each with a virulence score of 0 (Figure 6.11).

MDR *K. quasipneumoniae* isolates with a resistance score of 1 occurred in HRS (9), SSIs (4), UTIs (1), CMS (1), water (1), and flies (1), were distributed across 12 distinct STs (ST440, ST644, ST1473, ST1998, ST2355, ST3866, ST5681, ST5682, ST6303, ST7709, ST7727, ST7892). Clinical isolates from UTIs or SSIs each exhibited a distinct ST. The single isolate recovered from water with a resistance score of 2 (carried *bla*$_{\text{OXA-181}}$) belonged to ST3876, and the only hypervirulent isolate (virulence score 4, HRS) was ST3857.

In *K. variicola*, the dominant lineages exhibited low resistance and virulence potential. ST355 (HRS 7, CMS 1, DARS 1, BF 1), ST347 (HRS 5, CMS 2, water 1), and ST1562 (all HRS) each showed a resistance score of 0 and a virulence score of 0. ST1899 (HRS 3, flies 1, DARS 1) also had a resistance score of 0, with two isolates scoring 1 for virulence. The only *K. variicola* from HRS, with a resistance score of 1 (harbouring *bla*$_{\text{CTX-M-15}}$), was identified as ST6041. The single hypervirulent *K. variicola* (HRS, virulence score 4) was untypeable by MLST.

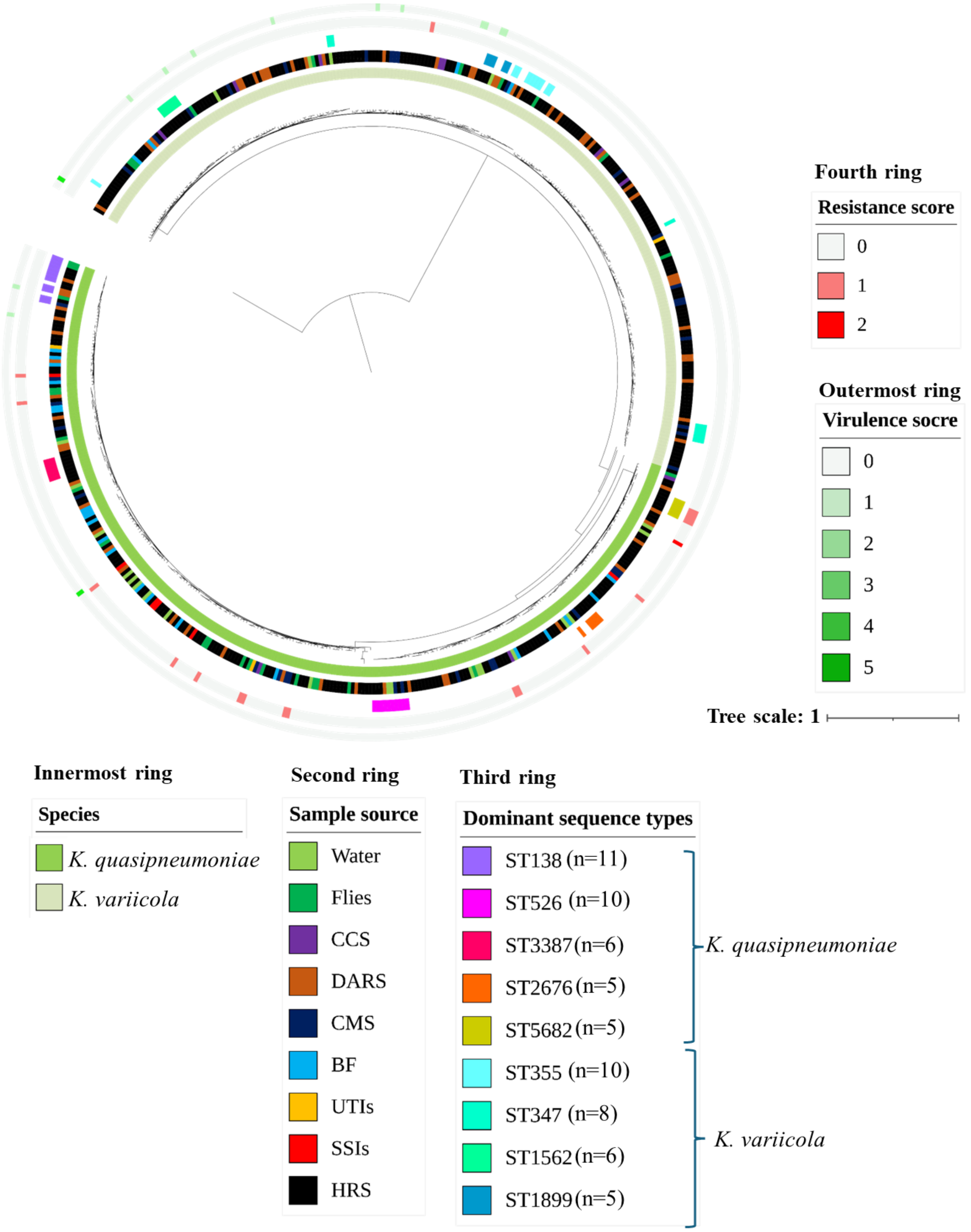


Figure 6.11 ML phylogenetic tree of *K. quasipneumoniae* and *K. variicola* isolates. This ML tree shows the phylogenetic distance between species, their source, resistance and virulence scores. It also shows the predominant STs identified in *K. quasipneumoniae* and *K. variicola*. The ML tree was constructed based on core-gene alignments (VeryFastTree v.4.0) and visualised in iTOL (v.6), with concentric rings indicating species, sample sources, predominant STs identified in *K. quasipneumoniae* and *K. variicola*, resistance and virulence scores (from inner to outermost ring).

6.3 Discussion

This cross-sectional carriage study conducted from December 2021 to March 2023 in Mymensingh, Bangladesh, extended beyond the well-studied *K. pneumoniae* to explore the prevalence, diversity, antimicrobial resistance and virulence determinants of understudied non-pneumoniae *Klebsiella* spp. through a One Health perspective.

This study identified eight recognised *Klebsiella* species from the multisource samples, including *K. pneumoniae*, *K. quasipneumoniae*, *K. variicola*, *K. oxytoca*, *K. michiganensis*, *K. grimontii*, *K. pasteurii*, and *K. aerogenes*. To the best of my knowledge, *K. michiganensis*, *K. grimontii*, and *K. pasteurii* reported in this study are the first to be identified in Bangladesh. Previous studies from Bangladesh have generally reported *Klebsiella* without species-level genomics, except for *K. pneumoniae*, *K. quasipneumoniae*, *K. variicola*, or *K. aerogenes*. (Farzana *et al*., 2019; Farzana *et al*., 2020; Ferdous *et al*., 2022; Mahmud *et al*., 2022; Hussain *et al*., 2023; Mazumder *et al*., 2023; Kawser *et al*., 2025; Tanni *et al*., 2025). The species diversity identified in this study aligns with recent One Health, WGS-based surveys from other settings, which typically detected 9–15 *Klebsiella* taxa across human, animal and environmental interfaces (Thorpe *et al*., 2022; Calland *et al*., 2023).

Within my dataset, the overall prevalence of *Klebsiella* spp. from nine ecological sources was 33.1% (1246/3759). *K. pneumoniae* alone dominated the dataset (43.4%, 541/1246) over other *Klebsiella* species. However, the non-pneumoniae *Klebsiella* spp. were also common, together comprising 56.6% (705/1246) of the isolates (Figure 6.2). Among the KpSC members, besides *K. pneumoniae*, *K. quasipneumoniae* (22.8%) and *K. variicola* (21.0%) were identified most frequently. These findings are consistent with recent species-level reports in South Asia, where an Indian study detected around 24% *K. quasipneumoniae* and 14% *K. variicola* along with *K. pneumoniae* (Mishra *et al*., 2025). Within the KoSC members, *K. michiganensis* dominated (around 60% of KoSC isolates, 66/111), which aligns with a study from China that also highlighted *K. michiganensis* as the leading KoSC pathogen over *K. oxytoca*, *K. grimontii*, and *K. pasteurii* (Li *et al*., 2024). In my cohort, *K. aerogenes* accounted for 3.9% (48/1246) of the isolates, a proportion concordant with recent One Health datasets from Italy and Ghana, reporting around 4-6% *K. aerogenes* (Thorpe *et al*., 2022; Calland *et al*., 2023).

The analysis of species-sources association revealed distinct ecological partitioning among non-pneumoniae *Klebsiella* members. A positive association was found between *K. quasipneumoniae* (z = 3.0) and *K. grimontii* (z = 3.3) and water samples. This finding aligns with recent environmental reports, including the recovery of *K. quasipneumoniae* from wastewater at a tertiary hospital in Bangladesh and other environmental surveys, as well as the detection of *K. grimontii* in water systems around the world (Hubbard *et al*., 2020; Ferdous *et al*., 2022; Mbanga *et al*., 2022; Altayb *et al*., 2023; Verburg *et al*., 2024). The over-representation of *K. variicola* in HRS (z = 3.1) is consistent with evidence that *K. variicola* is a recognised human gut coloniser as well as a pathogen in immunocompromised hosts. This is underscored by a high-mortality outbreak in neonates in Bangladesh, and by a recent clinical study associating *K. variicola* bloodstream infection with increased mortality (Farzana *et al*., 2019; Fostervold *et al*., 2024). The significant association of *K. aerogenes* with UTIs (z = 6.3) reflects its well-established role as a uropathogen, with a recent case report from Bangladesh further highlighting its clinical importance (Mazumder *et al*., 2023; González-Montalvo *et al*., 2024). The association of *K. oxytoca* with SSIs (z = 4.0) and flies (z = 3.8) is also supported by previous reports of hospital-associated *K. oxytoca* outbreaks and growing evidence of dissemination through houseflies (Neog *et al*., 2021; Cook *et al*., 2025; Obeng-Nkrumah *et al*., 2025). The source-species associations identified in this study indicate uneven species distributions across various sources, which is consistent with recent One Health genomic surveys (Thorpe *et al*., 2022; Calland *et al*., 2023). These findings emphasise the importance of species-level identification in South Asian surveillance efforts to better understand non-pneumoniae *Klebsiella* spp. ecology and transmission.

The overall AST findings of non-pneumoniae *Klebsiella* isolates (n=705) showed relatively low susceptibility to fosfomycin and fluoroquinolones. However, they generally exhibited high susceptibility to 3GCs, aminoglycosides, colistin, and tigecycline. All were susceptible to ceftazidime-avibactam and carbapenems. Species-level resistome analysis revealed a predominantly intrinsic-determinant resistome with some acquired AMR genes. ESBLs ($bla_{\text{CTX–M–15}}$, $bla_{\text{CTX–M–55}}$), aminoglycoside (*aadA2*, *aac(3)-IId*, *aph(6)–Id*, *aph(3")–Ib*, *aph(3')-Ia*), quinolone (*qnrB1*, *qnrS1*), tetracycline (*tetA*) and colistin resistance (*mcr-8*) genes were identified infrequently across the dataset, and carbapenemase genes were essentially absent. These findings align with recent reports from One Health studies, where severe resistance was concentrated in *K. pneumoniae* and hospital isolates, and was rarely detected in other *Klebsiella* spp. across community, animal, or environmental sectors (Thorpe *et al*., 2022; Calland *et al*., 2023).

In *K. quasipneumoniae* and *K. variicola*, high fosfomycin non-susceptibility (approximately 49% and 46%, respectively) coincided with the presence of the *fosA* gene, along with fluoroquinolone-resistant isolates carrying *oqxA/oqxB*. Among ESBLs, *bla*$_{\text{CTX–M–15}}$ (n=17) was predominantly found, particularly in *K. quasipneumoniae* isolates from water, flies, CMS, UTIs, SSIs and HRS (Figure 6.4). The only other *bla*$_{\text{CTX–M–15}}$ (n=1) was identified in a *K. variicola* from HRS. These findings are consistent with previous reports from Bangladesh, which also detected *bla*$_{\text{CTX–M–15}}$ commonly in *K. pneumoniae*, with less often in other *Klebsiella* spp. including *K. quasipneumoniae* and *K. variicola* (Farzana *et al*., 2019; Ferdous *et al*., 2022; Hussain *et al*., 2023).

Although *bla*$_{\text{OXA–181}}$ carbapenemase was detected in a single *K. quasipneumoniae*, it was phenotypically susceptible to carbapenemase. This discordance is well described for OXA-48-like enzymes, which frequently confer low-level carbapenem hydrolysis and may show susceptibility by routine AST in the absence of permeability defects, particularly for imipenem or meropenem and when ertapenem is not tested (Gelmez *et al*., 2021; Boyd *et al*., 2022). Sporadic detection of *mcr-8* in *K. quasipneumoniae* from water CCS, CMS and HRS highlight the potential for environmental reservoirs to seed high-risk alleles despite low colistin resistance in this dataset (Phetburom *et al*., 2021; Formenti *et al*., 2022).

Among KoSC members (*K. michiganensis*, *K. pasteurii*, *K. oxytoca* and *K. grimontii*), overall resistance remained low and was mainly explained by the presence of chromosomal OXY β-lactamases with limited acquired AMR genes, which is consistent with a recent study with taxonomical syntheses (Yang *et al*., 2021). Nearly all KoSC members scored 1 due to OXY β-lactamases flagged as ESBLs by the Kleborate algorithm. However, these isolates were phenotypically susceptible to 3GCs, which is in line with the literature that OXY β-lactamases are intrinsic to KoSC and only mimic ESBL phenotypes when hyperproduced or for certain variants (like OXY-2-5) (Nijhuis *et al*., 2015; Yang *et al*., 2021; Campos-Madueno *et al*., 2023). This finding highlights the limitations of Kleborate when applied beyond the

KpSC. Unlike other non-pneumoniae *Klebsiella* spp., *K. aerogenes* showed high resistance to amoxicillin-clavulanic acid (83.3%) (Table 6.3). This may be caused by the inherent β-lactam determinant, such as *ampC*, which confers resistance to aminopenicillins or β-lactamase inhibitor combinations (Tamma et al., 2019).

Virulence profiling of 705 non-pneumoniae *Klebsiella* isolates revealed a predominantly low virulence landscape in my dataset, as determined by VFDB screening and Kleborate (84% scored 0; 16% scored 1, with *ybt* only; and only two isolates scored 4 with both *iuc* and *ybt*). Species-specific analysis showed that core virulent loci (*entA*, *entB*, *fepC*, *fepG, ompA, yagV/W/X/Y/Z* and *ykgK*) were common in *K. quasipneumoniae* and *K. variicola*. They mostly scored 0 to 1 by Kleborate and lacked accessory virulent loci (Figure 6.8 and 6.9). The yersiniabactin gene clusters were present only in 1% of *K. quasipneumoniae* and 4% of *K. variicola* isolates, which aligns with the low prevalence of the ICEKp-borne *ybt* locus in these species (Lam *et al.*, 2018). Only two isolates, one from *K. quasipneumoniae* and one from *K. variicola*, both originating from HRS, carried high-risk virulence loci *iuc*, indicating a potential risk of hypervirulence traits in the community strains. These findings are consistent with recent studies, in which hypervirulence virulence loci (*iuc*, *clb* and *rmpA*) were uncommon outside hvKP lineages (Chew *et al*., 2021; Thorpe *et al*., 2022). However, the KoSC members frequently harboured *ybt* (virulence score 1) and lacked hypervirulence gene markers (*iuc*, *clb* or *rmpA*), aligning with previous hospital and community datasets, reporting *ybt* as the most common KoSC siderophore locus (Stewart *et al*., 2022). Interestingly, the *mgtB* gene (Mg^{2+} transporter) was found exclusively in *K. michiganensis*, indicating species-specific gene content. A multicentre study also reported that around 96% of *K. michiganensis* carried *mgtB* (related to enhanced survival within macrophages), and less often found in other KoSC species (Li *et al*., 2024). Additionally, *iroB* was frequently detected (41/48, 85%) in *K. aerogenes* but was rare or absent in other non-pneumoniae *Klebsiella* isolates. This finding aligns with a global analysis showing that the *iro* locus is highly prevalent in *K. aerogenes* (approximately 92%) without the *iuc* locus; however, the *iro* and *iuc* loci typically co-occur in *K. pneumoniae* (Lam *et al*., 2018; Morgado *et al*., 2024). This study found no convergence between MDR and hypervirulence. The two isolates identified as hypervirulent exhibited a low resistance score of 0.

This aligns with large cohort analyses, showing that hypervirulence typically occurs independently of MDR (Kochan *et al*., 2023; Jiang *et al*., 2025).

In the present study, a highly polyclonal population structure was observed in both *K. quasipneumoniae* (192 STs) and *K. variicola* (187 STs), with low-frequency dominant lineages, which is consistent with prior reports that these species are broadly diverse (Barrios-Camacho *et al*., 2019; Chew *et al*., 2021). The dominant *K. quasipneumoniae* lineages ST138, ST526, ST3387 and ST2676 exhibited lower resistance and virulence potential, whereas 4 out of 5 ST5682 isolates carried ESBLs (*bla*$_{\text{CTX-M-15}}$ or *bla*$_{\text{CTX-M-55}}$). However, overall, ESBLs were distributed among distinct STs from different sources, highlighting independent acquisition rather than expansion of a single high-risk clone. Interestingly, *K. quasipneumoniae* lineages ST138, which were most frequently found in this study, have been reported in diverse contexts (e.g., persistence on the International Space Station; in a hospital in Brazil carrying *bla*$_{\text{NDM-7}}$), underscoring its ecological diversity (Miliotis *et al*., 2023; Lobato *et al*., 2025). The hypervirulent *K. quasipneumoniae* identified in this study belonged to ST3857, which has also been reported as hypervirulent in a previous genome-wide analysis (Rodríguez-Medina *et al*., 2024). For *K. variicola*, ST355, ST347, ST1562, and ST1899 were the most common across sources, with resistance and virulence scores of 0. The only MDR *K. variicola* (carrying *bla*$_{\text{CTX-M-15}}$) from HRS belonged to ST6041. To the best of my knowledge, ST6041 has not been previously documented for MDR in *K. variicola*. The overall findings concerning KpSC pathogenic potential align with earlier One Health surveys, which documented a wide KpSC diversity and heightened resistance in hospital-adapted *K. pneumoniae* clones, rather than in *K. quasipneumoniae* or *K. variicola* (Thorpe *et al*., 2022; Calland *et al*., 2023).

This One Health study identified seven non-pneumoniae *Klebsiella* spp. comprising over half of the *Klebsiella* isolates, including the first detection of *K. michiganensis*, *K. grimontii*, and *K. pasteurii* in Bangladesh. Distinct species-source associations and a highly diverse population structure suggest niche partitioning and horizontal transmission events rather than clonal spread. Most strains carried intrinsic resistance determinants and exhibited low virulence scores, with scattered ESBLs (*bla*$_{\text{CTX-M-15}}$) and occasional high-risk loci (e.g., *mcr-8*, *bla*$_{\mathit{OXA-181}}$*, iuc*). These findings highlight the importance of species-level identification in clinical settings to detect non-pneumoniae *Klebsiella* contamination and support targeted surveillance at human-animal-environment interfaces for high-priority resistance genes, critical to preventing the clinical emergence of AMR in non-pneumoniae *Klebsiella* species.

Chapter 7

Emergence of Mobile Colistin Resistance in *Klebsiella* spp. Across the Human, Animal, and Environmental Sectors in Bangladesh

7.1 Introduction

Colistin is considered a last-resort antibiotic for treating serious infections caused by MDR Gram-negative bacteria, especially carbapenem-resistant *Enterobacteriaceae* (CRE) (Nang *et al.,* 2021; Liu *et al.,* 2024). However, its extensive use in livestock, particularly for treatment, prophylaxis and as a growth promoter, has led to the emergence and spread of mobile colistin resistance (*mcr*) in a wide host range globally (Liu *et al.,* 2016; Umair *et al.,* 2023). Since the emergence of the *mcr-1* gene in 2015 in China, there have been increasing reports of *mcr*-producing Enterobacterales being isolated from livestock, poultry, food of animal origin, humans, and the environment from more than 70 countries across six continents (Luo *et al.,* 2020; Mmatli *et al.,* 2022). So far, ten different *mcr* variants (*mcr-1* to *mcr-10*) have been reported from human, animal, and environmental sources, and among them, *mcr-1* is the most prevalent variant (Ling *et al.,* 2020; Liu *et al.,* 2024). Plasmids are considered the key drivers in the dissemination of *mcr* genes, with IncI2, IncH12, and IncX4 being reported as the major incompatibility groups associated with the global spread of *mcr* (Luo *et al.,* 2020; Liu *et al.,* 2024).

Recently, the widespread use of antibiotics has led to a significant increase in the dissemination of MDR *K. pneumoniae*, especially within clinical settings. To combat this challenge, particularly for the management of carbapenem-resistant strains, colistin is often used as a last treatment option (Liu *et al.,* 2024). However, *K. pneumoniae* is increasingly recognised as an important species within Enterobacteriaceae, contributing to the spread of colistin resistance, following *E. coli* (Wang *et al.,* 2020). Furthermore, in addition to *K. pneumoniae*, *mcr* genes have also been reported in other *Klebsiella* species from the human-animal-environment interface (Phetburom *et al.,* 2021; Liu *et al.,* 2022). Several *mcr* variants (*mcr-1*, *mcr-3*, *mcr-7*, *mcr-8*, *mcr-9* and *mcr-10*) have been identified, of which *mcr-1*, *mcr-8* and *mcr-9* have been reported to be the most prevalent in *Klebsiella* species. A recent geographical distribution study showed that *mcr-1* and *mcr-8* predominate in Asia, South America and Africa, whereas *mcr-9* is primarily found in Europe, North America and Oceania (Zhang *et al.,* 2025). In Bangladesh, routine *mcr* screening remains limited, yet plasmid-mediated colistin resistance has been documented in *K. pneumoniae*. Detection of *mcr-1* has been reported in *K. pneumoniae* from diarrhoeal patients and chicken gut samples (Sarker *et al.,* 2024; Islam *et al.,* 2020).

K. pneumoniae has also emerged as the primary host for *mcr-8* in Asia (Wang *et al.,* 2018; Zhang *et al.,* 2025). Farzana *et al.* (2020) reported the first detection of *mcr-8.1* in clinical *K. pneumoniae* ST15 isolated from a tertiary care hospital in Dhaka, Bangladesh. Recently, Mondol *et al.* (2025) reported *K. pneumoniae* harbouring *mcr-8.1* from canal water. These findings reveal multiple reservoirs of plasmid-mediated colistin resistance in Bangladesh, highlighting its circulation among human and animal populations.

In response to the global rise in AMR, the WHO, FAO, and OIE (WOAH) called for implementing global and national policies to improve antibiotic usage (WHO, 2015). They called to restrict critically important human antibiotics, e.g., colistin, for food-producing animals. Between 2016 and 2019, numerous countries, including China and India, implemented policies to restrict the use of colistin in livestock (Hennessey *et al.,* 2025). Colistin resistance was significantly reduced in China after the ban of colistin as an animal growth promoter (Wang *et al.,* 2020). The government of Bangladesh also enacted a law (Fish Feed and Animal Feed Act, 2010), banning the use of critical antibiotics, growth hormones and pesticides in fish and animal feed. Later, on 20 March 2022, the Directorate General of Drug Administration prohibited all veterinary colistin preparations (Directorate General of Drug Administration, 2022). However, doubts persist over the effective implementation of these measures in practice (Foysal *et al.,* 2024).

Although recent reports highlight the emergence of *mcr*-mediated colistin resistance in *Enterobacterales* from clinical infections, livestock and environmental settings in Bangladesh, the epidemiology of *mcr*-like mechanisms in *Klebsiella* spp. and the factors driving the dissemination of *mcr* are still largely unknown (Farzana *et al.,* 2019, 2020; Dutta *et al.,* 2020; Islam *et al.,* 2020; Ara *et al.,* 2021; Nath *et al.,* 2023; Munim *et al.,* 2024; Mondol *et al.,* 2025). Considering these facts, this study aims to investigate the molecular epidemiology of *mcr*-positive *Klebsiella* spp. (MCRPK) in Mymensingh, Bangladesh through a One Health lens.

In this chapter, I specifically aim to explore the following:

1. The prevalence of colistin resistance and *mcr* in *Klebsiella* spp. across One Health sectors in Bangladesh.
2. The resistance profile and clonal relatedness of MCRPK.
3. The genetic context of *mcr* genes and whether any dominant plasmid and/or strain type is associated with their dissemination.
4. Assessment of antibiotic usage practices, including colistin administration, on the poultry farms enrolled in this study.

7.2 Results

7.2.1 Prevalence of colistin resistance and MCRPK in the study area

A cross-sectional carriage study was conducted between December 2021 and March 2023 to determine the prevalence of MCRPK in Mymensingh, Bangladesh. Sampling was carried out using a One Health approach to assess the dissemination of MCRPK across human, animal, and environmental sectors.

Out of 3759 samples collected from humans (HRS, UTIs, SSIs), farms (CCS, CMS, DARS, BF) and the environment (water, flies) within Mymensingh, the overall prevalence of *Klebsiella* spp. was 33.1% (1246/3759), as detailed in Table 7.1. Eight distinct *Klebsiella* species were identified: *K. pneumoniae* (n=541), *K. quasipneumoniae* (n=284), *K. variicola* (n=262), *K. michiganensis* (n=66), *K. aerogenes* (n=48), *K. pasteurii* (n=25), *K. oxytoca* (n=18) and *K. grimontii* (n=2). All *Klebsiella* (n=1246) recovered from various samples were subjected to antimicrobial susceptibility testing. Antibiogram analysis revealed that 3.1% (39/1246) of the isolates across all samples exhibited phenotypic resistance to colistin (MIC > 2 mg/L, EUCAST 2024) (Table 7.1 and Figure 7.1). Colistin-resistant *Klebsiella* spp. (CRK) were identified in water (7.5%, 4/53), flies (5.6%, 7/125), CCS (15.4%, 12/78), CMS (3.6%, 4/111), DARS (5.5%, 5/91), BF (6.4%, 3/47) and HRS (0.6%, 4/618). However, all clinical isolates recovered from UTIs and SSIs were susceptible to colistin. Notably, all 39 CRK belonged exclusively to three species: *K. pneumoniae* (n=17), *K. variicola* (n=12) and *K. quasipneumoniae* (n=10).

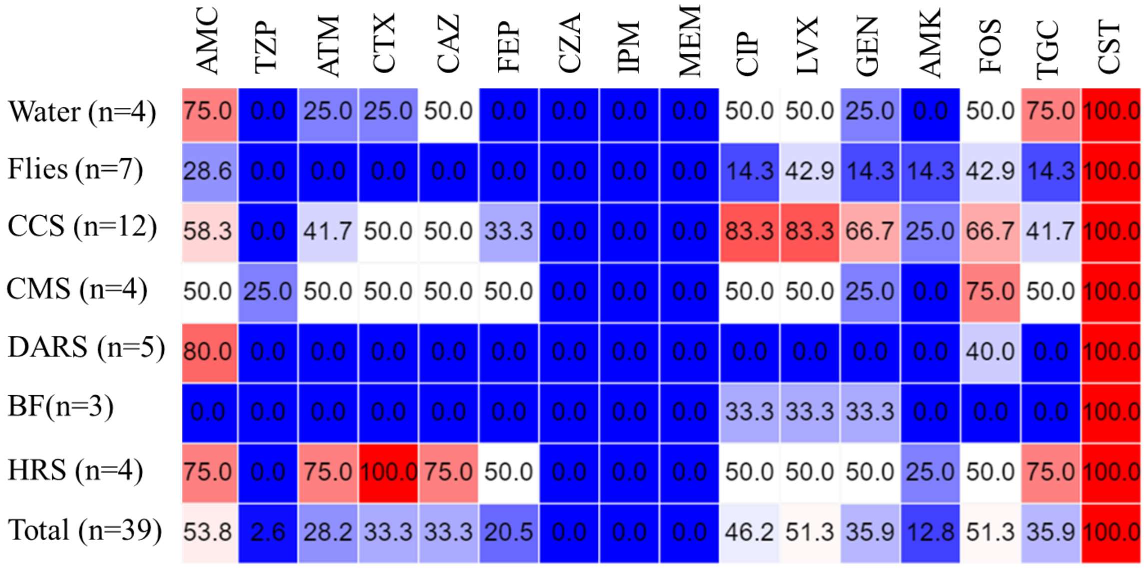
The antibiotic susceptibility pattern of CRK isolated from various sources is presented in Figure 7.1. Among the 39 CRK isolates, 53.8% exhibited resistance to amoxicillin-clavulanic acid, followed by fosfomycin (51.3%), levofloxacin (51.3%), ciprofloxacin (46.2%), gentamicin (35.9%, tigecycline (35.9%), cefotaxime (33.3%) and ceftazidime (33.3%). However, all CRK were susceptible to ceftazidime-avibactam, imipenem and meropenem.

The overall prevalence of MCRPK was 1.1% (14/1246), with the highest detection rates observed in samples from CCS (7.7%), followed by water (3.8%), BF (2.1%), CMS (1.8%), flies (0.8%) and HRS (0.3%) (Table 7.1). No MCRPK isolates were detected in samples from DARS, UTIs and SSIs. Two *mcr* gene variants, *mcr-1* and *mcr-8*, were detected among the MCRPK isolates. The *mcr-1* variant was exclusively detected in isolates originating from flies and HRS, whereas *mcr-8* was found across a broader range of samples, including CCS, CMS, BF, Water and HRS. One MCRPK isolated from HRS was found to co-harbour both *mcr-1* and *mcr-8*. All MCRPK (n=14) isolates were phenotypically resistant to colistin. Although CRK were found among three *Klebsiella* species, including *K. pneumoniae*, *K. quasipneumoniae*, and *K. variicola*, the *mcr* genes were found only in *K. pneumoniae* (n=10) and *K. quasipneumoniae* (n=4) isolates. Notably, a statistically significant association was found between MCRPK and *K. pneumoniae* (71.4%, 10/14) ($p<0.05$) (Table 7.2).

Table 7.1 Prevalence of CRK and MCRPK across various samples and the distribution of *mcr* genes**.**

Sample type	**Number of samples**	***Klebsiella* positive, number**	**CRK, n (prevalence, %)**	**MCRPK, n (prevalence, %)**	**Number of *mcr* variants**		
					mcr-1.1	***mcr-8.1***	***mcr-1.1 and mcr-8.1***
Water	220	53	4 (7.5%)	2 (3.8%)	0	2	0
Flies	220	125	7 (5.6%)	1 (0.8%)	1	0	0
CCS	200	78	12 (15.4%)	6 (7.7%)	0	6	0
CMS	200	111	4 (3.6%)	2 (1.8%)	0	2	0
DARS	200	91	5 (5.5%)	0 (0)	0	0	0
BF	75	47	3 (6.4%)	1 (2.1%)	0	1	0
UTIs	1170	22	0	0 (0)	0	0	0
SSIs	814	101	0	0 (0)	0	0	0
HRS	660	618	4 (0.6%)	2 (0.3%)	0	1	1
Total	**3759**	**1246**	**39 (3.1%)**	**14 (1.1%)**	**1**	**12**	**1**

CRK, colistin-resistant *Klebsiella* spp.; MCRPK, *mcr*-positive *Klebsiella* spp.; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; UTIs, urinary tract infections; SSIs, surgical site infections; HRS, human rectal swabs; prevalence of CRK (total number of CRK / total number of *Klebsiella* positive isolates); prevalence of MCRPK (total number of MCRPK / total number of *Klebsiella* positive isolates).



	AMC	TZP	ATM	CTX	CAZ	FEP	CZA	IPM	MEM	CIP	LVX	GEN	AMK	FOS	TGC	CST
Water (n=4)	75.0	0.0	25.0	25.0	50.0	0.0	0.0	0.0	0.0	50.0	50.0	25.0	0.0	50.0	75.0	100.0
Flies (n=7)	28.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.3	42.9	14.3	14.3	42.9	14.3	100.0
CCS (n=12)	58.3	0.0	41.7	50.0	50.0	33.3	0.0	0.0	0.0	83.3	83.3	66.7	25.0	66.7	41.7	100.0
CMS (n=4)	50.0	25.0	50.0	50.0	50.0	50.0	0.0	0.0	0.0	50.0	50.0	25.0	0.0	75.0	50.0	100.0
DARS (n=5)	80.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40.0	0.0	100.0
BF(n=3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	33.3	33.3	0.0	0.0	0.0	100.0
HRS (n=4)	75.0	0.0	75.0	100.0	75.0	50.0	0.0	0.0	0.0	50.0	50.0	50.0	25.0	50.0	75.0	100.0
Total (n=39)	53.8	2.6	28.2	33.3	33.3	20.5	0.0	0.0	0.0	46.2	51.3	35.9	12.8	51.3	35.9	100.0

Figure 7.1 Antibiotic susceptibility pattern of colistin-resistant *Klebsiella* spp. from various sources. The heatmap was generated according to the resistance percentage. AMC, Amoxicillin-clavulanic acid; TZP, Piperacillin-tazobactam; ATM, Aztreonam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime-avibactam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin; AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline; CST, Colistin; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; HRS, human rectal swabs.

Table 7.2 Univariate logistic regression analysis for MCRPK prevalence in different *Klebsiella* spp.

Species	**MCRPK (n=14)**	**MCRNK (n=1232)**	***p*-value**	**OR**	**95% CI**
K. pneumoniae (n=541)	10 (71.4%)	531 (41.3%)	0.033	3.300	1.029 - 10.581
K. quasipneumoniae (n=284)	4 (28.6%)	280 (22.7%)	0.604	1.360	0.423 - 4.369
K. variicola (n=262)	0 (0)	262 (21.3%)	-	NA	NA

Values in parentheses indicate column percentage. MCRPK, *mcr*-positive *Klebsiella* spp.; MCRNK, *mcr*-negative *Klebsiella* spp., NA, not applicable. Statistical significance was set at $p<0.05$. The cells in the table were highlighted to present whether any variable was significantly higher in MCRPK.

Univariate logistic regression analysis demonstrated a significantly higher occurrence of CRK in samples from Muktagachha layer farm (7.5%, 8/107) when compared to other sampling locations (2.7%, 31/1139) ($p<0.05$) (Table 7.3). Further analysis revealed a statistically significant association between CRK occurrence and samples obtained from CCS, with 15.4% (12/78) of isolates exhibiting resistance compared to 2.3% (27/1168) from all other sources ($p<0.05$) (Table 7.4). Figure 7.2 provides an overview of the distribution of CRK, categorised according to the specific species identified, the type of samples from which they were isolated, and the geographical locations.

Table 7.3 Univariate logistic regression analysis for CRK occurrence from different sampling locations.

Sampling location	**CRK (n=39)**	**CSK (n=1207)**	***p*-value**	**OR**	**95% CI**
MyB (n=142)	4 (10.3%)	138 (11.4%)	0.820	0.885	0.310 - 2.529
MyL (n=92)	2 (5.1%)	90 (7.5%)	0.587	0.671	0.159 - 2.829
MkB (n=104)	3 (7.7%)	101 (8.4%)	0.881	0.913	0.276 - 3.015
MkL (n=107)	8 (20.5%)	99 (8.2%)	0.01	2.888	1.293 - 6.453
TrB (n=98)	4 (10.3%)	94 (7.8%)	0.574	1.353	0.471 - 3.889
TrL (n=106)	6 (15.4%)	100 (8.3%)	0.125	2.013	0.824 - 4.919
GB (n=102)	4 (10.3%)	98 (8.1%)	0.633	1.293	0.450 - 3.714
GL (n=109)	6 (15.4%)	103 (8.5%)	0.143	1.949	0.798 - 4.760
TaB (n=93)	1 (2.6%)	92 (7.6%)	0.262	0.319	0.043 - 2.349
TaL (n=95)	1 (2.6%)	94 (7.8%)	0.252	0.312	0.042 - 2.295

Values in parentheses indicate column percentage. CRK, colistin-resistant *Klebsiella* spp.; CSK, colistin-sensitive *Klebsiella* spp.; total number of *Klebsiella* spp. isolates: n=1246; MyB, Mymensingh broiler; MyL, Mymensingh layer; MkB, Muktagachha broiler; MkL, Muktagachha layer; TrB, Trishal broiler; TrL, Trishal layer; GB, Gauripur broiler; GL, Gauripur layer; TaB, Tarakanda broiler; TaL, Tarakanda layer farms. Others indicate *Klebsiella* isolates from the remaining sampling locations. Statistical significance was set at $p<0.05$. The cells in the table were highlighted to present whether any variable was significantly higher with CRK.

Table 7.4 Univariate logistic regression analysis for CRK occurrence from different types of samples.

Sample type	**CRK (n=39)**	**CSK (n=1207)**	***p*-value**	**OR**	**95% CI**
Water (n=53)	4 (10.3%)	49 (4.1%)	0.07	2.701	0.923 - 7.900
Flies (n=125)	7 (17.9%)	118 (9.8%)	0.101	2.019	0.872 - 4.674
CCS (n=78)	12 (30.8%)	66 (5.5%)	<0.001	7.684	3.726 - 15.846
CMS (n=111)	4 (10.3%)	107 (8.9%)	0.764	1.175	0.410 - 3.369
DARS (n=91)	5 (12.8%)	86 (7.1%)	0.186	1.917	0.731 - 5.027
BF (n=47)	3 (7.7%)	44 (3.6%)	0.203	2.203	0.653 - 7.428
HRS (n=618)	4 (10.3%)	614 (50.9%)	<0.001	0.110	0.039 -0.312

Values in parentheses indicate column percentage. CRK, colistin-resistant *Klebsiella* spp.; CSK, colistin-sensitive *Klebsiella* spp.; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; HRS, human rectal swabs. Statistical significance was set at *p*<0.05. The cells in the table were highlighted to present whether any variable was significantly higher with CRK.

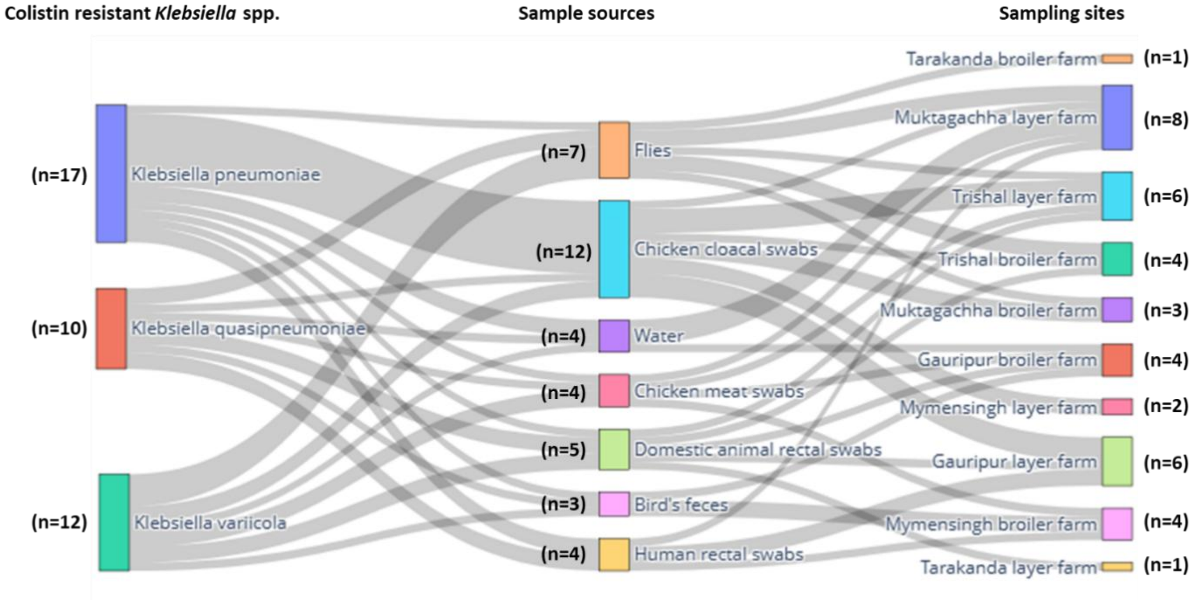


Figure 7.2 Distribution of colistin-resistant *Klebsiella* spp. by species, sample source and sampling location. This Sankey diagram was generated using Python (Colab notebook).

For MCRPK, a significantly higher prevalence was observed in samples from CCS, with 7.7% (6/78) of isolates exhibiting colistin resistance compared to 0.7% (8/1168) from all other sources (*p*<0.05)

(Table 7.5). Additionally, a significant association was observed between the prevalence of MCRPK and the Muktagachha layer farm, where 4.7% (5/107) of colistin-resistant isolates were identified, compared to 0.8% (9/1139) from other locations ($p<0.05$) (Table 7.6). Figure 7.3 illustrates the distribution of MCRPK, categorised according to the specific species identified, the type of samples from which they were isolated, and the sampling locations.

Table 7.5 Univariate logistic regression analysis for MCRPK prevalence in various sample types.

Sample type	**MCRPK (n=14)**	**MCRNK (n=1232)**	***p*-value**	**OR**	**95% CI**
Water (n=53)	2 (14.3)	51 (4.1%)	0.082	3.859	0.842 - 17.699
Flies (n=125)	1 (7.1%)	124 (10.1%)	0.719	0.687	0.089 - 5.299
CCS (n=78)	6 (42.9%)	72 (5.8%)	<0.001	12.083	4.083 - 35.758
CMS (n=111)	2 (14.3%)	109 (8.8%)	0.483	1.717	0.379 - 7.771
BF (n=47)	1 (7.1%)	46 (3.7%)	0.514	1.983	0.254 - 15.486
HRS (n=618)	2 (14.3%)	616 (50.0%)	0.019	0.167	0.037 - 0.748

Values in parentheses indicate column percentage. MCRPK, *mcr*-positive *Klebsiella* spp.; MCRNK, *mcr*-negative *Klebsiella* spp.; CCS, chicken cloacal swabs; CMS, chicken meat swabs; DARS, domestic animal rectal swabs; BF, bird faeces; HRS, human rectal swabs. Statistical significance was set at $p<0.05$. The cells in the table were highlighted to present whether any variable was significantly higher in MCRPK.

Table 7.6 Univariate logistic regression analysis for MCRPK prevalence from different sampling locations.

Sampling location	**MCRPK (n=14)**	**MCRNK (n=1232)**	***p*-value**	**OR**	**95% CI**
MyB (n=142)	2 (14.3%)	140 (11.4%)	0.733	1.300	0.288 - 5.869
MkB (n=104)	2 (14.3%)	102 (8.3%)	0.426	1.846	0.408 - 8.363
MkL (n=107)	5 (35.7%)	102 (8.3%)	<0.001	6.155	2.025 - 18.710
TrB (n=98)	1 (7.1%)	97 (7.9%)	0.920	0.900	0.117 - 6.953
TrL (n=106)	3 (21.4%)	103 (8.4%)	0.097	2.989	0.821 - 10.886
GL (n=109)	1 (7.1%)	108 (8.8%)	0.831	0.801	0.104 - 6.179

Values in parentheses indicate column percentage. MCRPK, *mcr*-positive *Klebsiella* spp.; MCRNK, *mcr*-negative *Klebsiella* spp.; MyB, Mymensingh broiler; MyL, Mymensingh layer; MkB, Muktagachha broiler; MkL, Muktagachha layer; TrB, Trishal broiler; TrL, Trishal layer; GB, Gauripur broiler; GL, Gauripur layer; TaB, Tarakanda broiler; TaL, Tarakanda layer farms. Statistical significance was set at *p*<0.05. The highlighted cells in the table present significantly higher variables for MCRPK.

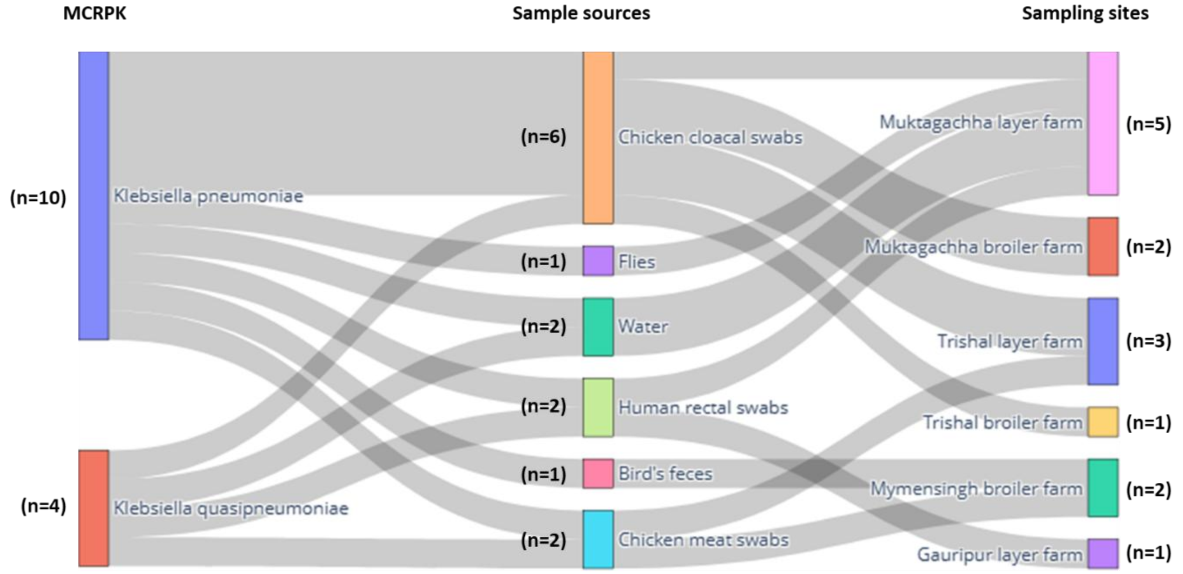


Figure 7.3 Distribution of *mcr*-positive *Klebsiella* spp. (MCRPK) by species, sample source and sampling location. This Sankey diagram was generated using Colab notebook.

7.2.2 AMR and virulence profile of MCRPK

Resistance patterns to tested antibiotics for MCRPK (n=14) and MCRNK (n=1232) were investigated. The resistance rates of MCRPK were significantly higher for ciprofloxacin (92.9%, 13/14), levofloxacin (92.9%, 13/14), amoxicillin-clavulanic acid (71.4%, 10/14), gentamicin (64.3%, 9/14), cefotaxime (57.1%, 8/14), ceftazidime (57.1%, 8/14) tigecycline (50.0%, 7/14) and aztreonam (42.9%, 6/14) compared with those of MCRNK (*p*<0.05) (Figure 7.4). However, MCRPK were 100% susceptible to ceftazidime-avibactam, imipenem and meropenem. The MIC values of colistin for MCRPK carrying the *mcr-8* gene (n=12) ranged from 4 to 16 mg/L, which are all above the EUCAST breakpoint for colistin resistance (>2 mg/L). One isolate carrying *mcr-1* exhibited an MIC of 8 mg/L, while one

Klebsiella carrying both *mcr-1* and *mcr-8* possessed an MIC of >64 mg/L, suggesting a synergistic resistance effect between the two *mcr* genes (Figure 7.5). Notably, the highest MIC values were observed among human-derived isolates, ranging from 16 to >64 mg/L. The distribution of colistin MICs for MCRPK stratified by sample origin is presented in Figure 7.6.

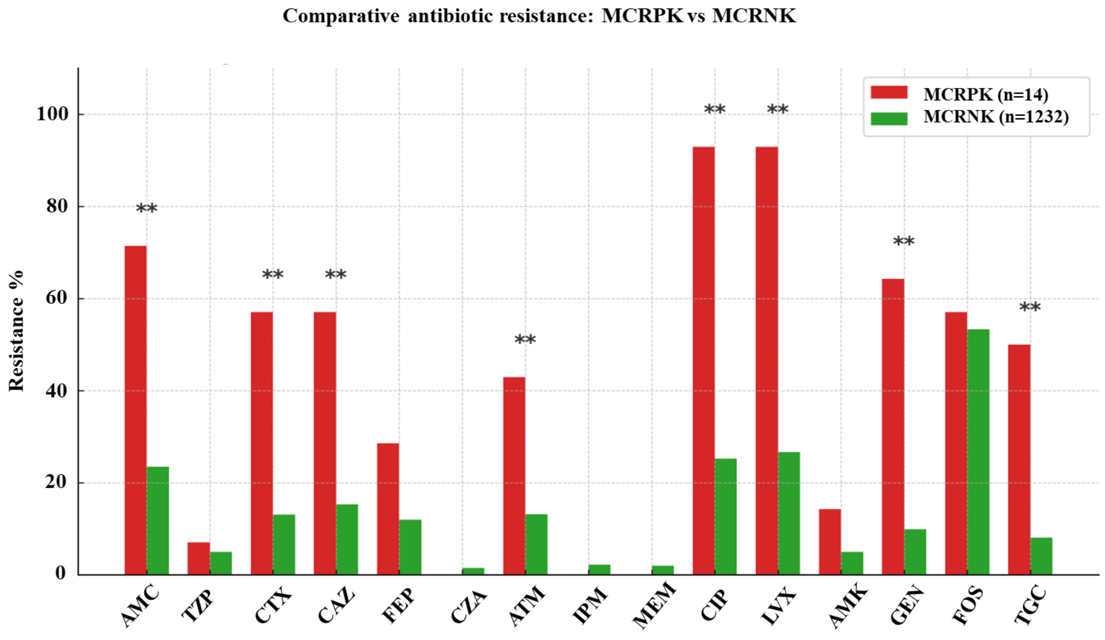


Figure 7.4 Bar chart illustrating resistance rates (%) for 15 antibiotics between MCRPK (n=14, in red) and MCRNK (n=1232, in green) isolates. Statistical significance was set at $p<0.05$, and asterisks above each antibiotic indicate statistical significance. AMC, Amoxicillin-clavulanic acid; TZP, Piperacillin-tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime-avibactam; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin; AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline.

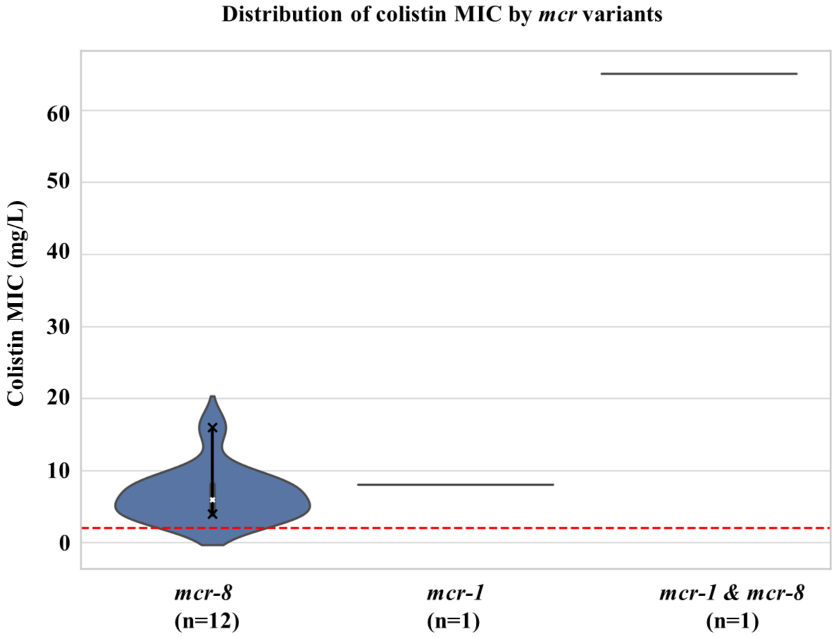


Figure 7.5 Violin plot shows the distribution and density of colistin MIC values grouped by *mcr* variant. The shape indicates the distribution of MIC values. The figure was generated in Python using Matplotlib (v.3.7). The red dashed line marks the EUCAST resistance breakpoint (2 mg/L). The figure illustrates how all variants exceed resistance levels, with the isolate carrying both *mcr-1* and *mcr-8*, resulting in high MICs. Since *mcr-1* was identified in only one isolate, and just one isolate harboured both *mcr-1* and *mcr-8*, the violin plot appears as a flat line due to the limited data points.

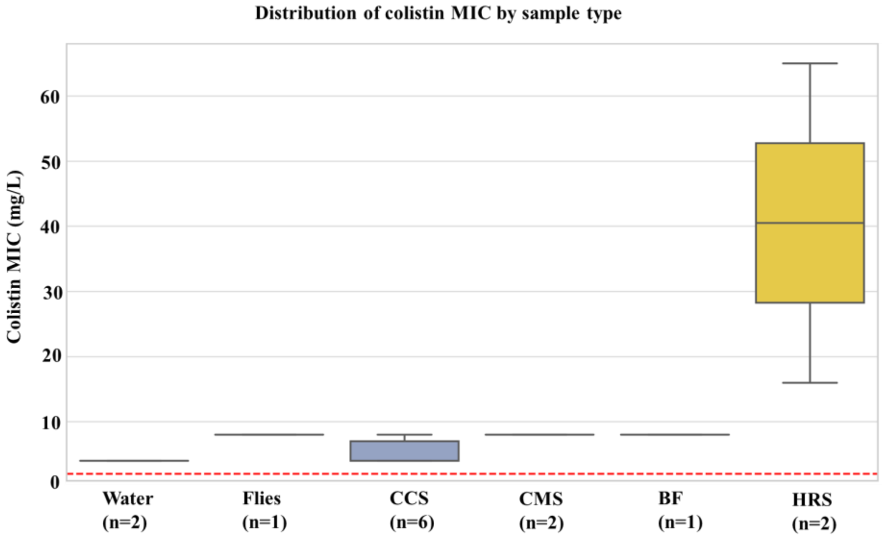


Figure 7.6 Boxplot shows the colistin MIC values for MCRPK isolates stratified by sample type. CCS, chicken cloacal swabs; CMS, chicken meat swabs; BF, bird's faeces; and HRS, human rectal swabs. The figure was generated in Python using Matplotlib (v.3.7).

The association of ARGs pertinent to different resistance mechanisms in MCRPK was also investigated. Aminoglycoside-resistance genes (*aadA2*), β-lactamase genes (bla_{OXA-1}, bla_{TEM-1B}), phenicol-resistance gene *floR*, fluoroquinolone-resistance genes (*aac(6')-Ib-cr*, *qnrS1*, *qnrB1*), sulfonamide-resistance genes (*sul1*, *sul2* and *sul3*), and the tetracycline resistance gene *tet(A)* were significantly associated with MCRPK ($p< 0.05$) (Table 7.7).

Table 7.7 Univariate logistic regression analysis for the distribution of ARGs in MCRPK and MCRNK.

ARGs	**MCRPK (n=14)**	**MCRNK (n=1232)**	***p*-value**	**OR**	**95% CI**
aac(6')-Ib-cr (n=95)	8 (57.1%)	87 (7.1%)	<0.001	17.548	5.955 - 57.710
aadA2 (n=93)	9 (64.3%)	84 (6.8%)	<0.001	24.600	8.063 - 64.052
bla_{OXA-1} (n=84)	4 (28.6%)	80 (6.5%)	0.001	5.760	1.767 - 28.773
bla_{TEM-1B} (n=153)	8 (57.1%)	145 (11.8%)	<0.001	9.995	3.420 - 57.216
floR (n=65)	5 (35.7%)	60 (4.9%)	<0.001	10.852	3.528 - 35.378
qnrB1 (n=71)	4 (28.6%)	67 (5.4%)	<0.001	6.955	2.126 - 28.757
qnrS1 (n=196)	8 (57.1%)	188 (15.3%)	<0.001	7.404	2.540 - 57.583
sul1 (n=203)	11 (78.6%)	192 (15.6%)	<0.001	19.861	5.490 - 78.852
sul2 (n=147)	7 (50%)	140 (11.4%)	<0.001	7.800	2.696 - 50.566
sul3 (n=38)	6 (42.9%)	32 (2.6%)	<0.001	28.125	9.221 - 42.786
tet(A) (n=219)	8 (57.1%)	211 (17.1%)	<0.001	6.452	2.216 - 57.787

Values in parentheses indicate column percentage. ARGs, antibiotic resistance genes; MCRPK, *mcr*-positive *Klebsiella* spp.; MCRNK, *mcr*-negative *Klebsiella* spp. Statistical significance was set at $p<0.05$. Only data that were significantly higher in MCRPK isolates are presented in the table.

Virulence-associated genes encoding enterobactins *entB* (99.8%, 1244/1246), *entA* (73.8%, 919/1246), *fepC* (81.5%, 1015/1246)) and the *ykgK* (86.3%, 1075/1246), *yagZ* (99.6%, 1241/1246), *yagY* (87.1%, 1085/1246), *yagX* (87.0%, 1084/1246), *yagW* (88.8%, 1107/1246), *yagV* (87.1%, 1085/1246) were highly prevalent among the *Klebsiella* spp. (n=1246). Furthermore, virulence genes encoding the virulence factors *ybt*, *iuc* and *iro* were also identified by the VFDB databases. These genes, along with *clb*, are considered virulent markers in *Klebsiella*. All MCRPK (n=14) recovered in this study possessed *entA*, *entB*, *fepC*, *ompA*, *ykgK*, *yagZ*, *yagX*, *yagW*, and *yagV* virulence determinants. However, no siderophore-associated virulence loci (*ybt*, *iuc*, *iro*) or the colibactin gene cluster (*clb*) were detected in any MCRPK isolate. K-antigen (capsular polysaccharide) and O-antigen (lipopolysaccharides) types of the 14 MCRPK were identified using ‘Kaptive’, a bioinformatics tool designed to type the capsule (K) and lipopolysaccharide (O) in *K. pneumoniae* and related species from WGS data. Four different K types were assigned to seven isolates: K45 (n=3), K49 (n=2), K28 (n=1), and K54 (n=1). Kaptive was unable to predict any K type for the remaining seven isolates. O1ab (n=6) was the most frequently found O serotype among the 14 MCRPK, followed by O3/3a (n=2), O3b (n=2), O5 (n=2), while two isolates could not be assigned any O type (Figure 7.7).

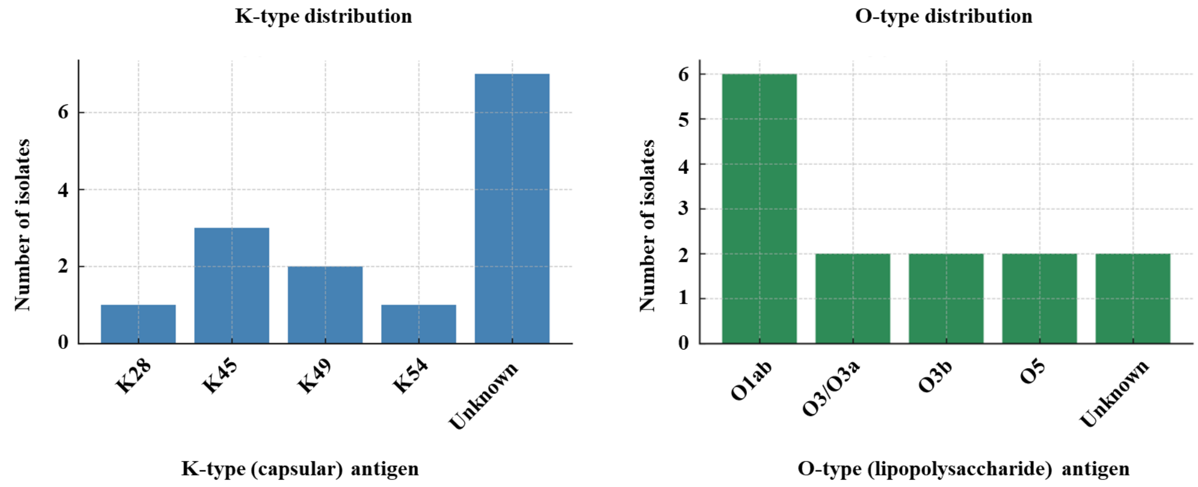


Figure 7.7 Distribution of K and O antigen types among MCRPK.

7.2.3 Clonal distribution of MCRPK

Clonal and Phylogenetic analyses were restricted to MCRPK isolates (n=14). Although additional isolates exhibited phenotypic resistance to colistin by antimicrobial susceptibility testing, these were excluded from phylogenetic reconstruction if no *mcr* gene was detected. This approach was adopted to specifically characterise the clonal diversity and genomic relatedness of mobile colistin resistance, as phenotypic colistin resistance in the absence of *mcr* is commonly mediated by chromosomal mutations (e.g., in *mgrB*, *pmrAB*, or *phoPQ*) and represents a distinct resistance mechanism.

Diverse sequence types (STs) were identified in MCRPK (n=14) from various sources and sampling locations within the study area. The most frequently occurring ST was ST1 (n=4), followed by single isolates from ST273, ST309, ST378, ST534, ST735, ST1844, ST1998, ST7730, ST7748, and ST7907 (Figure 7.8). The maximum-likelihood phylogeny shown in Figure 7.8 was constructed using core-genome alignments derived from Illumina short-read sequencing data. Reads were mapped and aligned using the Bactmap pipeline, and phylogenetic inference was performed on the resulting core-genome alignment using VeryFastTree (v.4.0). Sequence typing was assigned using the PubMLST database based on seven housekeeping loci. Antimicrobial resistance genes were identified using the ABRicate database, and resistance and virulence scores were determined using Kleborate (v.3.0, a dedicated genotypic tool for *K. pneumoniae* species complex) was used to assign resistance and virulence scores for MCRPK (described in Chapter 4 and 5) (Lam *et al.,* 2021).

Kleborate assigned four resistance scores (0 to 3) to *K. pneumoniae*, and an isolate with a score >0 was considered MDR. Similar to genotypic resistance profiles, Kleborate assigned six scores (0 to 5) to the *K. pneumoniae* isolates based on the presence of genes encoding the virulence factors *ybt*, *iuc*, and *clb*. Isolates with a score of ≥3 were considered highly virulent. Analysis indicated that 21.4% (3 out of 14) of MCRPK were MDR with a score of 1, while 78.6% (11 out of 14) showed lower levels of resistance, scoring 0. In the virulence study, all the MCRPK (n=14) were assigned a score of 0 by Kleborate, indicating a negative result for acquired virulence genes (Figure 7.8).

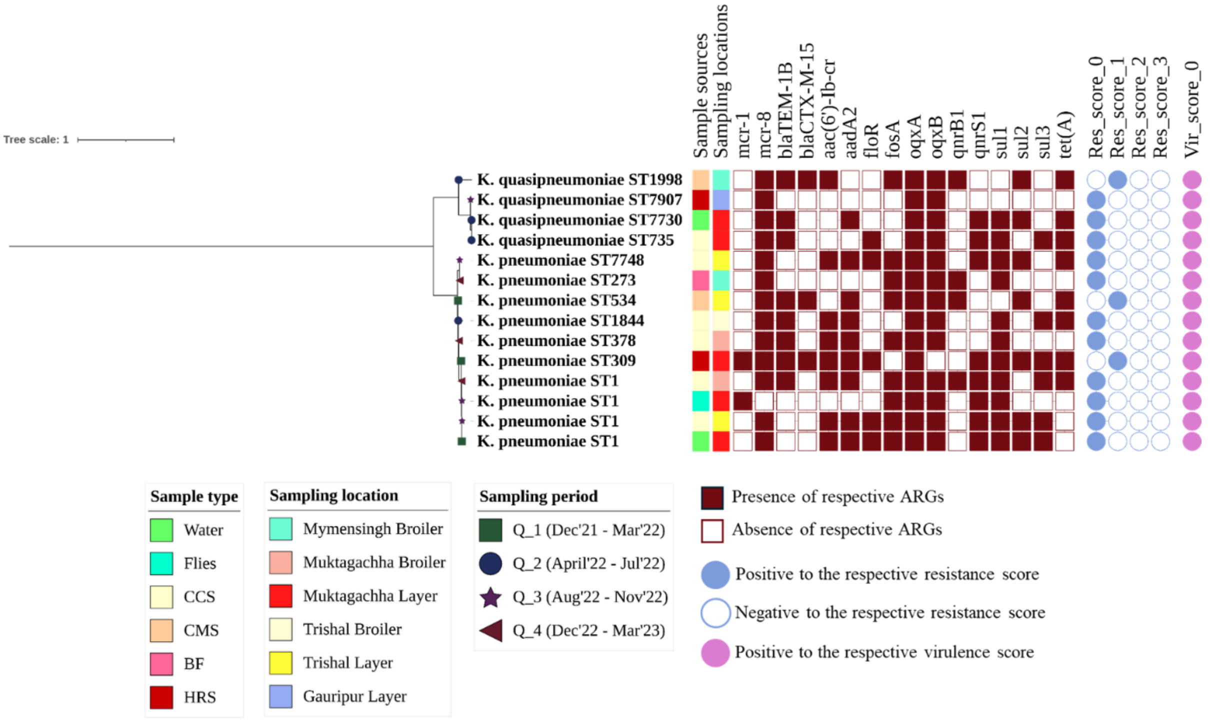


Figure 7.8 Clonal distribution of MCRPK across various sources. The ML tree was generated using VeryFastTree (v.4.0) and visualised in iTOL (v.6). Core-genome alignment was performed using the Bactmap pipeline (v.1.0.0). The PubMLST database was used for 7 loci MLST profiling. The latest ABRicate database was used to screen for ARGS, and Kleborate was used for resistance and virulence scores.

Phylogenetic analysis of MCRPK isolated in this study demonstrated that four *K. pneumoniae* isolates belonged to ST1 and clustered into a single clade (Figure 7.8). Subsequent core-genome SNP analysis of these four ST1 isolates (three *mcr-8* carriers and one *mcr-1* carrier) revealed that pairwise SNP distances spanned from 7 to 188 SNPs (Figure 7.9). The pairwise SNP difference between 261590_OHBC129KP (from CCS of Trishal layer farm) and 261476_OHBW25KP (from water of Muktagachha layer farm) showed the closest relationship with 7 SNP differences. The *mcr-1* isolate 261414_OHBF158KP (from flies of Muktagachha layer farm) was moderately related (79–83 SNPs) to the *mcr-8* pair (261476_OHBW25KP and 261590_OHBC129KP). 261789_OHBC165KP (from CCS of Muktagachha broiler farm) is the most divergent, differing by 183–188 SNPs from the other isolates.

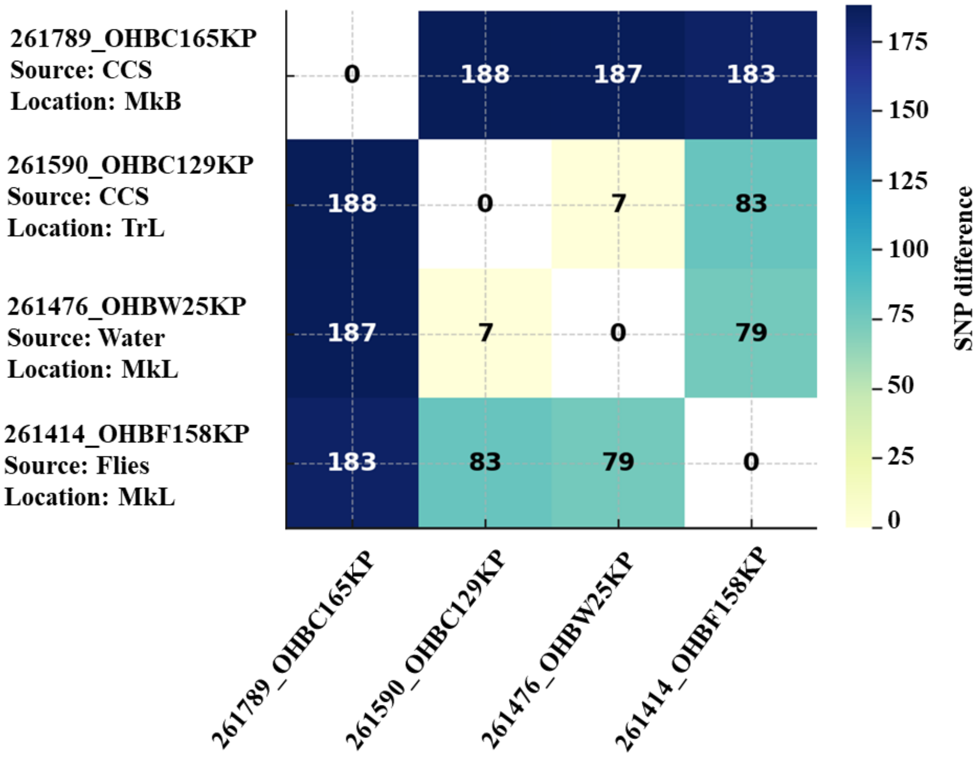


Figure 7.9 Pairwise SNP distance heatmap among ST1 MCRPK isolates. In this heatmap, all diagonal cells are white and labelled “0”, which reflects the self-comparisons resulting in no SNP differences.

7.2.4 Plasmid profile of MCRPK

To explore how plasmids contribute to the spread of *mcr* in *Klebsiella* across humans, animals, and the environment, all 14 MCRPK isolates were subjected to both short-read (Illumina NovaSeq 6000) and long-read sequencing (PromethION). Hybrid genome assemblies were generated by combining short- and long-read data to enable accurate plasmid reconstruction and localisation of *mcr* genes.

Plasmid replicon typing was performed using the PlasmidFinder database, which identified a diverse array of plasmid replicon markers and the presence of one or more plasmid replicons among the MCRPK isolates. IncFIB(K)Kpn3 (85.7%, 12/14), Col440I (71.4%, 10/14), IncFIA(HI1) (64.3%, 9/14), IncR (64.3%, 9/14), IncFII(pKP91) (57.1%, 8/14), ColRNAI (35.7%, 5/14) and IncFIB(pQil) (21.4%, 3/14) were the frequently found replicon types. Among the plasmid replicon types identified in MCRPK, *mcr-8* was associated with IncFIA(HI1) (n=3), IncR (n=3), IncFIB(pQil) (n=3) and IncFIB(K)Kpn3 (n=2) conjugative backbones, whereas the *mcr-1* determinant was associated with IncHI1A replicon (n=1) (Figure 7.10). The phylogenetic tree shown in Figure 7.10 is based on the same short-read-derived core-genome alignment used for Figure 7.8. However, in this figure, plasmid replicon types are overlaid onto the chromosomal phylogeny as metadata to visualise plasmid diversity and the association of mcr genes with specific plasmid replicon types. This approach demonstrates that genetically distinct MCRPK isolates may harbour similar *mcr*-carrying plasmids, while closely related isolates may differ in plasmid content.

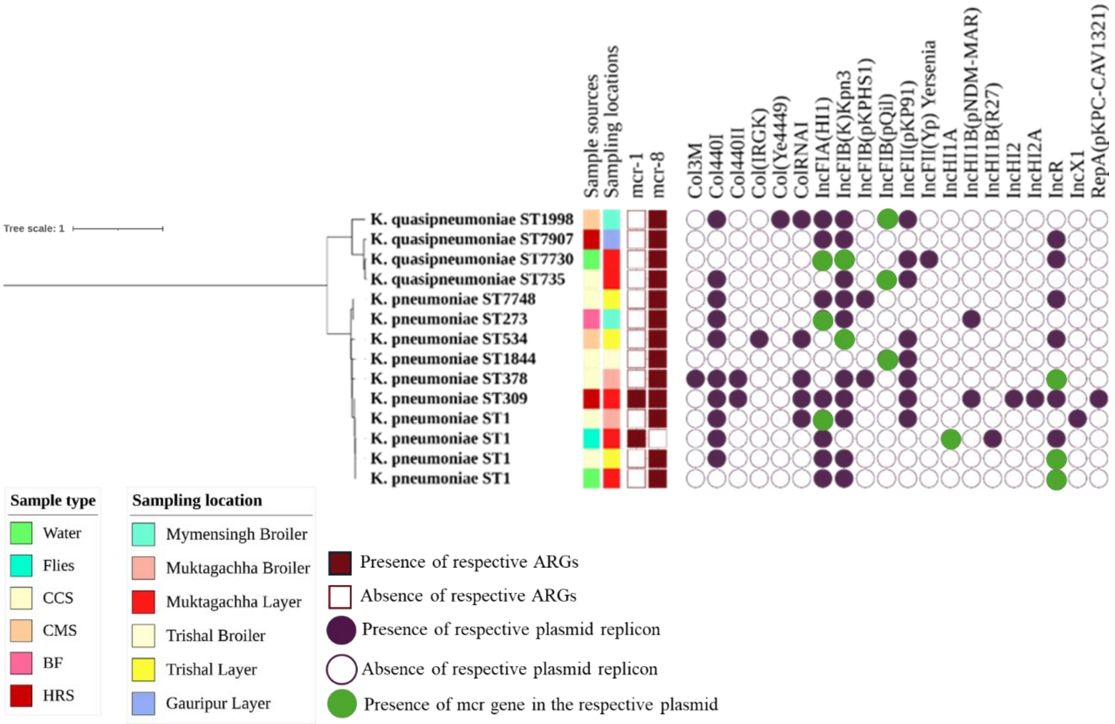


Figure 7.10 Distribution of plasmid replicon types in MCRPK across various sources. The ML tree was generated using VeryFastTree (v.4.0) and visualised in iTOL (v.6). Core-genome alignment was performed using the Bactmap pipeline (v.1.0.0). The PubMLST database was used for 7 loci MLST profiling. The ABRicate database was used to screen for ARGS, and the PlasmidFinder database was used for plasmid replicon typing.

7.2.4.1 Genetic context of plasmids carrying *mcr-8* and *mcr-1*

Complete circular plasmids carrying *mcr-8.1* belonging to IncFIA(HI1) of ~55kb (n=1) and IncR of ~57kb (n=1) were obtained in this study by hybrid assembly (Figure 7.11). Other plasmids carrying *mcr* genes were not successfully closed. Genome-wide analysis of circular IncFIA(HI1) revealed a conserved region of *mcr-8.1* (*gt-mcr-8.1-copR*) alongside additional resistance loci *qnrB1* and β-lactamase. The circular IncR plasmid also preserved *mcr-8.1* (*gt-mcr-8.1-copR*) while additionally encoding other resistance genes *bla*$_{\text{TEM-1}}$, *sul1* and *aac(6')-lb*. Further analysis of the *mcr*-harbouring plasmids showed that the ARGs (*aadA2*, *bla*$_{OXA\text{-}1}$, *bla*$_{TEM\text{-}1B}$, *floR*, *qnrB1*, *sul1*, *sul2*, *sul3* and *tet(A)*), which were significantly linked to MCRPK in this study (Table 7.7), were also variably found on these plasmids (Figure 7.12).

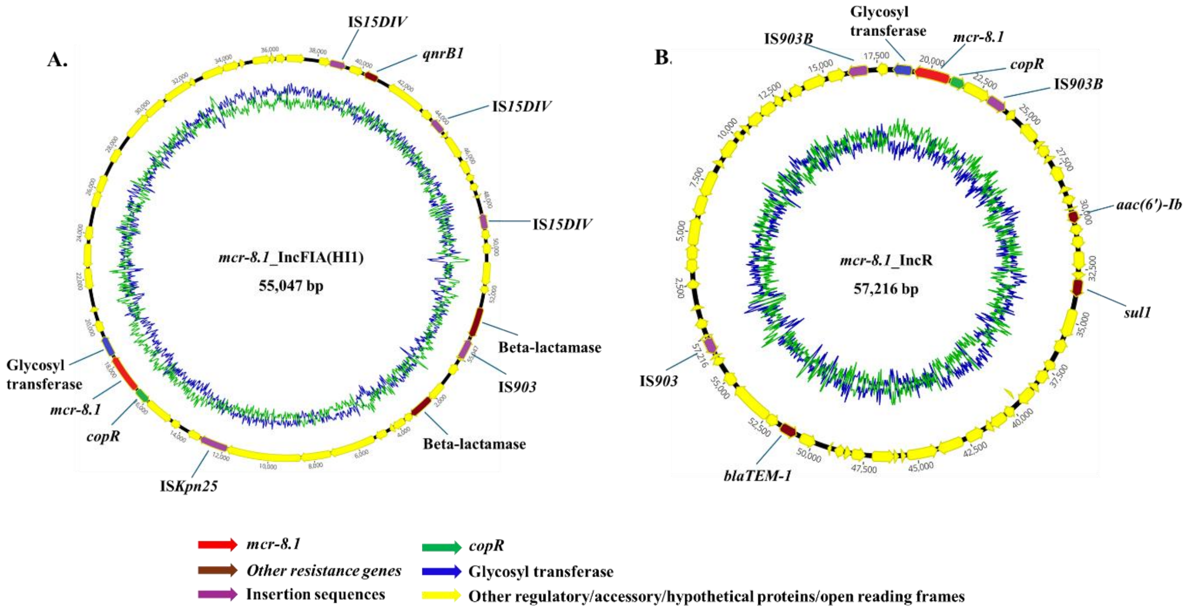


Figure 7.11 Schematic layout of IncFIA(HI1) (A) and IncR (B) plasmids carrying *mcr-8.1* identified in this study. Arrows represent the position and transcriptional direction of the open reading frames. The figures were produced using Geneious Prime (v.2025.1).

Genomic comparisons at the nucleotide level showed that *mcr-8.1* positive IncFIA(HI1) (n=3) plasmids identified in this study shared ≥ 99% similarity at ≥ 73% coverage, IncR (n=3) shared ≥ 99% similarity at ≥ 65% coverage, IncFIB(pQil) (n=3) shared ≥ 99% similarity at ≥ 69% coverage, and IncFIB(K)Kpn3 (n=2) shared ≥ 98% similarity at ≥ 67% coverage. The host origins of IncFIA(HI1), IncR, IncFIB(pQil), and IncFIB(K)Kpn3 plasmids are shown in Figure 7.12.

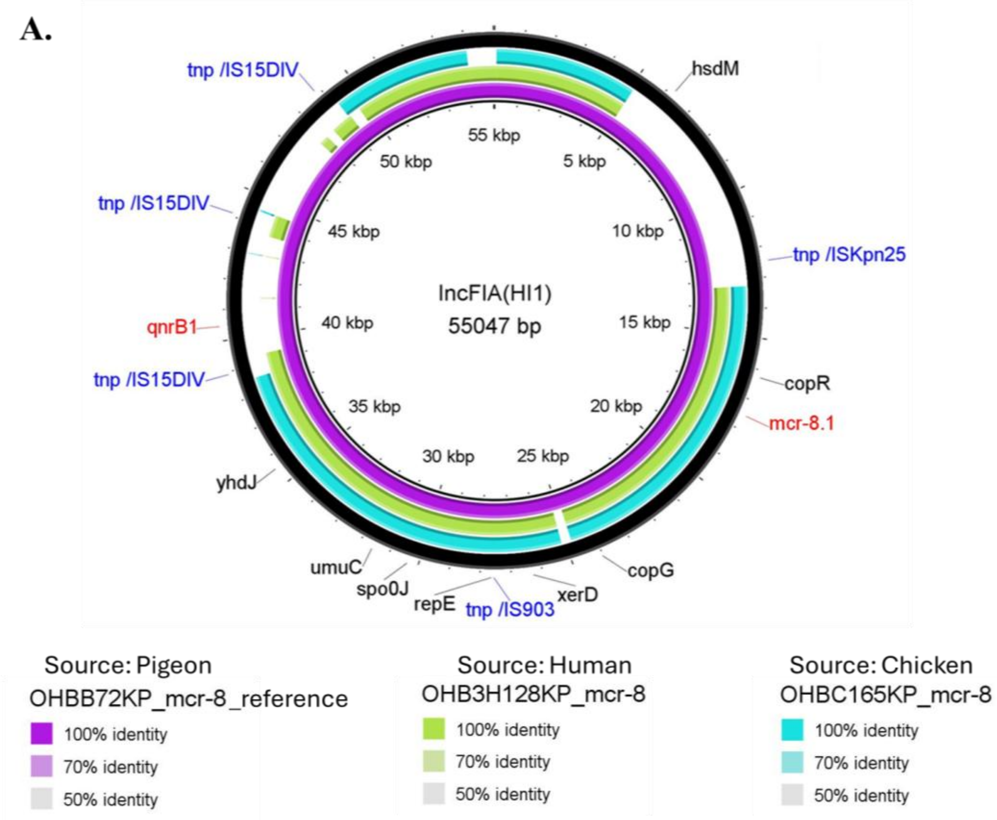
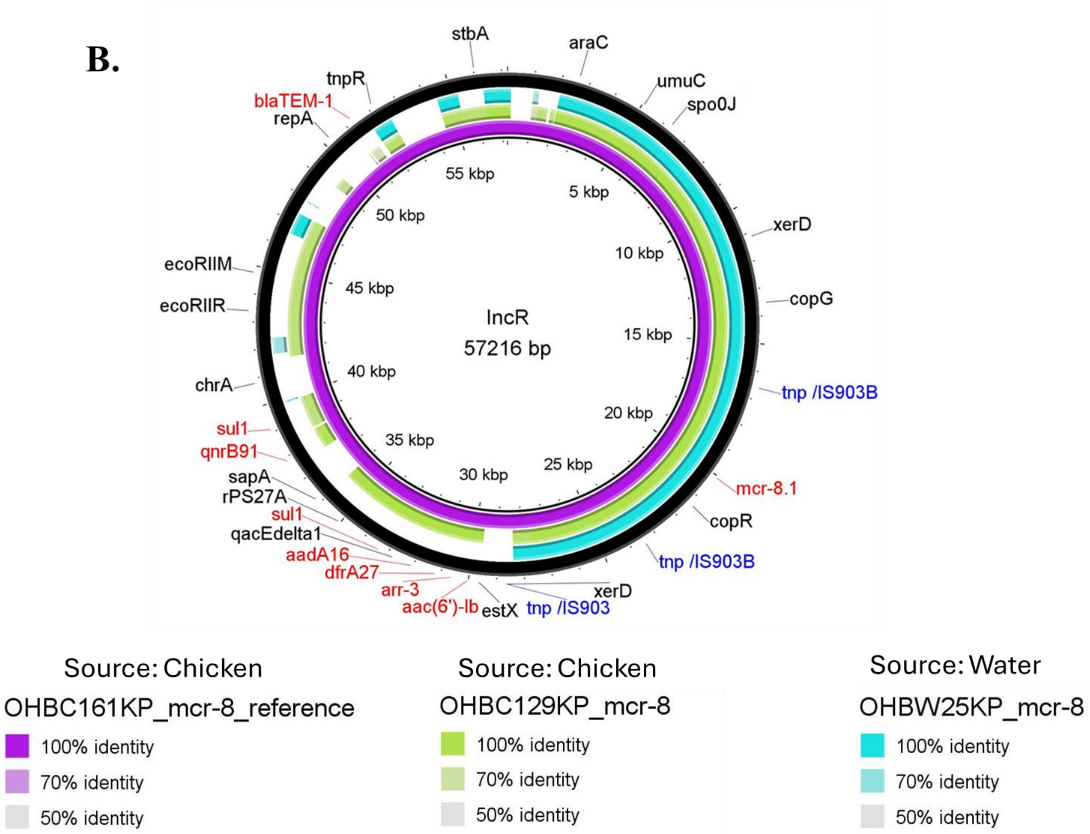
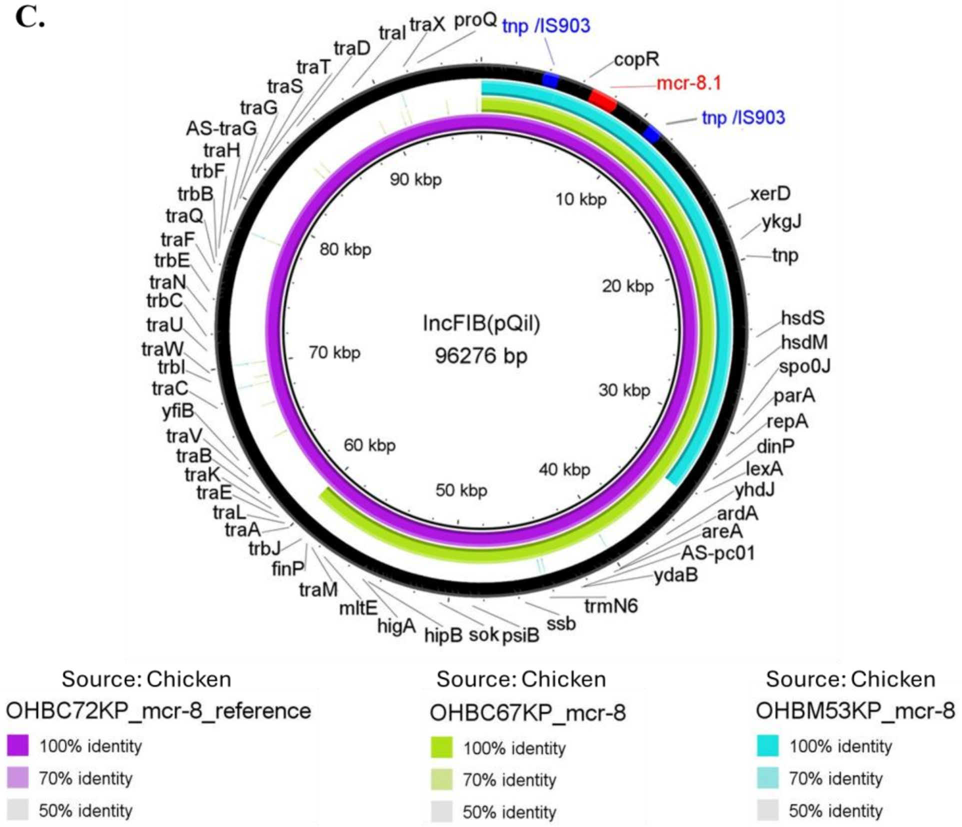


Figure 7.12 Circular sequence alignments of reference plasmids with homologous contigs of four groups of plasmids harbouring the *mcr-8* gene using BLASTn and BLAST Ring Image Generator (BRIG). The concentric rings display similarity between the reference sequence in the inner ring and the other sequences in the outer rings. The various colour levels indicate a BLAST result with a matched degree of shared regions, as shown at the bottom of the ring. (A) Comparative analysis of plasmids belonged to IncFIA(HI1), (B) Comparative analysis of plasmids belonged to IncR, (C) Comparative analysis of plasmids belonged to IncFIB(pQil), (D) Comparative analysis of plasmids belonged to IncFIB(K)Kpn3.

B.


stbA
araC
umuC
spo0J
tnpR
blaTEM-1
repA
xerD
copG
tnp /IS903B
mcr-8.1
copR
tnp /IS903B
xerD
tnp /IS903
estX
aac(6')-Ib
arr-3
dfrA27
aadA16
qacEdelta1
sul1
rPS27A
sapA
qnrB91
sul1
chrA
ecoRIIR
ecoRIIM
55 kbp
5 kbp
50 kbp
10 kbp
45 kbp
15 kbp
40 kbp
20 kbp
35 kbp
30 kbp
25 kbp
IncR
57216 bp
Source: Chicken
OHBC161KP_mcr-8_reference
100% identity
70% identity
50% identity
Source: Chicken
OHBC129KP_mcr-8
100% identity
70% identity
50% identity
Source: Water
OHBW25KP_mcr-8
100% identity
70% identity
50% identity

C.


traI
traX
proQ
tnp /IS903
copR
mcr-8.1
tnp /IS903
traD
traT
traS
traG
AS-traG
traH
trbF
trbB
traQ
traF
trbE
traN
trbC
traU
traW
trbI
traC
yfiB
traV
traB
traK
traE
traL
traA
trbJ
finP
traM
mltE
higA
hipB
sok
psiB
ssb
trmN6
ydaB
AS-pc01
areA
ardA
yhdJ
lexA
dinP
repA
parA
spo0J
hsdM
hsdS
tnp
ykgJ
xerD
90 kbp
10 kbp
80 kbp
20 kbp
70 kbp
30 kbp
60 kbp
40 kbp
50 kbp
IncFIB(pQil)
96276 bp
Source: Chicken
OHBC72KP_mcr-8_reference
100% identity
70% identity
50% identity
Source: Chicken
OHBC67KP_mcr-8
100% identity
70% identity
50% identity
Source: Chicken
OHBM53KP_mcr-8
100% identity
70% identity
50% identity

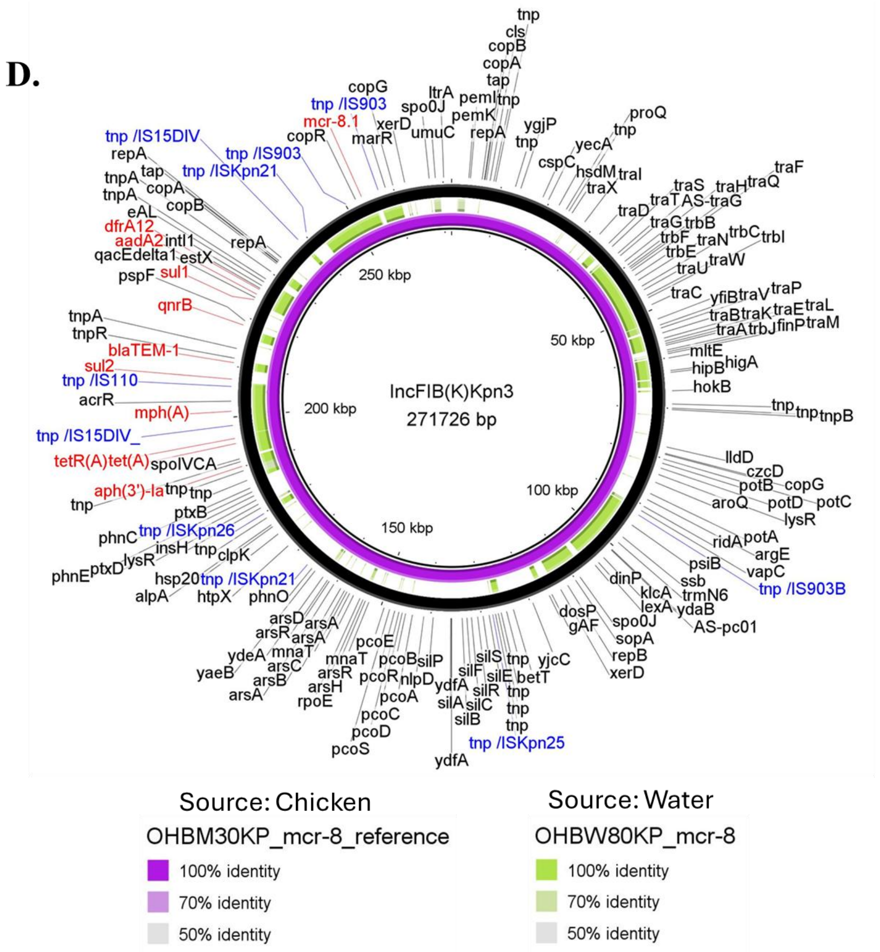
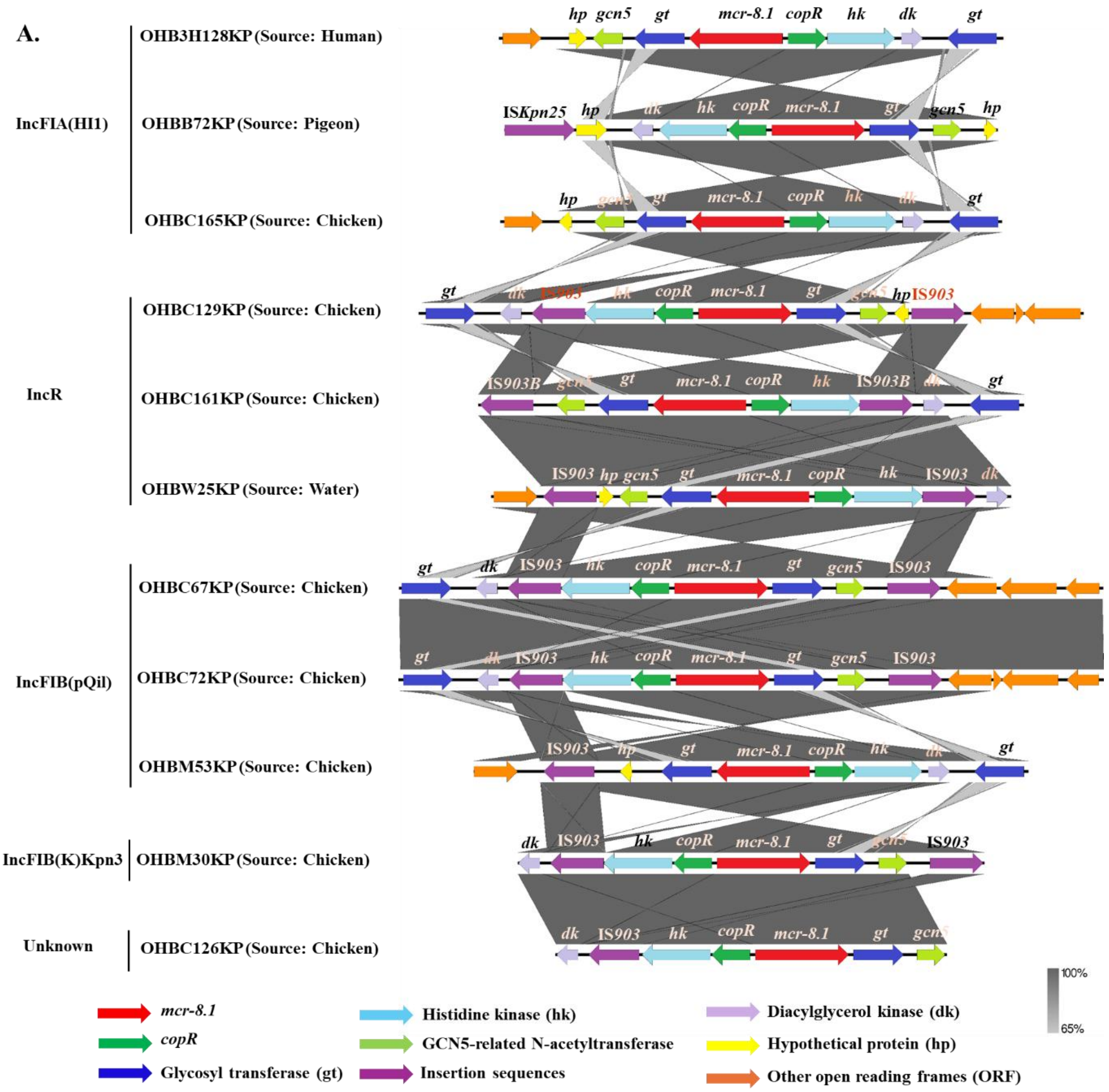


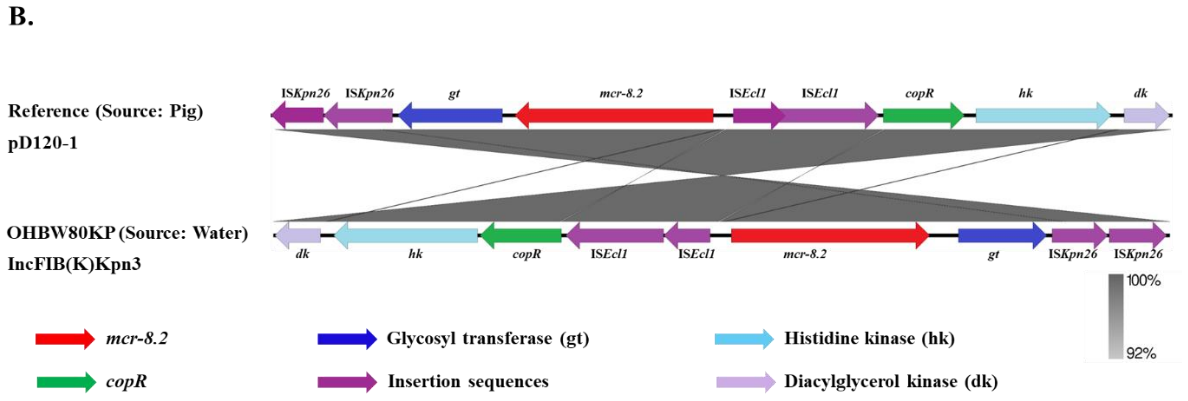
Figure 7.12 Circular sequence alignments of reference plasmids with homologous contigs of four groups of plasmids harbouring the *mcr-8* gene using BLASTn and BLAST Ring Image Generator (BRIG). The concentric rings display similarity between the reference sequence in the inner ring and the other sequences in the outer rings. The various colour levels indicate a BLAST result with a matched degree of shared regions, as shown at the bottom of the ring. (A) Comparative analysis of plasmids belonged to IncFIA(HI1), (B) Comparative analysis of plasmids belonged to IncR, (C) Comparative analysis of plasmids belonged to IncFIB(pQil), (D) Comparative analysis of plasmids belonged to IncFIB(K)Kpn3.

Genetic analysis of the plasmids carrying *mcr* genes revealed several variants, including *mcr-1.1*, *mcr-8.1*, and *mcr-8.2*. A detailed investigation of the genetic environment adjacent to *mcr-8.1* revealed that all plasmids had a highly conserved segment of ~3500 bp containing *copR* (downstream of *mcr-8.1*), *mcr-8.1* and glycosyl transferase (upstream of *mcr-8.1*) (Figure 7.13). Among IncFIA(HI1) plasmids isolated from both human and chicken, no insertion sequences were found flanking the conserved region. However, the IncFIA(HI1) from a pigeon isolate contained one complete IS*Kpn25* element downstream of *mcr-8.1*. In contrast, two complete copies of IS*903* bracketed the conserved region of *mcr-8.1* in IncR (n=3), IncFIB(pQil) (n=2), IncFIB(K)Kpn3 (n=1) plasmids. Additionally, one IncFIB(pQil) (OHBM53KP) plasmid had one complete IS*903* element upstream of *mcr-8.1,* while an unidentified plasmid (OHBC126KP) carried a single IS*903* downstream of the *mcr-8.1 gene* (Figure 7.13A).

The genetic environment surrounding *mcr-8.2* in IncFIB(K)Kpn3 shared identity (>90%) with a previously described *mcr-8.2* containing plasmid isolated from pigs in China (accession no: NZ_CP034679.1). In both plasmids, the conserved region of *mcr-8.2* encompassing glycosyl transferase and *mcr-8.2* was flanked upstream by two IS*Kpn26* and downstream by two IS*Ecl1* elements (Figure 7.13B).

A linear comparison of the genetic context of *mcr-1.1* on IncHI1 plasmid using a reference plasmid (accession no. CP064026.1) recovered from a human *E. coli* isolate in Bangladesh, revealed a similar conserved segment containing *mcr-1.1* and *pap2* (upstream of *mcr-1.1*). In the reference plasmid pRS571-MCR-1.1, the conserved segment was flanked downstream by IS*Apl1* (Figure 7.13C). IncHI1A plasmid in *K. pneumoniae*, identified from a fly sample in this study, contained two copies of *mcr-1* and one copy of *mcr-1.1*. In this plasmid, IS*Apl1* (downstream) and IS*Kpn26* (upstream) flanked the *mcr-1.1* conserved region, while IS*Apl1* (downstream) and IS*Kpn26* (upstream) flanked both *mcr-1* regions (Figure 7.13C).


A.
IncFIA(HI1)
OHB3H128KP (Source: Human)
OHBB72KP (Source: Pigeon)
OHBC165KP (Source: Chicken)
IncR
OHBC129KP (Source: Chicken)
OHBC161KP (Source: Chicken)
OHBW25KP (Source: Water)
IncFIB(pQil)
OHBC67KP (Source: Chicken)
OHBC72KP (Source: Chicken)
OHBM53KP (Source: Chicken)
IncFIB(K)Kpn3
OHBM30KP (Source: Chicken)
Unknown
OHBC126KP (Source: Chicken)
hp gcn5 gt mcr-8.1 copR hk dk gt
ISKpn25 hp dk hk copR mcr-8.1 gt gcn5 hp
hp gcn5 gt mcr-8.1 copR hk dk gt
gt dk IS903 hk copR mcr-8.1 gt gcn5 hp IS903
IS903B gcn5 gt mcr-8.1 copR hk IS903B dk gt
IS903 hp gcn5 gt mcr-8.1 copR hk IS903 dk
gt dk IS903 hk copR mcr-8.1 gt gcn5 IS903
gt dk IS903 hk copR mcr-8.1 gt gcn5 IS903
IS903 hp gt mcr-8.1 copR hk dk gt
dk IS903 hk copR mcr-8.1 gt gcn5 IS903
dk IS903 hk copR mcr-8.1 gt gcn5
100%
65%
mcr-8.1
copR
Glycosyl transferase (gt)
Histidine kinase (hk)
GCN5-related N-acetyltransferase
Insertion sequences
Diacylglycerol kinase (dk)
Hypothetical protein (hp)
Other open reading frames (ORF)


B.
Reference (Source: Pig)
pD120-1
ISKpn26 ISKpn26 gt mcr-8.2 ISEcl1 ISEcl1 copR hk dk
OHBW80KP (Source: Water)
IncFIB(K)Kpn3
dk hk copR ISEcl1 ISEcl1 mcr-8.2 gt ISKpn26 ISKpn26
100%
92%
mcr-8.2
copR
Glycosyl transferase (gt)
Insertion sequences
Histidine kinase (hk)
Diacylglycerol kinase (dk)

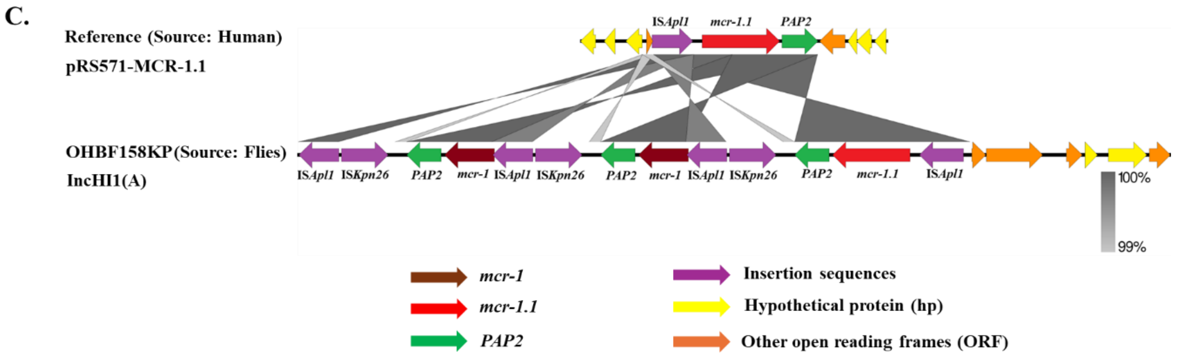


Figure 7.13 Linear comparison of the genetic context of *mcr* genes on various Inc-type plasmids. Arrows represent the position and transcriptional direction of the open reading frames. Grey cross-links between sequences demonstrate regions of sequence homology. The genomic comparison was performed by EasyFig (v2.2.5). A. Genetic environment of *mcr-8.1* on IncFIA(HI1), IncR (n=3), IncFIB(pQil), IncFIB(K)Kpn3 and one unknown plasmid. B. Genetic environment of *mcr-8.2* on IncFIB(K)Kpn3 (plasmid pD120-1, accession number: NZ_CP034679.1, was used as a reference for comparison). C. Genetic environment of *mcr-1.1* on IncHI1A plasmid (plasmid pRS571-MCR-1.1 from human, accession number: CP034390.1, was used as a reference for comparison).

7.2.5 Risk factors associated with the spread of *mcr* genes in *Klebsiella* spp.

MCRPK isolates were identified from six of the ten sampling sites on the poultry farms. However, their occurrence was significantly associated with the Muktagachha layer farm and with samples recovered from chicken cloacal swabs (Table 7.5 and 7.6). Based on these findings, antibiotic usage from farms and the demographic features of humans with MCRPK cases were further analysed to identify possible risk factors contributing to the spread of MCRPK.

7.2.5.1 Antibiotic usage practices on poultry farms

Among the ten poultry farms surveyed (five broiler and five-layer farms), all farmers reported some knowledge of AMR, while 80% (8/10) were aware of the antibiotic withdrawal period. Eight farms (80%) were operated under intensive rearing systems (confined indoor management), and two (20%) were operated under semi-intensive systems (partial housing and ranging). Poultry waste disposal practices included on-site disposal in the farm pits (5/10, 50%), manure application in the field (3/10, 30%) and other methods (2/10, 20%).

Antibiotic usage was observed during all four sample collection periods, including amoxicillin (used in 5 farms), doxycycline (5 farms), ciprofloxacin (3 farms), neomycin, trimethoprim and sulphadiazine (3 farms), and levofloxacin (3 farms). The use of gentamicin, tiamulin, tilmicosin, and colistin sulphate was also observed in at least one of the farms. Multiple farms reported using more than one antibiotic or a combination of antibiotics (Table 7.8). No antibiotic use was reported in the Mymensingh broiler farm. The highest number (5 out of 14) of MCRPK was recovered from Muktagachha layer farm, which used multiple antibiotics during sampling and practised intensive farming and manure-based waste disposal. In this study, MCRPK was more commonly isolated from layer farms, with 9 out of 14 positive cases: five from Muktagachha, three from Trishal, and one from Gauripur. In contrast, broiler farms showed five out of 14 positives, with two from Mymensingh, two from Muktagachha, and one from Trishal. Ten out of 14 (71.4%) MCRPK were isolated from farms where poultry wastes were repurposed either as manure (7/14) or through other means (3/14).

Table 7.8 Farm practices, antibiotic use and *mcr*-positive *Klebsiella* isolates in the study area.

Farm	**Rearing system**	**Knowledge of AMR**	**Antibiotic withdrawal knowledge**	**Farm waste disposal method**	**Antibiotics used (Q1/Q2/Q3/Q4)**	**MCRPK isolates**
Mymensingh broiler	Intensive	Yes	Yes	Poultry manure	– / – / – / –	2
Mymensingh layer	Intensive	Yes	Yes	On-site	Neo / Col / – / Cip, Amx	0
Muktagachha broiler	Intensive	Yes	No	Others	Amx / Amx + Enro / Amx / Dox	2
Muktagachha layer	Intensive	Yes	Yes	Poultry manure	– / Tilm + Trim + Sulfa / Amx / Dox	5
Trishal broiler	Semi-intensive	Yes	No	On-site	– / Trim + Sulfa + Genta / Cip + Azi / Amx + Enro	1
Trishal layer	Intensive	Yes	Yes	On-site	Nor / Lev / Lev + Tia / Dox	3
Gauripur broiler	Semi-intensive	Yes	Yes	On-site	Genta + Lev / Genta + Lev / – / Genta + Lev	0
Gauripur layer	Intensive	Yes	Yes	Others	– / Dox + Nor / – / Dox + Trim + Sulfa	1
Tarakanda broiler	Intensive	Yes	Yes	On-site	Lev / Neo + Cip / Cip / –	0
Tarakanda layer	Intensive	Yes	Yes	Poultry manure	– / Neo + Dox / Amx / Tia	0

AMR, antimicrobial resistance; MCRPK, mcr-positive *Klebsiella* spp.; Q-1, sampling quarter-1 (December 2021 – March 2022); Q-2, sampling quarter-2 (April 2022 – July 2022); Q-3, sampling quarter-3 (August 2022 – November 2022); Q-4, sampling quarter-4 (December 2022 – March 2023). Amx = Amoxicillin, Azi = Azithromycin, Cip = Ciprofloxacin, Col = Colistin, Dox = Doxycycline, Enro = Enrofloxacin, Genta = Gentamicin, Lev = Levofloxacin, Neo = Neomycin, Nor = Norfloxacin, Sulfa = Sulphadiazine, Tia =Tiamulin, Tilm = Tilmicosin, Trim = Trimethoprim. '–' indicates no use of antibiotics during that sampling time, and '+' indicates a combination of antibiotic use.

7.2.5.2 Description of human cases with MCRPK

In this study, MCRPK was isolated from rectal swabs of two healthy human volunteers from different sampling locations: one from the Muktagachha layer farm (Case 1, 269090_OHB1H230Kp) and another from the Gauripur layer farm (Case 2, 270276_OHB3H128Kp).

Case 1 was a 42-year-old male from Muktagachha who resided 300 meters away from the nearest poultry farm, raised chickens and goats, and disposed of household waste near his residence. Case 2 was a 35-year-old male from Gauripur who lived approximately 1000 meters from the nearest poultry farm, raised chickens in the household, and similarly disposed of waste nearby. Both cases have common demographic characteristics (e.g., gender, education, and occupation) and sanitation facilities in their houses (Table 7.9). Neither case reported antibiotic use within the past three months nor hospital admission within the previous six months before enrollment in this study.

The samples from Case 1 and Case 2 were collected approximately 11 months apart. The isolate from Case 1 was identified as *K. pneumoniae* ST309 and carried both *mcr-1* and *mcr-8* genes. The Case 1 isolate displayed resistance to multiple antibiotics, including β-lactams, aminoglycosides, fluoroquinolones, fosfomycin, colistin and tigecycline, but remained susceptible to carbapenems (meropenem and imipenem) and β-lactams/β-lactamase inhibitor combinations (ceftazidime-avibactam and piperacillin-tazobactam) (Table 7.10).

The isolate from Case 2 was identified as *K. quasipneumoniae* ST7907. Genetic analysis revealed the presence of the *mcr-8* gene, conferring colistin resistance. Unlike Case 1, Case 2 isolate showed susceptibility to most antibiotics tested, aside from resistance to colistin and ceftriaxone.

Table 7.9 Characteristics of the human cases with MCRPK.

Features	**Case 1**	**Case 2**
Sample ID	269090_OHB1H230Kp	270276_OHB3H128Kp
Sampling location	Muktagachha layer farm	Gauripur layer farm
Sample type	Rectal swab	Rectal swab
Date of sampling	03.01.2022	25.11.2022
Name of organism	*K. pneumoniae*	*K. quasipneumoniae*
***mcr*-variant**	*mcr-1* and *mcr-8*	*mcr-8*
ST type	309	7907
Gender	Male	Male
Age	42	35
Underlying diseases	No	No
Education	Primary	Primary
Occupation	Farmer	Farmer
Household waste disposal method	Disposed of near house	Disposed of near house
Raising domestic animals	Yes (Chicken, goat)	Yes (Chicken)
Distance to Nearest Farm (meters)	Approximately 300	Approximately 1000
Drinking water	Tube well water	Tube well water
Toilet facilities are available at home	Simple pit latrines	Simple pit latrines
Access to water and soap in the toilet	Yes	Yes
Antibiotic use (last 3 months)	No	No
Hospital admission (last 6 months)	No	No

Table 7.10 Antibiotic susceptibility test results for MCRPK isolates recovered from human cases.

Antibiotic	**Breakpoint**	**Case 1**	**Case 2**
AMC	(S≤8, R>8)	64 (R)	8 (S)
TZP	(S≤8, R>8)	0.5 (S)	2 (S)
ATM	(S≤1, R>4)	32 (R)	0.06 (S)
CTX	(S≤1, R>2)	8 (R)	8 (R)
CAZ	(S≤1, R>4)	32 (R)	0.25 (S)
FEP	(S≤1, R>4)	8 (R)	0.06 (S)
CZA	(S≤8, R>8)	0.06 (S)	0.125 (S)
IPM	(S≤2, R>4)	0.5 (S)	0.25 (S)
MEM	(S≤2, R>8)	0.06 (S)	0.06 (S)
CIP	(S≤0.25, R>0.5)	64 (R)	0.06 (S)
LVX	(S≤0.5, R>1)	32 (R)	0.06 (S)
AMK	(S≤8, R>8)	64 (R)	2 (S)
GEN	(S≤2, R>2)	64 (R)	0.5 (S)
FOS	(S≤8, R>8)	32 (R)	2 (S)
CST	(S≤2, R>2)	>64 (R)	16 (R)
TGC	(S≤0.5, R>0.5)	4 (R)	0.5 (S)

MIC breakpoints for antimicrobial susceptibility were interpreted according to the guidelines by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2022) (v12.0). AMC, Amoxicillin–clavulanic acid; TZP, Piperacillin–tazobactam; CTX, Cefotaxime; CAZ, Ceftazidime; FEP, Cefepime; CZA, Ceftazidime–avibactam; ATM, Aztreonam; IPM, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; LVX, Levofloxacin; AMK, Amikacin; GEN, Gentamicin; FOS, Fosfomycin; TGC, Tigecycline; CST, Colistin.

7.3 Discussion

Third-generation cephalosporin and carbapenem-resistant *Klebsiella* are a growing global concern due to the scarcity of effective treatment options and are listed as a top-priority pathogen by the WHO (WHO BBPL, 2024). To tackle complicated infections caused by carbapenem-resistant *Klebsiella*, colistin is recommended as a last-resort antibiotic. However, its widespread use in both livestock and for treating complex human infections has led to the development of colistin resistance (Luo *et al.,* 2020). Colistin (polymyxin E) is a polypeptide antibiotic of the polymyxin class with broad-spectrum efficacy against MDR Enterobacteriaceae infections, including sepsis caused by *Klebsiella*. Colistin binds to the outer membrane lipopolysaccharide (LPS) of the Gram-negative bacteria, leading to increased permeability and cell lysis. The resistance mechanism of *Klebsiella* spp. to colistin is via chromosomal mutations (e.g., *pmrA*/*pmrB*, *phoP*/*phoQ*, and *mgrB* gene) and/or acquisition of plasmid-mediated *mcr* genes. The *mcr* genes encode phosphoethanolamine (pEtN) transferases that alter the lipid A component of LPS, resulting in resistance (Liu *et al.,* 2016; Sabnis *et al.,* 2021; Liu *et al.,* 2024; Zhang *et al.,* 2025). This study focused particularly on the molecular epidemiology of plasmid-mediated colistin resistance driven by *mcr* genes in *Klebsiella* spp. isolated from human, animal and environmental sources.

In my study, phenotypic colistin resistance was observed in 3.1 % (39/1246) of *Klebsiella* spp. isolated from human-animal-environment interfaces, with 1.1% (14/1246) prevalence of *mcr* genes (Table 7.1). This disparity reflects a recognised genotype–phenotype discordance for polymyxins, where plasmid-mediated resistance determinants are not detected in all phenotypically resistant isolates (Liu *et al*., 2016; Poirel *et al*., 2017). Since genomic analyses in this study primarily focused on acquired resistance genes and plasmid-associated determinants, systematic assessment of chromosomal mutations associated with colistin resistance was not performed, representing a limitation of the current analysis. In *K. pneumoniae*, colistin resistance is predominantly mediated by chromosomal alterations, most commonly through inactivation of the negative regulator *mgrB* or activating mutations in the two-component regulatory systems *pmrA*/*pmrB* and *phoP*/*phoQ*. These changes lead to lipid A modification and reduced polymyxin binding independently of horizontally acquired genes (Olaitan *et al*., 2014;

Cannatelli *et al*., 2015; Poirel *et al*., 2017). Such mutations are strain-specific and are not detectable using standard resistance gene screening tools, providing a biologically plausible explanation for the observed discordance. In addition to biological mechanisms, technical factors may also contribute to discordant findings. Phenotypic susceptibility testing for colistin is technically challenging and subject to methodological variability due to factors such as poor diffusion, cation interactions, and adsorption to laboratory plastics (EUCAST, 2016; Humphries *et al*., 2019). Similarly, sequencing depth, assembly fragmentation, and the absence of targeted mutational analyses of *mgrB*, *pmrA/pmrB*, and *phoP/phoQ* may therefore limit resolution of chromosomal resistance mechanisms (Ellington *et al*., 2017; Wyres *et al*., 2020). Together, these considerations highlight the need to interpret phenotypic and genotypic data in a complementary manner rather than a deterministic manner when assessing colistin resistance in *Klebsiella* spp.

In this study, *mcr*-positive *Klebsiella* isolates were recovered from CCS, CMS, BF, water, flies, and human flora. However, a significant association was observed only with CCS (7.7%) (Table 7.5). Previous studies from Bangladesh have also reported the sporadic occurrence of colistin resistance and *mcr* genes in *Klebsiella* from human, animal and environmental sources (Farzana *et al.,* 2020; Islam *et al.,* 2020; Sarker *et al.,* 2024; Mondol *et al.,* 2025). The association between *mcr*-positive *Klebsiella* and CCS may be attributed to the unregulated use of colistin in poultry farming in Bangladesh. Although the Bangladesh government banned the use of colistin in veterinary practices, studies have reported that it is still frequently used in poultry farms as a feed additive or for treatment (Islam *et al.,* 2020; Foysal *et al.,* 2024). This study also found evidence of using colistin for treating birds in one of the surveyed poultry farms. These practices may create selective pressure for the emergence of plasmid-mediated *mcr* genes among Enterobacteriaceae, including *Klebsiella* spp., within the gut microbiota of chickens (Anyanwu *et al.,* 2023). In this study, the absence of colistin-resistant or *mcr*-positive *Klebsiella* in clinical samples, including UTIs and SSIs, suggests a low burden of plasmid-mediated colistin resistance in clinical *Klebsiella* infections since colistin is not often used to treat such infections. This finding is consistent with a previous report from Bangladesh, where Farzana *et al.* (2020) also reported a very low prevalence of only 0.3% (3/1097) of *mcr-8.1* in clinical *K. pneumoniae* isolates.

In the present study, *mcr* genes were identified in both *K. pneumoniae* and *K. quasipneumoniae* with two different gene variants, *mcr-1* and *mcr-8*. Notably, *mcr-8* predominated, found in 13 of 14 MCRPK, where *mcr-1* was identified in two isolates. These findings highlight the emerging evidence of *mcr-8* in Bangladesh, which is further supported by studies involving *mcr-8.1* in clinical *K. pneumoniae* and canal water from other regions of the country (Farzana *et al.,* 2020; Mondol *et al.,* 2025). In contrast, *mcr-1* has been largely documented in *E. coli* from Bangladesh (Farzana *et al.,* 2019; Ahmed *et al.,* 2020; Dutta *et al.,* 2020; Islam *et al.,* 2020; Johura *et al.,* 2020; Ara *et al.,* 2021; Sarker *et al.,* 2024). Although *mcr-1* has been reported in *Klebsiella* spp. from India and Pakistan, no *mcr-8* has been reported in *Klebsiella* from these neighbouring countries (Hameed *et al.,* 2020; Singh *et al.,* 2021; Zhou *et al.,* 2024). However, a recent review by Zhang *et al.* (2025) showed that *mcr-8* positive *K. pneumoniae* was found to be endemic in several Asian countries, particularly China and Thailand. These findings highlight the importance of studying species-specific gene dynamics and plasmid ecology.

All the MCRPK isolated in this study were resistant to colistin and exhibited increased resistance to clinically important antibiotics, including β-lactams, aminoglycosides, quinolones and tetracyclines, but susceptible to carbapenems (Figure 7.4). A study on MDR *K. pneumoniae* harbouring the *mcr* gene reported significant resistance to colistin and multiple antibiotics but remained susceptible to carbapenems, consistent with my findings (Liu *et al.,* 2022). Aminoglycoside-resistance genes (*aadA2*), β-lactamase gene ($bla_{OXA\text{-}1}$, $bla_{TEM\text{-}1B}$), fluoroquinolone-resistance genes (*aac(6')-Ib-cr*, *qnrS1*, *qnrB1*), and tetracycline resistance gene *tet(A)* were significantly associated with MCRPK, which is consistent with the phenotypic resistance findings of my study (Table 7.7). In my study, the MIC of colistin in MCRPK ranged from 4 to >64 mg/L, where isolates carrying both *mcr-1* and *mcr-8* exhibited higher MIC values (>64 mg/L) than the isolates with single *mcr* genes (Figure 7.5). This finding suggests that the co-occurrence of multiple *mcr* genes in a single *Klebsiella* isolate may have an additive effect, increasing colistin resistance (Hadjadj *et al.,* 2019; Yu *et al.,* 2020; Wang et al., 2023).

Genomic data analysis indicated that the MCRPK recovered in my study were avirulent due to the absence of key virulence markers such as *ybt*, *iuc*, *iro, clb,* and *rmpA* genes (Lam *et al.,* 2021). Furthermore, characterisation of MCRPK revealed a diverse set of K and O antigen types (Figure 7.7).

Although the K-antigens identified in my study, including K45, K28, and K49, are primarily associated with MDR *Klebsiella* lineages, K54 is found to be linked with hypervirulent strains. This finding indicates that capsular type alone is not a reliable indicator for potential pathogenicity (Lam *et al.,* 2021). O1ab type was the most frequently detected O-antigen in my study and is commonly associated with MDR strains, whereas O3/O3a, O3b, and O5 were less frequent and not linked to enhanced virulence (Wyres *et al.,* 2020). The predominance of K45 and O1ab, along with the absence of hypervirulent markers, suggests that the MCRPK isolates in this study are likely to be of low virulence and associated with resistance lineages. Perhaps these lineages stem from environmental or animal sources, rather than being derived from hypervirulent clinical clones.

In this study, MCRPK isolates were found across a diverse array of sequence types, including both common (ST1, ST273) and uncommon (ST309, ST378, ST534, ST735, ST1844, ST1998, ST7730, ST7748, and ST7907) lineages (Figure 7.8). Previous studies from Bangladesh reported *mcr-8.1* in *K. pneumoniae* ST15 and ST656 (Farzana *et al.,* 2020; Mondol *et al.,* 2025). This wide distribution highlights the growing genetic diversity of plasmid-mediated colistin resistance in Bangladesh. These findings also suggest ongoing horizontal gene transfer of *mcr* genes across *Klebsiella* populations (Wyres *et al.,* 2020; Ge *et al.,* 2022). This study also found a small cluster of ST1 (n=4) from various sources, where pairwise SNP analysis revealed 7 to 188 SNP differences between isolates (Figure 7.9). This spectrum of genetic divergence indicates both recent (e.g., the 7-SNP pair of *mcr-8* isolates) and distant diversification events within the ST1 lineage. These results suggest a broader genetic landscape of *mcr* dissemination in Bangladesh, beyond established MDR clones such as ST15 and ST147.

Plasmid analysis revealed substantial heterogeneity among MCRPK isolates, with *mcr* genes associated with multiple plasmid backbones. Genomic characterisation of plasmids in MCRPK isolates through a hybrid assembly of short- and long-read sequencing data revealed *mcr-8* was localised on IncFIA(HI1), IncFIB(pQil), IncR and IncFIB(K)Kpn3 plasmids, whereas the *mcr-1* determinant was carried on IncHI1A plasmid. This plasmid heterogeneity aligns with the earlier studies; for instance, Farzana *et al.* (2020) identified *mcr-8.1* on IncFIB(pQil) plasmid in an ST15 *K. pneumoniae* from Bangladesh, while Salloum *et al.* (2020) described a multi-replicon IncFIA–IncR–IncHI1B plasmid carrying *mcr-8.1* in

Lebanon. The ability of *mcr-8* to associate with multiple plasmid backbones highlights its mobilisation capacity via many potential vectors, significantly enhancing its dissemination across different species and settings. Detection of *mcr-1* on IncHI1A plasmid aligns with the well-documented role of IncHI1-type plasmids in spreading *mcr-1* globally (Farzana *et al.,* 2019; Martino *et al.,* 2024). Two plasmids, IncFIA(HI1) of ~55kb (n=1) and IncR of ~57kb (n=1) carrying *mcr-8.1* were successfully closed and characterised in this study (Figure 7.11). Along with *mcr* genes, these plasmids also carried ARGs (*aadA2*, bla_{OXA-1}, bla_{TEM-1B}, *floR*, *qnrB1*, *sul1*, *sul2*, *sul3* and *tet(A)*), which aligns with previous reports (Sheng *et al.,* 2025). The distribution of highly similar *mcr*-bearing plasmids in different sampling sectors (e.g., IncFIA(HI1) found in isolates from human, pigeon and chicken (Figure 7.12A); IncR in isolates from chickens and water (Figure 7.12B); IncFIB(pQil) in isolates from chickens (Figure 7.12C) and IncFIB(K)Kpn3 in isolates of chicken and water (Figure 7.12D) suggests possible plasmid-mediated inter-host transmission.

Analysis of the genetic environment surrounding the *mcr* genes revealed that all plasmids carrying *mcr-8.1* featured a highly conserved region of ~3.5 kb (*gt-mcr-8.1-copR*). This conserved region was flanked by IS*903* in all plasmids (IncR, IncFIB(pQil), IncFIB(K)Kpn3), except for IncFIA(HI1) (Figure 7.13A). This IS*903* flanked structure is characteristic of a stable mobile resistance cassette, facilitating horizontal gene transfer. This arrangement is consistent with the first reported *mcr-8* in *K. pneumoniae* from China (Wang *et al.,* 2018) and a subsequent study from Bangladesh (Farzana *et al.,* 2020), both of which identified similar genetic context surrounding the *mcr-8* gene. In IncFIA(HI1) plasmids, two isolates lacked insertion sequences, while in one isolate, IS*Kpn25* was located downstream of *mcr-8.1*. The absence of insertion sequences in some cases may indicate that plasmid rearrangements have occurred towards a more stable, non-transposable form (Che *et al.,* 2021). This study also reports the first identification of *mcr-8.2* in IncFIB(K)Kpn3 plasmid in Bangladesh, recovered from a water sample. It shared over 92% identity with a previously described *mcr-8.2* containing plasmid isolated from pigs in China (accession no: NZ_CP034679.1), suggesting a potential zoonotic or environmental link (Yang *et al.,* 2019). The conserved region of *mcr-8.2* (*gt-mcr-8.2*) showed differences with *mcr-*

8.1 and was flanked upstream by two IS*Kpn26* and downstream by two IS*Ecl1* elements (Figure 7.13B), which is consistent with a report from China (Zhao *et al.,* 2022).

In the case of *mcr-1.1* carried on IncHI1, IS*Apl1* (downstream) and IS*Kpn26* (upstream) flanked the *mcr-1.1* conserved region (*mcr-1.1- pap2*), forming the classic *mcr-1.1* unit. Farzana *et al.* (2019) also reported a similar genetic environment of *mcr-1* recovered from a human *E. coli* isolate in Bangladesh. Remarkably, this plasmid contained three copies of the *mcr-1* gene, which could potentially increase colistin resistance. The duplication of *mcr-1* genes on a single plasmid may be driven by selective antibiotic pressure in both human and animal settings, thereby maintaining the resistance phenotype, even if one copy of the gene is mutated or lost (Huang *et al.,* 2013; Silva & Khare, 2024).

This study highlights the association between farm management practices and the occurrence of MCRPK in poultry farms. Data showed that the enrolled farmers were aware of the development of AMR due to inappropriate antibiotic use and the term 'drug withdrawal time'. Antibiotic use varied and remained widespread, including critically important antimicrobials such as ciprofloxacin, gentamicin and colistin (Table 7.8). However, data collected on antibiotic usage history in this study had some limitations. The antibiotic usage data provided by the farm owners was self-reported, which may be biased towards underreporting. Sometimes, they hesitated to share their information due to market value concerns. This can lead to underreporting of colistin usage data, especially since its use is banned by the Bangladesh government (Islam *et al.,* 2020).

MCRPK were more common in layer farms (9/14) than broiler farms (5/14), with the Muktagachha layer farm accounting for the highest number of positive isolates (5/14). This farm was characterised by intensive rearing, multiple antibiotic use, and manure-based waste disposal. The findings suggest that a combination of high antimicrobial exposure and environmental contamination may contribute to the spread of this resistant determinant in layer farms (Ferdous *et al.,* 2019). Furthermore, 71.4% (10/14) of MCRPK isolates originated from farms where poultry waste was repurposed, either as manure or through other means, practices that may promote further environmental contamination and lead to the flow of pathogens into soil and water systems. These findings highlight the need for enhanced food biosecurity, antibiotic stewardship, and safe waste disposal practices in poultry farms to limit the spread of *mcr* (Ferdous *et al.,* 2019; Foysal *et al.,* 2024).

The isolation of MCRPK from rectal swabs of two healthy individuals from the community highlights the potential for silent gastrointestinal colonisation and environmental acquisition of colistin-resistant bacteria. Notably, both cases had no recent antibiotic exposure or hospitalisation, suggesting that they are non-clinical carriers. Livestock contact, poor waste disposal, and proximity to poultry farms may be contributing factors to the acquisition. Case 1, who lived near a layer farm and raised poultry and goats, harboured a *K. pneumoniae* ST309 strain carrying both *mcr-1* and *mcr-8*, which corresponded with multidrug resistance across several antimicrobial classes. In contrast, Case 2, carrying a *K. quasipneumoniae* ST7907 strain with *mcr-8* only, exhibited lower resistance. These findings are worrisome as the coexistence of *mcr-1* and *mcr-8* in a single isolate, especially from the community, raises concerns about horizontal gene transfer and the emergence of highly colistin-resistant strains.

Despite the limitations, this study provides the first comprehensive genomic insight into the spread of *mcr* genes in *Klebsiella* spp. across poultry farms, their surrounding environment and among people living near those farms in Mymensingh, Bangladesh. The findings highlight the expanding reservoirs of plasmid-mediated colistin-resistant bacteria and underline a potential threat to public health. To effectively address this emerging threat and mitigate further dissemination, it is crucial to establish a genomic surveillance system for regular monitoring of community members, livestock and associated environmental reservoirs. Immediate actions such as strengthening policies, stringent regulations on colistin use in farming, community awareness of appropriate animal husbandry practices and campaigns on antibiotic stewardship must be implemented to mitigate the spread of the highly resistant bacteria from animal reservoirs to humans.

Chapter 8

General Discussion

8.1 Why does One Health AMR surveillance matter in Bangladesh?

The One Health framework is recognised as essential to AMR surveillance to identify the risks posed by non-clinical reservoirs of AMR and their relative contribution to human infections (Thorpe *et al.*, 2022). Bangladesh is confronting a growing, cross-sectoral AMR problem with increased reports of MDR infections among human, animal, and environmental domains (Figure 8.1). This is driven by high population density, frequent infectious disease burden, widespread misuse of antibiotics in both humans and animals, inadequate healthcare infrastructures and gaps in diagnostics and stewardship capacity (Hoque *et al.*, 2020; Imam *et al.*, 2021; Hassan *et al.*, 2021; Raihan *et al.*, 2024; Al Masud *et al.*, 2025). Furthermore, densely populated communities living in close proximity to livestock, combined with poor sanitation and waste management systems, and recurrent natural disasters such as floods and cyclones, facilitate microbial dispersal and spread of AMR (Hossain *et al.*, 2021; Fernández Salgueiro *et al.*, 2024).

National surveillance data and recent clinical studies indicate high AMR rates among Enterobacterales. Particularly, *E. coli* and *K. pneumoniae* show widespread resistance to 3GCs (over 70% resistant), fluoroquinolones, and aminoglycosides, with an increasing detection of carbapenem resistance, which severely limits treatment options (Farzana *et al.*, 2023; IEDCR, 2024). Outbreaks of CR-KP have been documented in neonatal intensive care units (NICUs) and surgical wards, raising concerns of untreatable infections (Rahman *et al.*, 2020; Rashid *et al.*, 2022). Moreover, the use of antimicrobials in animal feed as growth promoters and prophylaxis in poultry, aquaculture and agriculture has intensified selection pressure. Reports show colistin resistance genes (*mcr-1*, *mcr-8*) in farm-associated Enterobacterales, highlighting a direct link between agricultural misuse and human health risk (Hameed *et al.*, 2020). The AMR action plan of FAO in LMICs identified weak drug regulation, limited infrastructure and inadequate biosecurity as factors rapidly facilitating the emergence and cross-species transmission, elevating AMR risk (FAO, 2016; Al Amin *et al.*, 2020; Islam *et al.*, 2020; Chowdhury *et al.*, 2022). Environmental reservoirs further complicate the picture. The discharge of untreated hospital wastewater, agricultural effluents, and domestic sewage into open water bodies creates environmental reservoirs where AMR bacteria can persist and exchange ARGs (Hossain *et al.*, 2021). Environmental studies from Bangladesh have also reported MDR bacteria with clinical relevance from surface water and hospital wastewater (McInnes *et al*., 2021; Siddique *et al*., 2024). These dynamics mean that even if clinical stewardship succeeds, the broader resistome can continue to reseed hospitals unless the environmental and veterinary aspects are addressed simultaneously.

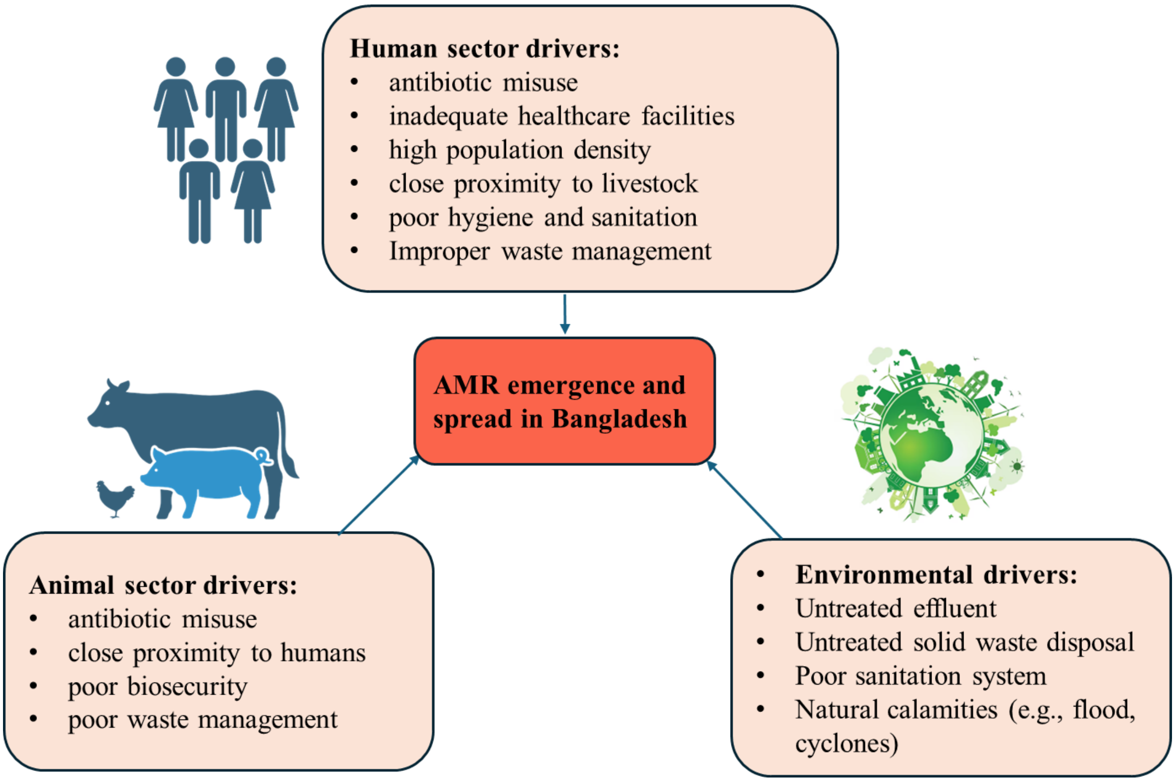


Figure 8.1 Schematic presentation of interconnected sectoral drivers contributing to the emergence and spread of AMR in Bangladesh.

In response to the growing AMR burden, Bangladesh adopted a National Action Plan (2017-2022) and reports data to the WHO's Global Antimicrobial Resistance and Use Surveillance System (GLASS). However, reports from the national human health AMR surveillance conducted by IEDCR show that case-based human surveillance has only been conducted at 9–11 sentinel sites since 2017. This surveillance has relied on international funding and is limited to tertiary healthcare facilities (IEDCR, 2024). Although human health surveillance is partially established, the veterinary and environmental sectors remain relatively underdeveloped and underrepresented (WHO, 2022; IEDCR, 2024). A scoping review of AMR research in Bangladesh (2010–2019) also revealed a predominant human health focus

(36 studies), with far fewer in animals (5), aquaculture (8), and the environment (6), and only three studies adopting a One Health approach (integrating ≥2 compartments with genomic studies) (Figure 8.2) (Hoque *et al*., 2020; Chowdhury *et al*., 2022; Islam *et al*., 2023; Flatgard *et al*., 2024). Furthermore, genomic epidemiology capacity, critical for tracking AMR transmission dynamics, remains limited in Bangladesh, leaving significant gaps in One Health integrated AMR surveillance (Sujan *et al*., 2023; Flatgard *et al*., 2024).

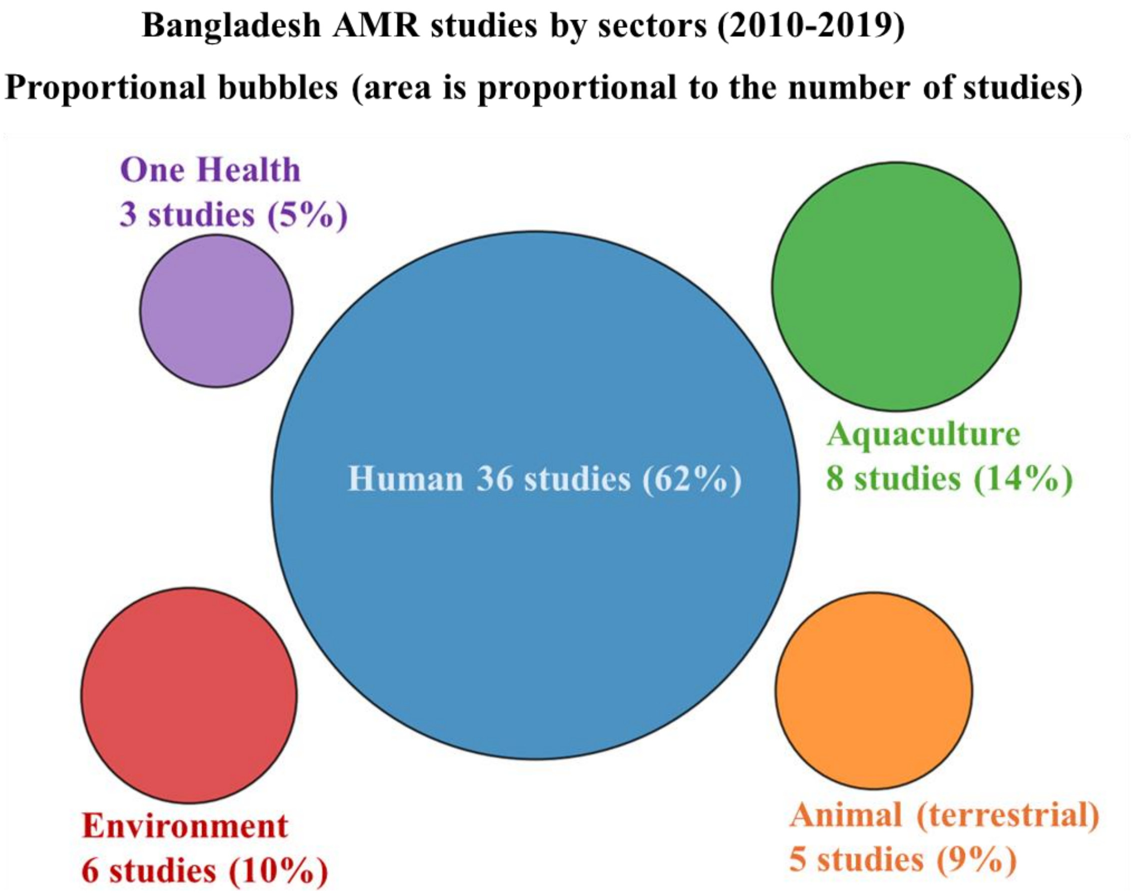


Figure 8.2 Distribution of studies by sector, highlighting the underrepresentation of veterinary, aquaculture, environmental, and integrated One Health research compared to human-focused studies (data adapted from Hoque *et al*., 2020; Chowdhury *et al*., 2022; Islam *et al*., 2023; Flatgard *et al*., 2024).

Together, these pieces of evidence show that although Bangladesh sectoral AMR surveillance observes resistant infections in hospitals, it lacks cross-sector links to explain how those phenotypes and genotypes emerge, persist and circulate across the hospital, farm, and environmental sectors. This thesis addresses these gaps for *Klebsiella* spp. by applying a One Health design that combines field sampling, phenotypic resistance profiling, epidemiological analysis, clonal relatedness, and plasmid characterisation across the hospital–farm–environment to provide a comprehensive view of AMR in Bangladesh. This study also demonstrates the importance of adopting a One Health framework for AMR surveillance, aligning with global calls for integrated approaches to tackle this public health threat.

8.2 From context to contribution: how does this thesis extend the AMR picture?

My thesis presents the first large-scale One Health genomic analysis of *Klebsiella* spp. in Bangladesh. With more than 1,200 isolates from hospitals, community, farms, and the environment, the study reveals three central insights from the study area (Figure 8.3).

Firstly, hospitals serve as the primary hotspot of MDR, significantly associated with hospital-derived SSIs. The analyses reveal the highest MDR burdens, the broadest β-lactamase repertoires (including predominant $bla_{\text{CTX-M-15}}$, $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-48-like}}$), circulation of global high-risk clones (ST37, ST490, ST15, ST147, ST48, ST23) and the signals of putative clonal transmission of MDR ST48 and ST490 from surgical wards, with occasional detection in hospital wastewater. These findings align with the regional and global findings reporting the highest MDR burden in clinical *K. pneumoniae* (Islam *et al*., 2020; Aminul *et al*., 2021; Tanni *et al.,* 2021; Aslam *et al.,* 2022; Thorpe *et al*., 2022).

The detection of CR-hvKP carrying hypervirulence loci highlights a concerning global trend, including the plasmid-mediated assembly of "convergent" strains that are both difficult to treat and well-equipped to cause severe disease (Shankar *et al*., 2022; Jiang *et al*., 2025). In South and Southeast Asia, OXA-232 on small ColKP3 plasmids and bla_{NDM} variants co-circulate with IncF-type virulence plasmids carrying *iuc* and *rmpA2*, creating the genetic backdrop for convergence (Nagaraj *et al*., 2021; Shankar *et al*., 2022; Chen *et al*., 2023; Spadar *et al*., 2023; Li *et al*., 2025). The fact that such strains in my study were confined to hospital sources reinforces the centrality of the healthcare environment in knitting together resistance and virulence, with wastewater may be acting as an outward conduit.

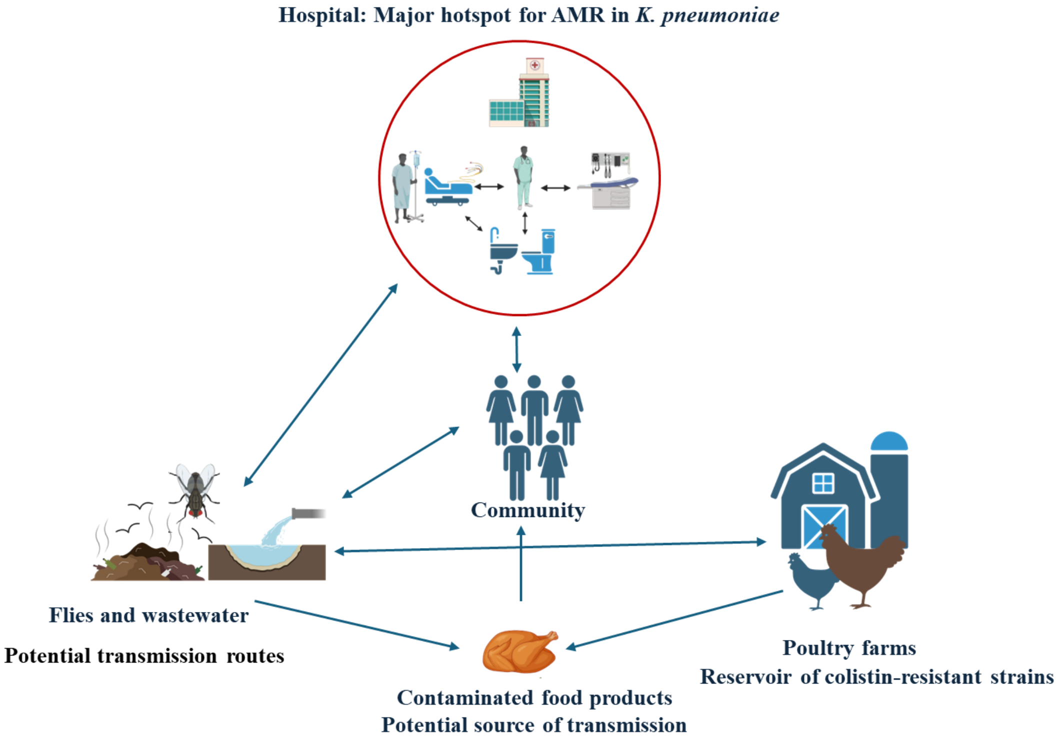


Figure 8.3 A schematic for potential transmission pathways and reservoirs of AMR *K. pneumoniae* at the human-animal-environment interface in Mymensingh. The figure was created with BioRender.com.

Secondly, poultry farms serve as the reservoirs of colistin resistance. High colonisation in chickens and retail meat, together with farm-associated colistin resistance, indicate that farm practices and poor biosecurity at farms can drive resistance selection and onward spread. Furthermore, although no *mcr-8* has been reported in *Klebsiella* spp. from close neighbouring countries, a previous report of *mcr-8.1* in clinical isolates, together with my findings, indicates that *mcr-8.1* predominates over *mcr-1.1* among circulating *K. pneumoniae* in Bangladesh. (Hameed *et al.,* 2020; Singh *et al.,* 2021; Zhou *et al.,* 2024). However, a recent review shows *mcr-8*-positive *K. pneumoniae* is endemic in several Asian countries, highlighting the importance of studying species-specific gene dynamics and plasmid ecology (Zhang *et al.*, 2025).

Thirdly, the contaminated environment (e.g., untreated waste, hospital wastewater) and vectors (flies) may serve as a connector that shuttles the pathogenic bacteria between sectors. Flies exhibited the highest prevalence of *K. pneumoniae* in my sampling, with significant enrichment during the summer and monsoon seasons, consistent with international studies showing houseflies as mechanical carriers of MDR Enterobacterales from markets and animal facilities to households and health-care settings (Akter *et al*., 2020; Mourão *et al*., 2024). In Bangladesh's dense urban markets, where organic waste accumulates and WASH are poor, fly-borne transfer and waterborne contamination reinforce each other, particularly during monsoons when surface water disseminates faecal matter (Furlan *et al*., 2024). These lines of evidence suggest that vector control and wastewater management are not peripheral but central to AMR mitigation.

These findings highlight the significance of adopting an integrated One Health genomic surveillance to monitor and contain the high-risk strains before they become entrenched. Collectively, these insights add to the global evidence that effective AMR mitigation necessitates transdisciplinary monitoring and policies tailored to local needs.

8.3 Why does the hospital–farm–environment triad amplify AMR risks in *Klebsiella*?

The observations of this study are not surprising when viewed against systemic constraints.

8.3.1 Lack of effective IPC (infection prevention and control) programmes at hospitals

WHO defines IPC as evidence-based measures that prevent infections in patients and health workers. The Core Components and minimum requirements set national and facility standards for programme design, monitoring, and improvement. Effective IPC programmes (e.g., multimodal hand hygiene strategies, surveillance with feedback, and environmental hygiene) reduce HAIs and are central to combating AMR (WHO, 2016).

During my study, I observed that the only government tertiary hospital in Mymensingh division, MMCH (a 1,000-bed tertiary facility), was severely overcrowded with poor waste management practices (Figure 8.4). A government report in 2021 showed that its bed-occupancy rate was 234.8%, the highest among Bangladeshi medical college hospitals, creating conditions in which basic IPC falters (DGHS, 2021). Such congestion is a recognised driver of HAI risk. Another report of WHO/UNICEF showed that only 38% of facilities nationally meet basic hygiene standards, with lower coverage in government facilities. Recent IPCAF (Infection Prevention and Control Assessment Framework) assessments have placed most tertiary hospitals at the "basic" level of IPC, characterised by the absence of HAI surveillance, limited audits, toilet and washbasin shortages, and chronic bed-space constraints (Figure 8.5). Hand hygiene compliance in tertiary hospitals has been documented at around 25%, reflecting constraints in supplies, infrastructure, and safety culture (Harun *et al*., 2022). Empirical broad-spectrum therapy and limited stewardship compound the effects of overcrowding and understaffing. These micro-ecologies are ideal for *K. pneumoniae* to flourish, facilitating the clonal spread and persistence of outbreak-prone lineages within and between wards, while high antibiotic pressure favours the emergence of resistant clones.

Figure 8.4 Evidence of patient overcrowding and waste management challenges at Mymensingh Medical College Hospital. Photographs showing corridor boarding, crowded wards, poor hospital management, and improper management of hospital wastes. Images were taken during sampling, and consents were obtained while capturing in-ward photographs.

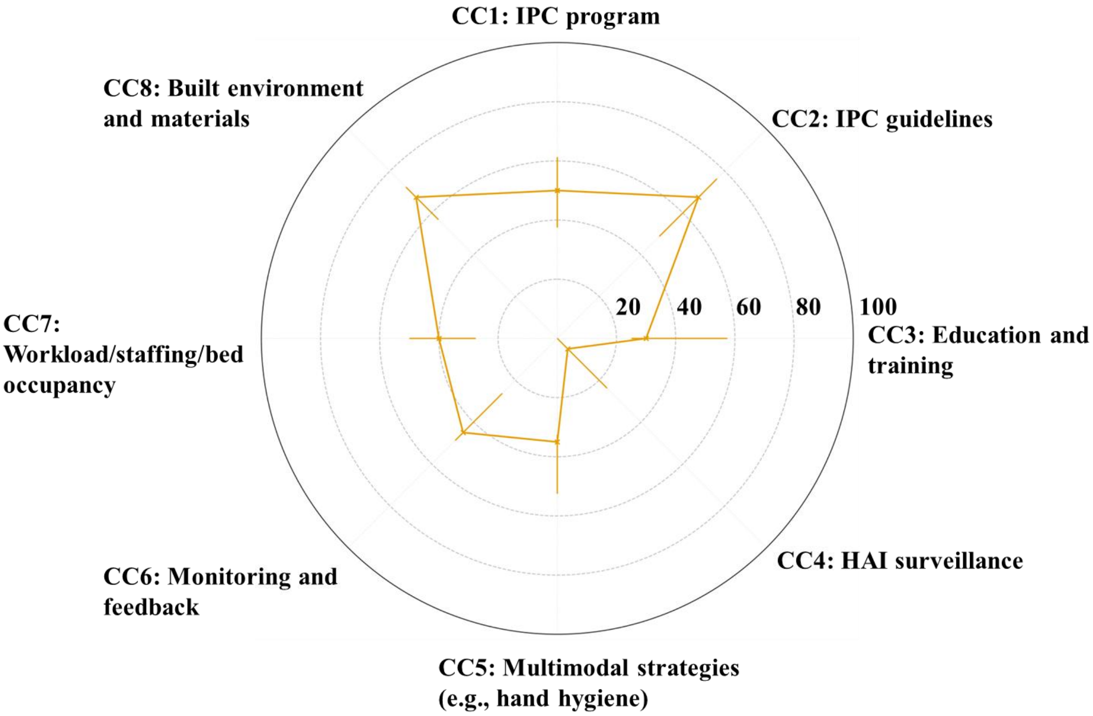


Figure 8.5 IPCAF core component scores (CC1–CC8) of Bangladesh tertiary hospitals (n=11) in 2020 (adapted from Harun *et al*., 2022). Results from the WHO IPCAF assessments conducted from September to December 2020 in 11 tertiary hospitals in Bangladesh. Radar chart showing median scores for the eight WHO IPC core components with interquartile-range whiskers (0–100 scale). Concentric rings are at 20-point intervals (20, 40, 60, 80, outer ring = 100).

8.3.2 Weak antimicrobial stewardship (AMS) across One Health sectors

In Bangladesh, the widespread misuse of antibiotics in human health, livestock and aquaculture presents one of the most pressing challenges in tackling AMR. In the human sector, over-the-counter (OTC) availability without prescription, empirical use of broad-spectrum agents, and inadequate AMS in hospitals contribute to high and often unnecessary exposure. A recent survey reported that 56.6% of antibiotics sold in community pharmacies in Bangladesh were dispensed without a prescription, highlighting easy access and weak regulatory enforcement (Al Masud *et al*., 2024). In rural settings, care is often initiated by unqualified village doctors and drug vendors, leading to inappropriate antibiotic use and incomplete treatment courses (Bepari *et al*., 2023).

Similar challenges are evident in the livestock and aquaculture sectors (Figure 8.6). Antimicrobials are routinely used for prophylaxis, metaphylaxis, and growth promotion, often without veterinary oversight or adherence to dosing guidelines, resulting in residues and effluents that further contaminate the surrounding environment (Sarker *et al*., 2024). Widespread over-the-counter access to veterinary drugs has facilitated routine use, with surveys showing that up to 70% of poultry farmers administer antibiotics without prescription, primarily for disease prevention or growth promotion (Imam *et al*., 2021; Hassan *et al*., 2021). This may contribute to the presence of drug residues in food products and effluents, thereby amplifying environmental contamination. Consistent with this picture, my findings show high-burden carbapenem resistance in hospital isolates, reflecting pressure from inappropriate and prolonged use of last-line agents, while colistin resistance was found on farms and in surrounding matrices, aligning with historical use patterns and environmental spillover (Sobur *et al*., 2019).

Recognising these risks, Bangladesh has introduced regulatory measures in recent years. The Fish Feed and Animal Feed Act (2010), and Animal Feed Rules (2013) prohibited antibiotic growth promoters in livestock production; in 2019, combination veterinary products containing colistin were cancelled, and in 2022, the Directorate General of Drug Administration (DGDA) formally banned colistin use in food animals (DGDA, 2023). Colistin (critically important for treating human infections) was commonly used until its ban, explaining the dominance of *mcr-8* in poultry isolates. Weak enforcement of regulations and poor biosecurity remain major challenges, similar to observations in Vietnam and India, where colistin resistance rapidly spread in poultry (Olaitan *et al.,* 2021). Overall, these findings highlight a One Health nexus where weak enforcement and limited stewardship exacerbate ARGs to circulate across hospitals, food-animal systems, and the environment, despite progressive policy frameworks.

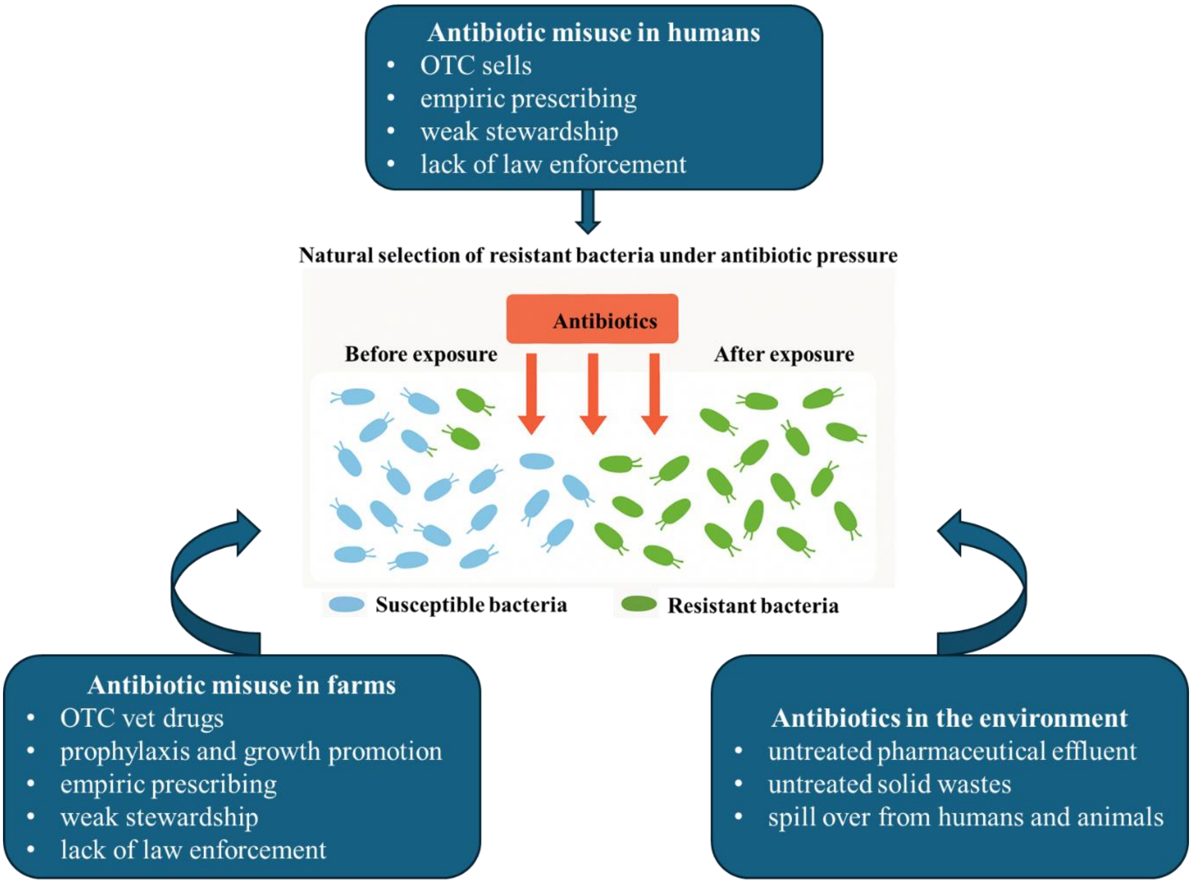


Figure 8.6 One Health perspective of antibiotic misuse and its role in resistance selection. Antibiotics eliminate susceptible bacteria, allowing resistant strains to dominate.

8.3.3 Improper waste management as a driver of AMR transmission

In recent years, Bangladesh has expanded basic water and sanitation coverage, but universal safe services remain out of reach. Nearly 98% of people have access to at least basic drinking water, while only 42–43% receive a safe and managed supply due to arsenic and microbial contamination (UNICEF & WHO, 2021). Despite improvements in basic sanitation (e.g., toilet access), waste management systems remain poorly developed in both rural and urban areas, creating distinct risks for environmental contamination and sustaining transmission pathways relevant to AMR.

Structural gaps in solid-waste handling, effluent management, faecal-sludge management, and uneven WASH access create dense "exchange zones" where hospital effluents, industrial effluents, market refuse, and household wastewater converge, allowing *Klebsiella* (and its resistance genes) to circulate between people, animals, and the wider environment. Empirical studies in Bangladesh have consistently recovered MDR bacteria, including *Klebsiella*, from municipal wastewater and urban surface waters, underscoring the role of environmental reservoirs linked to both healthcare and community sources (Mondol *et al*., 2025; Siddique *et al*., 2024). In Dhaka alone, it is estimated that more than 230 tonnes of untreated faecal waste are discharged daily into open water bodies, with severe implications for public health and environmental contamination (UNICEF & WaterAid, 2025).

Seasonal hazards further amplify these risks. During the 2024 monsoon and Cyclone Remal, UNICEF reported widespread faecal contamination of surface ponds that remain critical domestic water sources. More broadly, monsoon flooding has long been shown to degrade household water quality and accelerate the redistribution of pathogens and ARGs across sectors (Broyles *et al*., 2024; UNICEF, 2024). These conditions not only increase community exposure but also heighten the likelihood of MDR bacteria being reintroduced into farms and hospitals. These findings are consistent with my observations during fieldwork in Mymensingh as well as the study findings. I observed humans living in close proximity to livestock farms with suboptimal hygiene and biosecurity, manure mismanagement and improper disposal of household and hospital waste (Figure 8.7).

Figure 8.7 Field observations in Mymensingh showing close human and livestock proximity, manure mismanagement, and improper disposal of household and hospital wastes. Images were taken during sampling.

In One Health terms, the failure to manage these wastes properly tightly couples the hospital–farm–environment triad, where untreated hospital and other waste discharges seed ARGs into drainage networks; animal waste and food markets provide niches for vectors (e.g., flies, rodents, etc.) to facilitate vector-borne transmission; and seasonal flooding redistributes contamination into shared water sources. Collectively, these dynamics drive the selection, circulation, and re-entry of AMR *Klebsiella* into the community, patients, caregivers, livestock, and food chains (Figure 8.8).

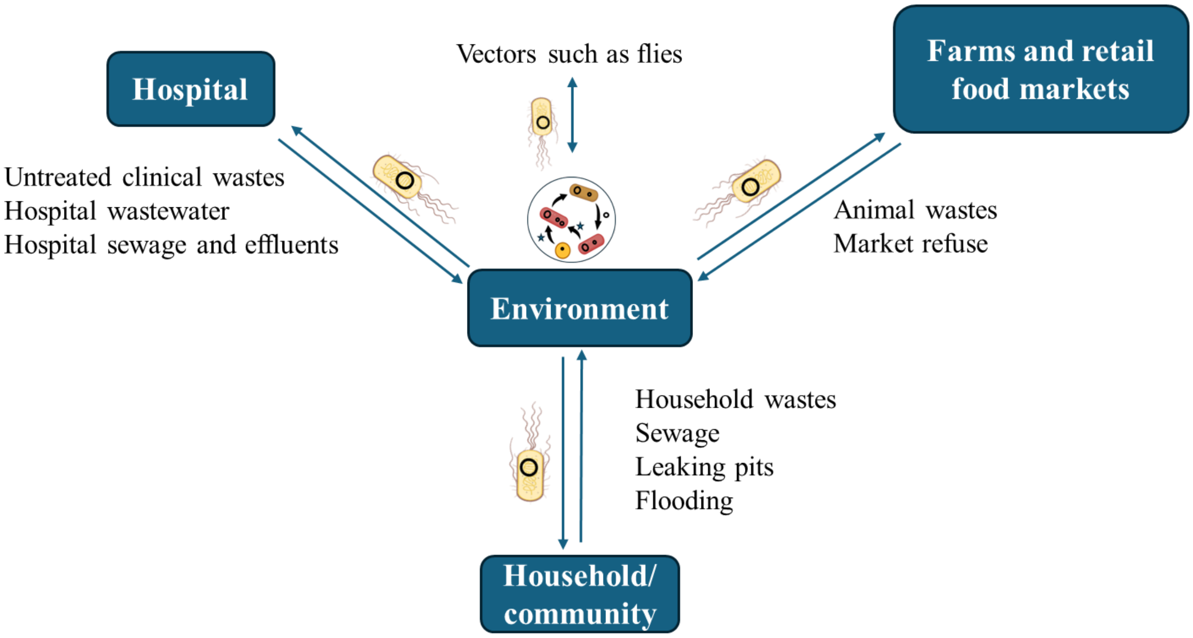


Figure 8.8 Conceptual pathways linking hospitals, farms/markets, and the environment in Bangladesh. Improper waste and faecal-sludge management create exchange zones in the environment that amplify the movement of *Klebsiella*/AMR genes between sectors, underscoring waste governance and WASH as core One Health interventions.

My observational findings and the dataset suggest that prioritising wastewater treatment at both facility and municipal levels, improving faecal-sludge management, strengthening solid-waste and effluent controls in farms and markets, and addressing inequities in WASH services need to be integrated into AMR control strategies in Bangladesh.

8.4 What should change: policy and practice implications aligned to Bangladeshi realities?

The implications point to a coordinated suite of interventions rather than a single measure.

8.4.1 Strengthening IPC and AMS in Bangladeshi hospitals

Bangladesh has a national IPC guideline, but to achieve meaningful reductions in HAIs, this must be translated into standardised, hospital-wide implementation (CDC, 2020). The priority is to elevate IPC from a “basic” to an “intermediate/advanced” level by institutionalising the WHO multimodal hand hygiene strategy, ensuring uninterrupted access to alcohol-based handrub (ABHR) at every point of care in the hospital and embedding routine WGS-based HAI surveillance with feedback to clinical teams to identify high-risk lineages circulating in tertiary hospitals. Evidence shows that hand hygiene alone can prevent nearly 50% of avoidable HAIs, making it one of the most cost-saving measures (WHO, 2023). Figure 8.9 illustrates the measures prioritised by my study findings. Together, this integrated, resource-sensitive suite of measures offers a feasible roadmap for strengthening IPC and mitigating AMR threats in Bangladeshi hospitals.

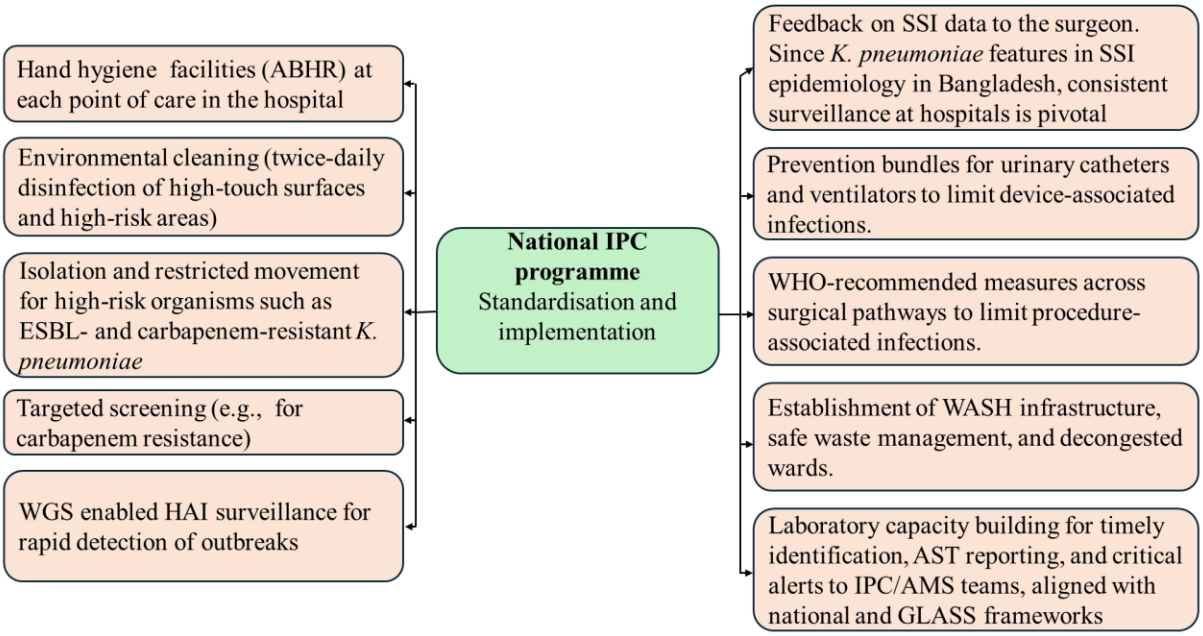


Figure 8.9 Integrated IPC Programme interventions for strengthening hospital infection prevention in Bangladesh.

Recent point-prevalence data from Bangladeshi hospitals show that 64% of inpatient antibiotic use is from AWaRe 'Watch' agents (broad-spectrum antibiotics) and 0.1% from 'Reserve' group (last-line antibiotics), while WHO recommends that ≥60% of national antibiotic use should be from 'Access' agents (narrow spectrum antibiotics), underscoring how far current hospital use in Bangladesh leans toward 'Watch' (Chowdhury *et al*., 2025; Rashid *et al*., 2022). Therefore, stewardship must include microbiology capacity building, local prescribing guideline development, regular audit and feedback and formulary restrictions. Surgical pathways deserve special attention: SSI prevention bundles, perioperative antibiotic timing, and device-associated infection bundles reduce the ecological opportunities for *K. pneumoniae* transmission in crowded wards (WHO, 2018; WHO, 2023).

8.4.2 Farm level interventions

The FAO and WHO supported stewardship initiatives in Bangladesh to promote prudent antimicrobial use in veterinary practice (WHO, 2022; FAO, 2023). In 2024, Bangladesh AMR Response Alliance (BARA) expanded its in-service AMR training programme to include veterinarians and has been training farmers on the rational use of antibiotics and alternative husbandry to reduce prophylactic and growth-promotion use. However, uneven enforcement and thriving informal markets marked by OTC sales, coupled with limited public awareness and weak monitoring capacity, continue to undermine progress. The findings of this study highlight the consequences of unregulated antibiotic misuse, with the emergence of *mcr*-mediated colistin resistance in poultry and associated environments.

Therefore, at the farm–food interface, enforcement of the 2022 prohibition of colistin in animals must be visible and consistent, paired with farmer-centred stewardship, including prescription-only access, practical biosecurity measures that incorporate rodent and insect control, and simple manure-treatment options that fit smallholder economics. Behaviour changes demand reconfiguring incentives in the supply chain, including restricting sales by informal prescribers and aligning feed and drug distribution practices with veterinary oversight (Imam *et al.*, 2021). These steps align with the Quadripartite (FAO/UNEP/WHO/WOAH) One Health guidance that prioritises IPC and WASH, better wastewater governance, and prudent antimicrobial use across sectors (FAO/UNEP/WHO/WOAH, 2024).

8.4.3 Measure to control environmental contamination

Strengthening environmental controls should include strict segregation of hospital wastes, basic pre-discharge treatment of hospital effluents, and improvements to drainage and solid-waste collection across households, farms, and markets to mitigate the breeding of filth flies, and integrating environmental AMR indicators into urban water monitoring. Recent studies from Dhaka's municipal wastewater and surface waters demonstrate the feasibility and value of such monitoring, which can provide early warning of hotspot emergence and track the impact of upstream interventions (Siddique *et al*., 2024; Nisa *et al*., 2024).

8.4.4 An alternative long-term measure to curb antibiotic use and AMR

Finally, this vision should include measures that reduce the demand for antibiotic use, providing a tangible opportunity to mitigate the continuous selection of AMR. Vaccines targeting *K. pneumoniae* O antigens (notably O1, O2, O3, O5 serotypes) that account for the majority of invasive disease have progressed to early-phase human trials with encouraging immunogenicity (Miller *et al*., 2024; Wantuch *et al*., 2024; LimmaTech, 2021–2024). Although no vaccine is yet licensed, policy roadmaps anticipate prioritising high-risk patients (ICU, surgical, transplant), older adults and selected inpatient groups (e.g., given the burden of neonatal sepsis, pregnant women from LMICs) once efficacy is proven (Dangor *et al*., 2024). In parallel, bacteriophage therapy is re-emerging as a plausible adjunct for MDR Enterobacterales; while clinical implementation is nascent, the technology warrants local evaluation given the AMR landscape (Pirnay *et al*., 2024; Sawa *et al*., 2024).

8.4.5 Translating evidence into policy for advancing One Health AMR surveillance

The study highlights the interconnected nature of AMR across the human, animal and environmental settings in Bangladesh. High-end threats CR-KP, *mcr*-mediated colistin resistance, and, notably, convergent CR-hvKP are unlikely to be detected reliably without an integrated, One Health-based genomic surveillance system, emphasising its importance. Although Bangladesh has adopted the National Action Plan on AMR (aligned with the WHO Global Action Plan) and the National AMR Surveillance Strategy 2020–2025, the approach lacks integration across human, animal, and environmental sectors (Figure 8.10) (IEDCR, 2024). The GLASS report also highlights the variability in inputs and limited specimen coverage from Bangladesh. Furthermore, integration of genomic approaches into AMR surveillance is still in progress (Ahmed *et al*., 2022). However, complementary initiatives have begun to close the gaps and build capacity, such as CAPTURA's (Collecting Antibiotic Prescription data for Universal Access and Use) multi-year effort (funded by Fleming Fund, UK) compiled retrospective AMR/AMU datasets, produced a Bangladesh country report to inform policy and data quality improvement, and in 2025 supported a national monitoring-and-evaluation framework (AMRSurME) to consolidate indicators for a more integrated, nationally owned system (Prifti *et al*., 2023).

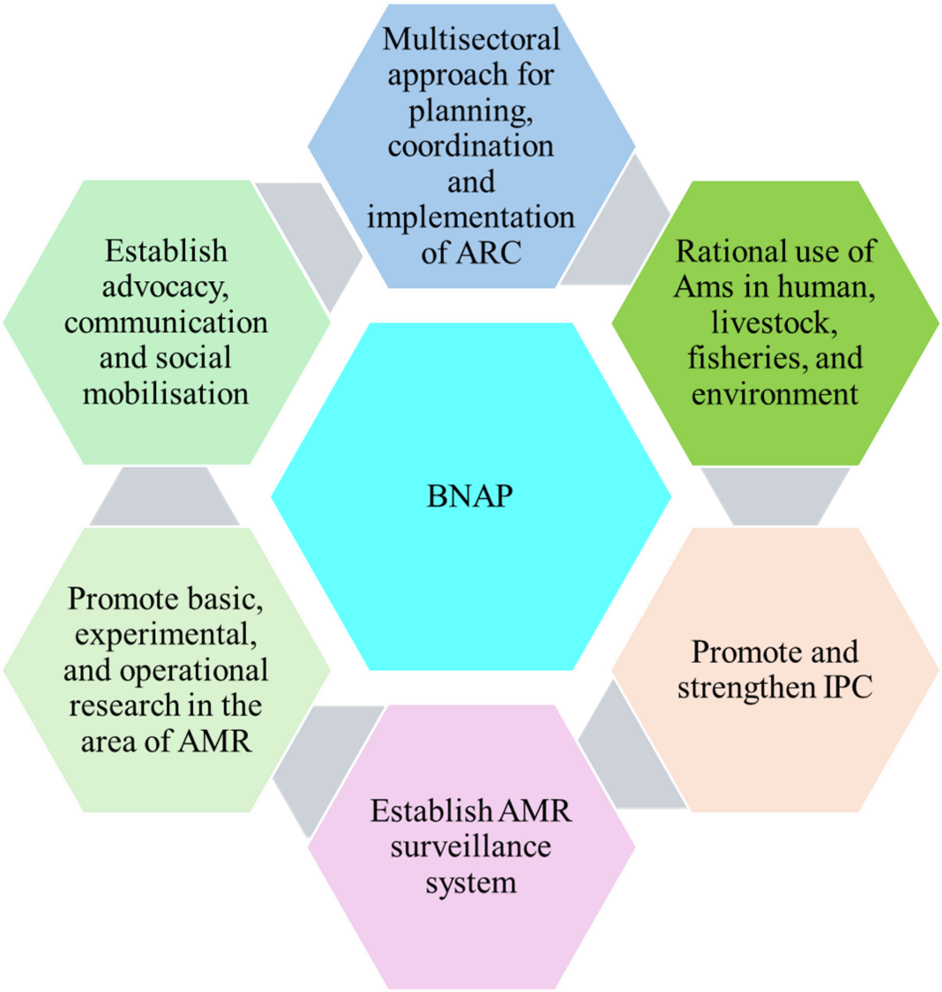


Figure 8.10 Bangladesh National Action Plan (BNAP): Antimicrobial Resistance Containment (ARC) in Bangladesh 2017–2022 (adapted from Ahmed *et al*., 2022).

In parallel, BARA, profiled by FAO/Quadripartite as a notable workforce and stewardship platform, has mobilised clinicians and veterinarians to disseminate One Health guidance, complementing government surveillance with practice-change at the farm–food interface. Building on these efforts, FAO and its partners have highlighted the need to combine hospital data with environmental and animal health intelligence, and to strengthen wastewater governance and WASH within a One Health framework (CDC, 2023). Within this policy landscape, the present thesis contributes a large *Klebsiella* species-specific One Health dataset and informs cross-sectoral evidence that the NAP and CAPTURA identify as missing and that BARA/FAO seek to operationalise. Nationally, these data can be incorporated into the IEDCR-led GLASS reporting and the emerging AMRSurME (Antimicrobial Resistance Surveillance Monitoring and Evaluation) indicators to guide IPC/AMS, farm biosecurity, and wastewater management priorities, while internationally, they can extend South Asia's comparative baselines for *Klebsiella* clonality, resistance genes, plasmid ecology, and convergence (CR-hvKP), enabling regional risk assessment and shared intervention design.

8.5 Field challenges in implementing One Health AMR research in LMICs: Insights from Bangladesh

Carrying out large-scale One Health AMR research in Bangladesh has shown me how scientific ambition often collides with practical and social realities. Although my study was based on a rigorous One Health framework, translating this into the field revealed multiple layers of challenges, including administrative and regulatory challenges, cultural sensitivities, and operational constraints. These challenges are also documented by AMR surveillance in other LMICs (Peters *et al.*, 2024).

In this study, I was involved in every step in establishing the entire project under the guidance of my research supervisors and a Bangladeshi co-investigator, from securing ethical approval to managing sampling and data collection. Obtaining ethical clearance was more time-consuming than expected as the study involved multiple domains, including hospital patients, community members, farm animals, and environmental sources.

Once the approvals and risk assessments were in place, sampling presented further challenges. I personally visited every sampling site to collect samples and epidemiological data. It soon became clear that community awareness of AMR is still very limited. Collecting human rectal swabs required patience, careful explanation and reassurance to build trust. Gender and occupational dynamics also complicated recruitment; women frequently expressed hesitation, while men were often absent during working hours, creating risks of sampling bias.

This study began during the 2nd wave of COVID-19 in late 2021, exacerbating these challenges. Communities were understandably cautious at that time, which slowed the procedures. One incident remains vivid in my memory: during a community visit around the farm, a group of people confronted us, accusing my team of spreading COVID-19 and claiming that the equipment we carried contained COVID germs. Fortunately, others in the community intervened to protect us, but the experience highlighted how easily fear and misinformation can escalate into hostility.

Despite protective measures, one team member got COVID-19 during the first period of sampling, underlining the occupational risks associated with One Health AMR research in high-exposure environments.

Sampling in food markets posed a different challenge. Many shopkeepers refused to sell meat samples, fearing adverse findings and reputational or economic harm. This interaction shows how economic vulnerability and stigma can constrain research access.

Despite these obstacles, I successfully collected the targeted samples for my study. However, these experiences shift the focus of this thesis from the laboratory to the ground realities of conducting One Health research. These experiences demonstrate that AMR surveillance in LMICs such as Bangladesh is not only influenced by technical skills but also deeply influenced by socio-economic vulnerabilities, local cultural sensitivities, infrastructures and global health disruptions. Recognising and addressing these challenges is critically important for successful implementation and reliable data generation from such AMR surveillance (Figure 8.11).

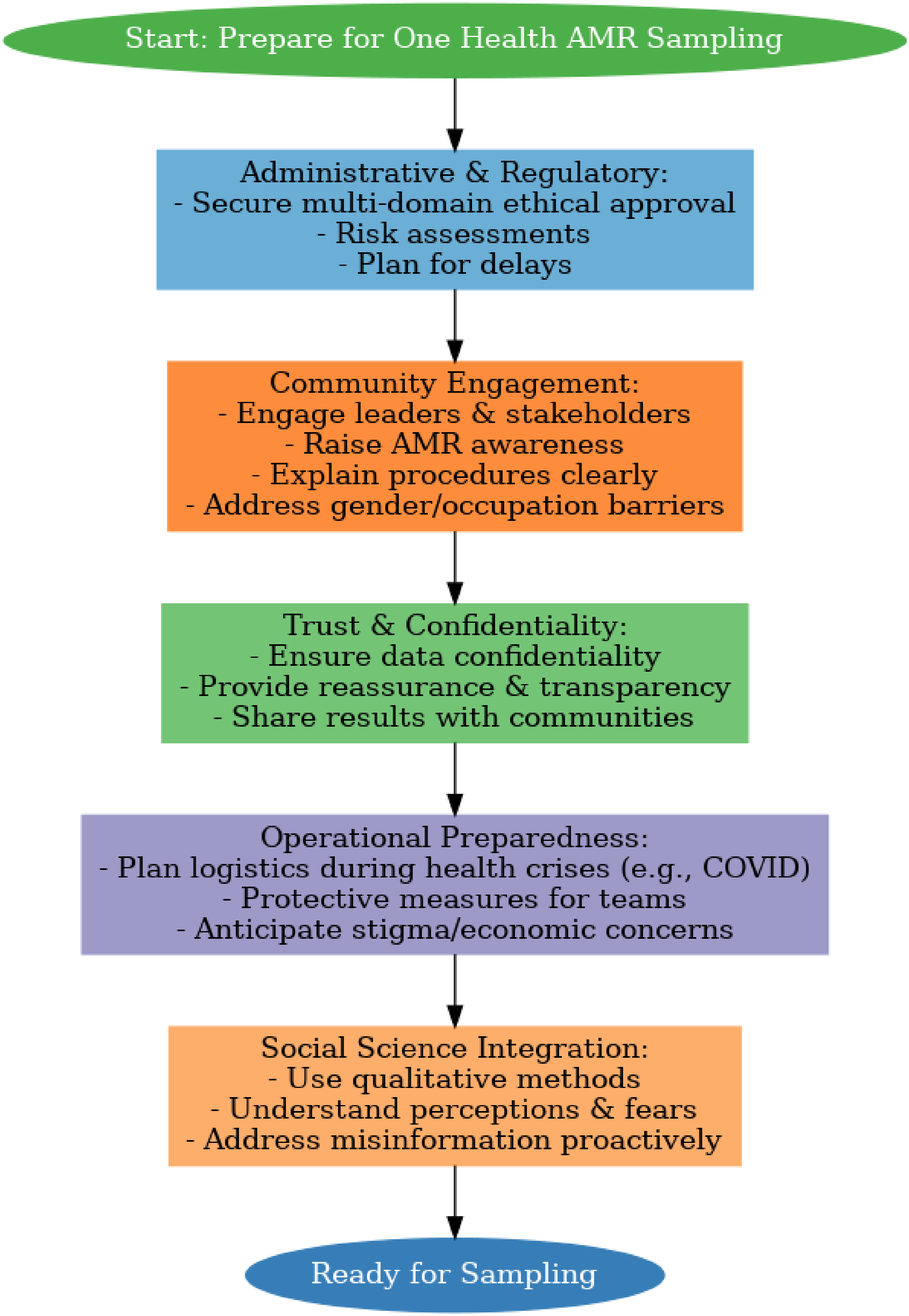


Figure 8.11 The key measures to consider before starting One Health AMR sampling in LMICs such as Bangladesh.

To address these barriers, engagement strategies should be developed jointly with community leaders, healthcare workers, farmers, and other relevant stakeholders. Recent reviews emphasise that community and environmental AMR surveillance in LMICs must tackle trust deficits, coordination gaps and resource constraints (Delpy *et al*., 2024; Peters *et al*., 2024). Ensuring confidentiality and sharing the outcome of the project with communities through local workshops could help to minimise mistrust. Additionally, incorporating social science approaches within the One Health fieldwork would further support this process by identifying community perceptions, fears, and motivations.

8.6 Reflection on study limitations and future horizons

Although this study provides several insightful interventions, it has some limitations which should be acknowledged. Despite extensive sampling across humans, animals, and environmental sources, the geographic scope of this study was narrow. Sampling was limited to selected sites and a single tertiary hospital in Mymensingh, which may not reflect the national diversity of AMR in *Klebsiella* in Bangladesh. Human rectal swabs were self-collected, which may have led to reduced detection due to suboptimal sampling. Hospital- and community-based sampling were conducted independently, and no follow-up of patients, families, or communities was performed; therefore, direct transmission pathways between reservoirs could not be assessed. In addition, outbreak analyses did not include concurrent environmental sampling or source tracing, so links between reservoirs and transmission routes remain uncertain. As this work primarily focused on screening acquired antimicrobial resistance genes using ABRicate, resistance mediated by mutations in chromosomal loci (e.g., regulatory genes affecting colistin susceptibility) was not captured. Virulence profiling used Kleborate, which is calibrated for the *K. pneumoniae* species complex, so virulence in other *Klebsiella* species may be under-detected, and scores may not show true potential. Experimental confirmation of gene function, plasmid transferability, and trade-offs between fitness and persistence was beyond the scope of my studies. Antibiotic use in humans and animals was self-reported without verification, so underreporting and recall bias are possible.

Despite these limitations, the study offers one of the most comprehensive datasets on *Klebsiella* AMR in Bangladesh and establishes a strong foundation for future research. Beyond immediate findings, genomic data can be leveraged for deeper genomic analyses, including a detailed study of the genetic elements that drive resistance. Contextualisation of Bangladeshi *K. pneumoniae* genomes within global datasets would clarify where local lineages sit in the global phylogeny and flag the emerging high-risk clones. Finally, sharing these data on global platforms such as Pathogenwatch, PubMLST (BIGSdb), and WHO GLASS will anchor Bangladesh within the international genomic AMR surveillance network.

8.7 Conclusion

This comprehensive One Health analysis of *K. pneumoniae*, with a focus on AMR in Bangladesh, highlights the necessity for coordinated interventions across the interfaces of human, animal, and environmental health. The findings reveal a complex epidemiological landscape where hospitals serve as amplification hubs for MDR strains, poultry systems drive the selection of colistin resistance, and environmental vectors facilitate transmission across interconnected reservoirs. Although the emergence of CR-hvKP in healthcare settings represents an immediate clinical threat, the broader challenge lies in addressing the systemic drivers of AMR through strengthened IPC, AMS, improved farm biosecurity, and enhanced waste management infrastructure. The study demonstrates that effective AMR surveillance in LMICs such as Bangladesh must navigate not only technical and resource constraints but also complex social, cultural, and economic realities that can significantly impact implementation success. Moving forward, sustainable progress against *K. pneumoniae* AMR will depend on integrating genomic surveillance capabilities with evidence-based targeted interventions, community engagement strategies, and policy frameworks that can address the multifaceted nature of resistance emergence and spread in densely populated, resource-limited settings where human, animal, and environmental health are inextricably linked.

Finally, as an alumnus of the Prime Minister Fellowship Programme and a faculty member at a leading agricultural university in Bangladesh, I am committed to supporting the development of the next generation of microbiologists, contributing to the establishment of a sustainable, integrated AMR surveillance system throughout Bangladesh and influencing national policy on AMR, utilising my expertise in molecular microbiology, bioinformatics, and epidemiology, acquired through my DPhil studies at Oxford.

Bibliography

Abraham, E. P., & Chain, E. (1940). An enzyme from bacteria able to destroy penicillin [1]. Nature, 146(3713), 837. https://doi.org/10.1038/146837A0;KWRD

Abbas, R., Chakkour, M., Zein El Dine, H., Obaseki, E. F., Obeid, S. T., Jezzini, A., Ghssein, G., & Ezzeddine, Z. (2024). General Overview of *Klebsiella pneumonia*: Epidemiology and the Role of Siderophores in Its Pathogenicity. Biology, 13(2), 78. https://doi.org/10.3390/BIOLOGY13020078

Addae-Nuku, D. S., Kotey, F. C. N., Dayie, N. T. K. D., Osei, M. M., Tette, E. M. A., Debrah, P., & Donkor, E. S. (2022). Multidrug-Resistant Bacteria in Hospital Wastewater of the Korle Bu Teaching Hospital in Accra, Ghana. Environmental Health Insights, 16. https://doi.org/10.1177/11786302221130613

Adeolu, M., Alnajar, S., Naushad, S., & Gupta, R. S. (2016). Genome-based phylogeny and taxonomy of the 'Enterobacteriales': Proposal for enterobacterales ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. International Journal of Systematic and Evolutionary Microbiology, 66(12), 5575–5599. https://doi.org/10.1099/IJSEM.0.001485,

Aguilar-Bultet, L., García-Martín, A. B., Vock, I., Maurer Pekerman, L., Stadler, R., Schindler, R., Battegay, M., Stadler, T., Gómez-Sanz, E., & Tschudin-Sutter, S. (2023). Within-host genetic diversity of extended-spectrum beta-lactamase-producing Enterobacterales in long-term colonized patients. Nature Communications, 14(1), 1–14. https://doi.org/10.1038/S41467-023-44285-W;TECHMETA

Aguilar-Salazar, A., Martínez-Vázquez, A. V., Aguilera-Arreola, G., de Jesus de Luna-Santillana, E., Cruz-Hernández, M. A., Escobedo-Bonilla, C. M., Lara-Ramírez, E., Sánchez-Sánchez, M., Guerrero, A., Rivera, G., & Bocanegra-Garcia, V. (2023). Prevalence of ESKAPE Bacteria in Surface Water and Wastewater Sources: Multidrug Resistance and Molecular Characterization, an Updated Review. Water 2023, Vol. 15, Page 3200, 15(18), 3200. https://doi.org/10.3390/W15183200

Ahmad, N., Joji, R. M., & Shahid, M. (2023). Evolution and implementation of One Health to control the dissemination of antibiotic-resistant bacteria and resistance genes: A review. Frontiers in Cellular and Infection Microbiology, 12, 1065796. https://doi.org/10.3389/FCIMB.2022.1065796/FULL

Ahmed, I., Rabbi, M. B., & Sultana, S. (2019). Antibiotic resistance in Bangladesh: A systematic review. International Journal of Infectious Diseases, 80, 54–61. https://doi.org/10.1016/J.IJID.2018.12.017

Ahmed, S., Das, T., Islam, M. Z., Herrero-Fresno, A., Biswas, P. K., & Olsen, J. E. (2020). High prevalence of *mcr-1*-encoded colistin resistance in commensal *Escherichia coli* from broiler chicken in Bangladesh. Scientific Reports 2020 10:1, 10(1), 1–13. https://doi.org/10.1038/s41598-020-75608-2

Ahmed, S. M., Naher, N., Tune, S. N. B. K., & Islam, B. Z. (2022). The Implementation of National Action Plan (NAP) on Antimicrobial Resistance (AMR) in Bangladesh: Challenges and Lessons Learned from a Cross-Sectional Qualitative Study. Antibiotics 2022, Vol. 11, Page 690, 11(5), 690. https://doi.org/10.3390/ANTIBIOTICS11050690

Ahn, C., Yoon, S. S., Yong, T. S., Jeong, S. H., & Lee, K. (2016). The resistance mechanism and clonal distribution of tigecycline-nonsusceptible *Klebsiella pneumoniae* isolates in Korea. Yonsei Medical Journal, 57(3), 641–646. https://doi.org/10.3349/YMJ.2016.57.3.641,

Ajulo, S., & Awosile, B. (2024). Global antimicrobial resistance and use surveillance system (GLASS 2022): Investigating the relationship between antimicrobial resistance and antimicrobial consumption data across the participating countries. PLOS ONE, 19(2), e0297921. https://doi.org/10.1371/JOURNAL.PONE.0297921

Akter, S., Sabuj, A. A. M., Haque, Z. F., Rahman, M. T., Kafi, M. A., & Saha, S. (2020). Detection of antibiotic-resistant bacteria and their resistance genes from houseflies, Veterinary World, 13(2): 266-274

Al Amin, M., Hoque, M. N., Siddiki, A. Z., Saha, S., & Kamal, M. M. (2020). Antimicrobial resistance situation in animal health of Bangladesh. Veterinary World, 13(12), 2713. https://doi.org/10.14202/VETWORLD.2020.2713-2727

Al Masud, A., Walpola, R. L., Sarker, M., Asaduzzaman, M., Islam, M. S., Mostafa, A. T., Akhtar, Z., Kabir, A., & Seale, H. (2025). Antibiotic dispensing practices in community pharmacies: Implications for antimicrobial stewardship in resource-constrained settings. Exploratory Research in Clinical and Social Pharmacy, 19, 100606. https://doi.org/10.1016/J.RCSOP.2025.100606

Alam, M., Ali, M., Das, N., & Rahman, M. (2014). Present status of rearing backyard poultry in selected areas of Mymensingh district. Bangladesh Journal of Animal Science, 43(1), 30–37. https://doi.org/10.3329/BJAS.V43I1.19382

Alchalaby, A. Y., Al-Abedi, S. F., & Al-Aalim, A. M. (2024). Isolation and identification of *Klebsiella pneumoniae* from respiratory disease in chicken. Iraqi Journal of Veterinary Sciences, 38(3), 583–588. https://doi.org/10.33899/IJVS.2024.144877.3335

Al-Hasan, M. N., Lahr, B. D., Eckel-Passow, J. E., & Baddour, L. M. (2009). Seasonal variation in *Escherichia coli* bloodstream infection: a population-based study. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases, 15(10), 947–950. https://doi.org/10.1111/J.1469-0691.2009.02877.X

Alikhan, N. F., Petty, N. K., Ben Zakour, N. L., & Beatson, S. A. (2011). BLAST Ring Image Generator (BRIG): Simple prokaryote genome comparisons. *BMC Genomics*, *12*(1), 1–10. https://doi.org/10.1186/1471-2164-12-402/TABLES/3

Altayb, H. N., Elbadawi, H. S., Alzahrani, F. A., Baothman, O., Kazmi, I., Nadeem, M. S., Hosawi, S., & Chaieb, K. (2022). Co–occurrence of β–Lactam and Aminoglycoside Resistance Determinants among Clinical and Environmental Isolates of *Klebsiella pneumoniae* and *Escherichia coli*: A Genomic Approach. Pharmaceuticals, 15(8). https://doi.org/10.3390/PH15081011

Altayb, H. N., Hosawi, S., Baothman, O., Kazmi, I., Chaieb, K., Abu Zeid, I. M., Elbadawi, H. S., Lopes, B. S., & Moglad, E. (2023). Molecular insights into novel environmental strains of *Klebsiella quasipneumoniae* harboring different antimicrobial-resistance genes. Frontiers in Public Health, 10, 1068888. https://doi.org/10.3389/FPUBH.2022.1068888/BIBTEX

Alvarez, C., Nastro, M., Goyheneche, B., Potente, N., Ledesma, M., Vay, C., Naas, T., Foccoli, M., de Gregorio, S., Famiglietti, A., Dabos, L., & Rodriguez, C. H. (2024). NDM–5–producing *Klebsiella pneumoniae* ST258 in a university hospital in Argentina. Journal of Antimicrobial Chemotherapy, 79(12), 3174–3177. https://doi.org/10.1093/JAC/DKAE337

Aminul, P., Anwar, S., Molla, M. M. A., & Miah, M. R. A. (2021). Evaluation of antibiotic resistance patterns in clinical isolates of *Klebsiella pneumoniae* in Bangladesh. Biosafety and Health, 3(6), 301–306. https://doi.org/10.1016/J.BSHEAL.2021.11.001

AMR Bangladesh - dashboard.iedcr.gov.bd. Accessed on 10 February 2025.

Anderson, D. J., Richet, H., Chen, L. F., Spelman, D. W., Hung, Y. J., Huang, A. T., Sexton, D. J., & Raoult, D. (2008). Seasonal Variation in *Klebsiella pneumoniae* Bloodstream Infection on 4 Continents. The Journal of Infectious Diseases, 197(5), 752–756. https://doi.org/10.1086/527486

Andrews JM. (2001). Determination of minimum inhibitory concentrations. *J Antimicrob Chemother.* 48 (1):5-16.

Antoniadou, A., Kontopidou, F., Poulakou, G., Koratzanis, E., Galani, I., Papadomichelakis, E., Kopterides, P., Souli, M., Armaganidis, A., & Giamarellou, H. (2007). Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: First report of a multiclonal cluster. Journal of Antimicrobial Chemotherapy, 59(4), 786–790. https://doi.org/10.1093/JAC/DKL562,

Anyanwu, M. U., Jaja, I. F., Okpala, C. O. R., Njoga, E. O., Okafor, N. A., & Oguttu, J. W. (2023). Mobile Colistin Resistance (*mcr*) Gene-Containing Organisms in Poultry Sector in Low- and Middle-Income Countries: Epidemiology, Characteristics, and One Health Control Strategies. Antibiotics, 12(7), 1117. https://doi.org/10.3390/ANTIBIOTICS12071117/S1

Anzai, E. K., de Souza Júnior, J. C., Peruchi, A. R., Fonseca, J. M., Gumpl, E. K., Pignatari, A. C. C., Hirano, Z. M. B., & Silveira, A. C. de O. (2017). First case report of non-human primates (Alouatta clamitans) with the hypervirulent *Klebsiella pneumoniae* serotype K1 strain ST 23: A possible emerging wildlife pathogen. Journal of Medical Primatology, 46(6), 337–342. https://doi.org/10.1111/JMP.12296,

Ara, B., Urmi, U. L., Haque, T. A., Nahar, S., Rumnaz, A., Ali, T., Alam, M. S., Mosaddek, A. S. M., Rahman, nor A. a., Haque, M., & Islam, S. (2021). Detection of mobile colistin-resistance gene variants (*mcr-1* and *mcr-2*) in urinary tract pathogens in Bangladesh: the last resort of infectious disease management colistin efficacy is under threat. Expert Review of Clinical Pharmacology, 14(4), 513–522. https://doi.org/10.1080/17512433.2021.1901577

Araújo, S., Silva, V., de Lurdes Enes Dapkevicius, M., Pereira, J. E., Martins, Â., Igrejas, G., & Poeta, P. (2024). Comprehensive Profiling of *Klebsiella* in Surface Waters from Northern Portugal: Understanding Patterns in Prevalence, Antibiotic Resistance, and Biofilm Formation. Water (Switzerland), 16(9), 1297. https://doi.org/10.3390/W16091297/S1

Araújo, S., Silva, V., Quintelas, M., Martins, Â., Igrejas, G., & Poeta, P. (2025). From soil to surface water: exploring *Klebsiella*'s clonal lineages and antibiotic resistance odyssey in environmental health. BMC Microbiology, 25(1), 1–18. https://doi.org/10.1186/S12866-025-03798-8/TABLES/1

Arredondo-Alonso, S., Willems, R. J. L., van Schaik, W., & Schürch, A. C. (2017). On the (im)possibility of reconstructing plasmids from whole-genome short-read sequencing data. Microbial Genomics, 3(10), e000128. https://doi.org/10.1099/mgen.0.000128

Arena, F., De Angelis, L. H., Pieralli, F., Di Pilato, V., Giani, T., Torricelli, F., D'Andrea, M. M., & Rossolini, G. M. (2015). Draft Genome Sequence of the First Hypermucoviscous *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* Isolate from a Bloodstream Infection. Genome Announcements, 3(5). https://doi.org/10.1128/GENOMEA.00952-15

Aslam, B., Asghar, R., Muzammil, S., Shafique, M., Siddique, A. B., Khurshid, M., Ijaz, M., Rasool, M. H., Chaudhry, T. H., Aamir, A., & Baloch, Z. (2024). AMR and Sustainable Development Goals: at a crossroads. Globalization and Health, 20(1), 1–21. https://doi.org/10.1186/S12992-024-01046-8/FIGURES/13

Aslam, B., Chaudhry, T. H., Arshad, M. I., Muzammil, S., Siddique, A. B., Yasmeen, N., Khurshid, M., Amir, A., Salman, M., Rasool, M. H., Xia, X., & Baloch, Z. (2022). Distribution and genetic diversity of multi-drug-resistant *Klebsiella pneumoniae* at the human–animal–environment interface in Pakistan. Frontiers in Microbiology, 13, 898248. https://doi.org/10.3389/FMICB.2022.898248

Assoni, L., Couto, A. J. M., Vieira, B., Milani, B., Lima, A. S., Converso, T. R., & Darrieux, M. (2024). Animal models of *Klebsiella pneumoniae* mucosal infections. Frontiers in Microbiology, 15, 1367422. https://doi.org/10.3389/FMICB.2024.1367422/XML

Azarian T, & Black A. (2024). Genomic epidemiology of bacteria. In The Applied Genomic Epidemiology Handbook (1st ed., pp. 22). Chapman and Hall/CRC. https://doi.org/10.1201/9781003409809-8

Bangladesh Bureau of Statistics. (2022). Mymensingh (City Corporation, Bangladesh) - Population Statistics, Charts, Map and Location. Accessed on 10 February 2025. https://citypopulation.de/en/bangladesh/admin/mymensingh/6140__mymensingh

Barrios-Camacho, H., Aguilar-Vera, A., Beltran-Rojel, M., Aguilar-Vera, E., Duran-Bedolla, J., Rodriguez-Medina, N., Lozano-Aguirre, L., Perez-Carrascal, O. M., Rojas, J., & Garza-Ramos, U. (2019). Molecular epidemiology of *Klebsiella variicola* obtained from different sources. Scientific Reports, 9(1), 10610. https://doi.org/10.1038/S41598-019-46998-9

Bassis, C. M., Seekatz, A., Moore, N. M., Lolans, K., Weinstein, R. A., Young, V. B., & Hayden, M. K. (2015). Comparison of Stool Versus Rectal Swab Samples From Long-Term Acute Care Hospital (LTACH) Patients for Microbiota Analysis. Open Forum Infectious Diseases, 2(suppl_1). https://doi.org/10.1093/OFID/OFV133.472

Bepari, A. K., Rabbi, G., Shaon, H. R., Khan, S. I., Zahid, Z. I., Dalal, K., & Reza, H. M. (2023). Factors Driving Antimicrobial Resistance in Rural Bangladesh: A Cross-Sectional Study on Antibiotic Use-Related Knowledge, Attitude, and Practice Among Unqualified Village Medical Practitioners and Pharmacy Shopkeepers. Advances in therapy, 40(8), 3478–3494. https://doi.org/10.1007/s12325-023-02547-5

Beyrouthy, R., Dalmasso, G., Birer, A., Robin, F., & Bonnet, R. (2020). Carbapenem Resistance Conferred by OXA-48 in K2-ST86 Hypervirulent *Klebsiella pneumoniae*, France. Emerging Infectious Diseases, 26(7), 1529. https://doi.org/10.3201/EID2607.191490

Bobbadi, S., Chinnam, B. K., Reddy, P. N., & Kandhan, S. (2021). Analysis of antibiotic resistance and virulence patterns in *Klebsiella pneumoniae* isolated from human urinary tract infections in India. Letters in Applied Microbiology, 73(5), 590–598. https://doi.org/10.1111/LAM.13544

Boolchandani, M., D'Souza, A. W., & Dantas, G. (2019). Sequencing-based methods and resources to study antimicrobial resistance. Nature Reviews Genetics, 20(6), 356–370. https://doi.org/10.1038/s41576-019-0108-4

Bowring, B. G., Fahy, V. A., Morris, A., & Collins, A. M. (2017). An unusual culprit: *Klebsiella pneumoniae* causing septicaemia outbreaks in neonatal pigs? Veterinary Microbiology, 203, 267–270. https://doi.org/10.1016/j.vetmic.2017.03.018

Boyd, S. E., Holmes, A., Peck, R., Livermore, D. M., & Hope, W. (2022). OXA-48-Like β-Lactamases: Global Epidemiology, Treatment Options, and Development Pipeline. Antimicrobial Agents and Chemotherapy, 66(8). https://doi.org/10.1128/AAC.00216-22

Brisse S, Grimont F, Grimont PAD. (2006). The genus Klebsiella. In: Dworkin M, Brisse S, Grimont F, Grimont PD. (2006). Prokaryotes. New York, NY: Springer New York. pp. 159–196. ISBN 9783540325246.

Broyles, L. M. T., Pakhtigian, E. L., & Mejia, A. (2024). Estimating effects of monsoon flooding on household water access. Environmental research letters: ERL [Web site], 19(9), 094038. https://doi.org/10.1088/1748-9326/ad6ce9

Brown, M. F., Gupta, R. R., Kuhn, M., Flanagan, M. E., & Mitton–Fry, M. (2011). New analyses of MIC 90 data to aid antibacterial drug discovery. MedChemComm, 2(8), 735–742. https://doi.org/10.1039/C1MD00095K

Butaye, P., Stegger, M., Moodley, A., Damborg, P., Williams, A., Halliday-Simmonds, I., & Guardabassi, L. (2021). One Health Genomic Study of Human and Animal *Klebsiella pneumoniae* Isolated at Diagnostic Laboratories on a Small Caribbean Island. Antibiotics, 11(1), 42. https://doi.org/10.3390/ANTIBIOTICS11010042/S1

Byarugaba, D. K., Erima, B., Wokorach, G., Alafi, S., Kibuuka, H., Mworozi, E., Najjuka, F., Kiyengo, J., Musinguzi, A. K., & Wabwire–Mangen, F. (2023). Genome Analysis of *Klebsiella pneumoniae*

Reveals International High–Risk Pandemic MDR Clones Emerging in Tertiary Healthcare Settings in Uganda. Pathogens, 12(11), 1334. https://doi.org/10.3390/PATHOGENS12111334/S1

Cabrera, A., Mason, E., Mullins, L. P., & Sadarangani, M. (2025). Antimicrobial resistance and vaccines in Enterobacteriaceae including extraintestinal pathogenic *Escherichia coli* and *Klebsiella pneumoniae*. Npj Antimicrobials and Resistance 2025 3:1, 3(1), 1–14. https://doi.org/10.1038/s44259-025-00100-8

Calderon-Gonzalez, R., Lee, A., Lopez-Campos, G., Hancock, S. J., Sa-Pessoa, J., Dumigan, A., McMullan, R., Campbell, E. L., & Bengoechea, J. A. (2023). Modelling the Gastrointestinal Carriage of *Klebsiella pneumoniae* Infections. MBio, 14(1), e03121-22. https://doi.org/10.1128/MBIO.03121-22

Calland, J. K., Haukka, K., Kpordze, S. W., Brusah, A., Corbella, M., Merla, C., Samuelsen, Ø., Feil, E. J., Sassera, D., Karikari, A. B., Saba, C. K. S., Thorpe, H. A., & Corander, J. (2023). Population structure and antimicrobial resistance among *Klebsiella* isolates sampled from human, animal, and environmental sources in Ghana: a cross-sectional genomic One Health study. The Lancet Microbe, 4(11), e943–e952. https://doi.org/10.1016/S2666-5247(23)00208-2

Calvo-Villamañán, A., Sastre-Dominguez, J., Barrera-Martín, Á., Costas, C., & San Millan, Á. (2025). Dissecting pOXA-48 fitness effects in clinical Enterobacterales using plasmid-wide CRISPRi screens. Nature Communications 2025 16:1, 16(1), 1–13. https://doi.org/10.1038/s41467-025-63082-1

Campos-Madueno, E. I., Mauri, C., Meroni, E., Paseiro, P. P., Consonni, A., Luzzaro, F., & Endimiani, A. (2022). Simultaneous gut colonization by *Klebsiella grimontii* and *Escherichia coli* co-possessing the blaKPC-3-carrying pQil plasmid. European Journal of Clinical Microbiology & Infectious Diseases, 41(7), 1087. https://doi.org/10.1007/S10096-022-04462-Z

Campos-Madueno, E. I., Moser, A. I., Risch, M., Bodmer, T., & Endimiani, A. (2021). Exploring the Global Spread of *Klebsiella grimontii* Isolates Possessing blaVIM-1 and mcr-9. Antimicrobial Agents and Chemotherapy, 65(9). https://doi.org/10.1128/AAC.00724-21

Cannatelli, A., Giani, T., D'Andrea, M. M., Di Pilato, V., Arena, F., Conte, V., & Rossolini, G. M. (2015). Molecular mechanisms of colistin resistance in Klebsiella pneumoniae clinical isolates. Antimicrobial Agents and Chemotherapy, 59(8), 4640–4642. https://doi.org/10.1128/AAC.01030-15

Carattoli, A. (2009). Resistance plasmid families in Enterobacteriaceae. Antimicrobial Agents and Chemotherapy, 53(6), 2227–2238. https://doi.org/10.1128/AAC.01707–08

Casey, J. A., Rudolph, K. E., Robinson, S. C., Bruxvoort, K., Raphael, E., Hong, V., Pressman, A., Morello-Frosch, R., Wei, R. X., & Tartof, S. Y. (2021). Sociodemographic Inequalities in Urinary Tract Infection in 2 Large California Health Systems. Open Forum Infectious Diseases, 8(6), ofab276. https://doi.org/10.1093/OFID/OFAB276

Castanheira, M., Simner, P. J., & Bradford, P. A. (2021). Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC-Antimicrobial Resistance, 3(3). https://doi.org/10.1093/JACAMR/DLAB092

CDC. (2020). 02-IPC-guideline-v6.pdf. Accessed on 10 August 2025. https://amr.cdc.gov.bd/wp-content/uploads/2022/03/02-IPC-guideline-v6.pdf

CDC. (2023). https://amr.cdc.gov.bd/wp-content/uploads/2023/03/AMR-Newsletter-7-Draft-13-16-Feb-23.pdf

Cha, E., Kristensen, A. R., Hertl, J. A., Schukken, Y. H., Tauer, L. W., Welcome, F. L., & Gröhn, Y. T. (2014). Optimal insemination and replacement decisions to minimize the cost of pathogen-specific clinical mastitis in dairy cows. Journal of Dairy Science, 97(4), 2101–2117. https://doi.org/10.3168/JDS.2013-7067, Chapman, P., Forde, B. M., Roberts, L. W., Bergh, H., Vesey, D., Jennison, A. V., Moss, S., Paterson, D. L., Beatson, S. A., & Harris, P. N. A. (2020). Genomic Investigation Reveals Contaminated Detergent as the Source of an Extended-Spectrum-β-Lactamase-Producing *Klebsiella michiganensis* Outbreak in a Neonatal Unit. Journal of Clinical Microbiology, 58(5), e01980-19. https://doi.org/10.1128/JCM.01980-19

Chawla, K., Piveteau, P., & Sharma, S. (2025). *Klebsiella pneumoniae*: a connecting link in the One Health concept. Pathogens and Global Health, 119(5–6). https://doi.org/10.1080/20477724.2025.2506042

Che, Y., Yang, Y., Xu, X., Brinda, K., Polz, M. F., Hanage, W. P., & Zhang, T. (2021). Conjugative plasmids interact with insertion sequences to shape the horizontal transfer of antimicrobial resistance genes. Proceedings of the National Academy of Sciences of the United States of America, 118(6), e2008731118. https://doi.org/10.1073/PNAS

Chellat, M. F., Raguž, L., & Riedl, R. (2016). Targeting Antibiotic Resistance. Angewandte Chemie (International Ed. in English), 55(23), 6600. https://doi.org/10.1002/ANIE.201506818

Chen, T., Xu, H., Chen, Y., Ji, J., Ying, C., Liu, Z., Xu, H., Zhou, K., Xiao, Y., & Shen, P. (2023). Identification and Characterization of OXA-232-Producing Sequence Type 231 Multidrug Resistant *Klebsiella pneumoniae* Strains Causing Bloodstream Infections in China. Microbiology Spectrum, 11(2), e02607-22. https://doi.org/10.1128/SPECTRUM.02607-22

Chen, T., Ying, L., Xiong, L., Wang, X., Lu, P., Wang, Y., Shen, P., & Xiao, Y. (2024). Understanding carbapenem-resistant hypervirulent *Klebsiella pneumoniae*: Key virulence factors and evolutionary convergence. HLife, 2(12), 611–624. https://doi.org/10.1016/J.HLIFE.2024.06.005

Cherak, Z., Loucif, L., Moussi, A., & Rolain, J. M. (2021). Epidemiology of mobile colistin resistance (mcr) genes in aquatic environments. Journal of Global Antimicrobial Resistance, 27, 51–62. https://doi.org/10.1016/J.JGAR.2021.07.021

Chew, K. L., Octavia, S., Lai, D., Lin, R. T. P., & Teoa, J. W. P. (2021). Genomic characterization of *Klebsiella quasipneumoniae* from clinical specimens in Singapore. Antimicrobial Agents and Chemotherapy, 65(8). https://doi.org/10.1128/AAC.00412-21/SUPPL_FILE/AAC00412-21_SUPP_S1_SEQ4.PDF

Chiaverini, A., Cornacchia, A., Centorotola, G., Tieri, E. E., Sulli, N., Del Matto, I., Iannitto, G., Petrone, D., Petrini, A., & Pomilio, F. (2022). Phenotypic and Genetic Characterization of *Klebsiella pneumoniae* Isolates from Wild Animals in Central Italy. Animals: An Open Access Journal from MDPI, 12(11), 1347. https://doi.org/10.3390/ANI12111347

Choby, J. E., Howard-Anderson, J., & Weiss, D. S. (2019). Hypervirulent *Klebsiella pneumoniae* – clinical and molecular perspectives. Journal of Internal Medicine, 287(3), 283. https://doi.org/10.1111/JOIM.13007

Chong, C. E., Pham, T. M., Carey, M. E., Wee, B. A., Taouk, M. L., Favieres, J. F., Moore, C. E., Dyson, Z. A., Lim, C., Brown, C. L., Williamson, D., Opatowski, L., Outterson, K., Mukiri, K. M., Sherry, N. L., Essack, S. Y., Brisse, S., Grad, Y. H., & Baker, K. S. (2024). Conference report of the 2024 Antimicrobial Resistance Meeting. Npj Antimicrobials and Resistance 2024 2:1, 2(1), 1–6. https://doi.org/10.1038/S44259-024-00058-Z

Chouduri, A. U., Biswas, M., Haque, M. U., Arman, M. S. I., Uddin, N., Kona, N., Akter, R., & Haque, A. (2018). Cephalosporin–3G, Highly Prescribed Antibiotic to Outpatients in Rajshahi, Bangladesh: Prescription Errors, Carelessness, Irrational Uses are the Triggering Causes of Antibiotic Resistance. Journal of Applied Pharmaceutical Science, 8,(6), 105–112. https://doi.org/10.7324/JAPS.2018.8614

Chowdhury, F., Bhuiya, S., Abdul Aleem, M., Shuvo, T. A., Mamun, G. M. S., Kumar Ghosh, P., Shahrin, L., Khan, S. Y., Islam, M. A., & Rahman, M. (2025). Decade-Long Trends in Antibiotic

Prescriptions According to WHO AWaRe Classification Among Severe Acute Respiratory Infection Patients at Tertiary Hospitals in Bangladesh (2011–2020). Antibiotics, 14(2), 199. https://doi.org/10.3390/ANTIBIOTICS14020199/S1

Chowdhury, S., Fournié, G., Blake, D., Henning, J., Conway, P., Hoque, M. A., Ghosh, S., Parveen, S., Biswas, P. K., Akhtar, Z., Islam, K., Islam, M. A., Rashid, M. M., Pelligand, L., Khan, Z. H., Rahman, M., Tomley, F., Debnath, N., & Chowdhury, F. (2022). Antibiotic usage practices and its drivers in commercial chicken production in Bangladesh. PLOS ONE, 17(10), e0276158. https://doi.org/10.1371/JOURNAL.PONE.0276158

Chowdhury, S., Rheman, S., Debnath, N., Delamare-Deboutteville, J., Akhtar, Z., Ghosh, S., Parveen, S., Islam, K., Islam, M. A., Rashid, M. M., Khan, Z. H., Rahman, M., Chadag, V. M., & Chowdhury, F. (2022). Antibiotics usage practices in aquaculture in Bangladesh and their associated factors. One Health, 15, 100445. https://doi.org/10.1016/J.ONEHLT.2022.100445

Christensen, S. B. (2021). Drugs That Changed Society: History and Current Status of the Early Antibiotics: Salvarsan, Sulfonamides, and β-Lactams. Molecules, 26(19), 6057. https://doi.org/10.3390/MOLECULES26196057

Cimmino T., Le Page, S., & Raoult, D. (2016). Contemporary challenges and opportunities in the diagnosis and outbreak detection of multidrug-resistant infectious disease. Expert Rev Mol Diagn. 16(11):1163-1175.

Clinical and Laboratory Standards Institute. (2023). Performance standards for antimicrobial susceptibility testing (33rd ed.; CLSI supplement M100). CLSI.

Coates, A. R., Halls, G., & Hu, Y. (2011). Novel classes of antibiotics or more of the same? British Journal of Pharmacology, 163(1), 184. https://doi.org/10.1111/J.1476-5381.2011.01250.X

Collins, A. M., & Mizzi, R. (2025). Virulence determinants in *Klebsiella pneumoniae* associated with septicaemia outbreaks in neonatal pigs. Veterinary Microbiology, 302. https://doi.org/10.1016/j.vetmic.2025.110409

Cook, K., Premchand-Branker, S., Nieto-Rosado, M., Portal, E. A. R., Li, M., Rubio, C. O., Mathias, J., Aziz, J., Iregbu, K., Afegbua, S. L., Aliyu, A., Mohammed, Y., Nwafia, I., Oduyebo, O., Ibrahim, A., Tanko, Z., Walsh, T. R., Achi, C., & Sands, K. (2025). Flies as carriers of antimicrobial resistant (AMR) bacteria in Nigerian hospitals: A workflow for surveillance of AMR bacteria carried by arthropod pests in hospital settings. Environment International, 196, 109294. https://doi.org/10.1016/J.ENVINT.2025.109294

Croucher, N. J., Page, A. J., Connor, T. R., Delaney, A. J., Keane, J. A., Bentley, S. D., Parkhill, J., & Harris, S. R. (2015). Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Research*, *43*(3), e15–e15. https://doi.org/10.1093/NAR/GKU1196

Dangor, Z., Benson, N., Berkley, J. A., Bielicki, J., Bijsma, M. W., Broad, J., Buurman, E. T., Cross, A., Duffy, E. M., Holt, K. E., Iroh Tam, P. Y., Jit, M., Karampatsas, K., Katwere, M., Kwatra, G., Laxminarayan, R., Le Doare, K., Mboizi, R., Micoli, F., … Madhi, S. A. (2024). Vaccine value profile for *Klebsiella pneumoniae*. Vaccine, 42(19), S125–S141. https://doi.org/10.1016/J.VACCINE.2024.02.072

Davies, A. R., Chisnall, T., Akter, S., Afrad, M. M. H., Sadekuzzaman, M., Badhy, S. C., Hasan, M. Z., Rahman, M. T., Smith, R. P., Card, R. M., Brum, E., & Chowdhury, M. G. A. (2024). Genomic characterisation of *Escherichia coli* isolated from poultry at retail through Sink Surveillance in Dhaka, Bangladesh reveals high levels of multi-drug resistance. Frontiers in Microbiology, 15, 1418476. https://doi.org/10.3389/FMICB.2024.1418476/BIBTEX

De-Majumdar, S., Veleba, M., Finn, S., Fanning, S., & Schneiders, T. (2013). Elucidating the regulon of multidrug resistance regulator rarA in *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy, 57(4), 1603–1609. https://doi.org/10.1128/AAC.01998-12,

De Maio, N., Shaw, L. P., Hubbard, A., George, S., Sanderson, N. D., Swann, J., Wick, R., AbuOun, M., Stubberfield, E., Hoosdally, S. J., Crook, D. W., Peto, T. E. A., Sheppard, A. E., Bailey, M. J., Read, D. S., Anjum, M. F., Walker, A. S., Stoesser, N., & REHAB Consortium. (2019). Comparison of long-read sequencing technologies in the hybrid assembly of complex bacterial genomes. Microbial Genomics, 5(9), e000294. https://doi.org/10.1099/mgen.0.000294

Delpy, L., Astbury, C. C., Aenishaenslin, C., Ruckert, A., Penney, T. L., Wiktorowicz, M., Ciss, M., Benko, R., & Bordier, M. (2024). Integrated surveillance systems for antibiotic resistance in a One Health context: a scoping review. BMC Public Health, 24(1), 1–21. https://doi.org/10.1186/S12889-024-19158-6/FIGURES/2

Denissen, J., Reyneke, B., Waso-Reyneke, M., Havenga, B., Barnard, T., Khan, S., & Khan, W. (2022). Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. International Journal of Hygiene and Environmental Health, 244, 114006. https://doi.org/10.1016/J.IJHEH.2022.114006

Dereeper, A., Gruel, G., Pot, M., Couvin, D., Barbier, E., Bastian, S., Bambou, J.–C., Gelu–Simeon, M., Ferdinand, S., Guyomard–Rabenirina, S., Passet, V., Martino, F., Piveteau, P., Reynaud, Y., Rodrigues, C., Roger, P.–M., Roy, X., Talarmin, A., Tressieres, B., … Breurec, S. (2022). Limited Transmission of *Klebsiella pneumoniae* among Humans, Animals, and the Environment in a Caribbean Island, Guadeloupe (French West Indies). Microbiology Spectrum, 10(5). https://doi.org/10.1128/SPECTRUM.01242–22,

Dey, T., Chakrabortty, A., Kapoor, A., Warrier, A., Nag, V. L., Sivashanmugam, K., & Shankar, M. (2022). Unusual Hypermucoviscous Clinical Isolate of *Klebsiella pneumoniae* with No Known Determinants of Hypermucoviscosity. Microbiology Spectrum, 10(3). https://doi.org/10.1128/SPECTRUM.00393-22

DGDA. (2023). Bangladesh National Veterinary Formulary 2023. Accessed on 30 August 2025. https://dgda.portal.gov.bd/sites/default/files/files/dgda.portal.gov.bd/policies/f29f9e48_5de0_4721_bbd6_af9819b4e734/2024-10-28-15-43-19c52408cb076df5f8f64cdeafe16a1d.pdf

DGHS. (2021). Government Hospital Services Details. Accessed on 30 August 2025. https://dashboard.dghs.gov.bd/pages/dashboard_hospital_services_details.php?type=5&year=2021

Di Pilato, V., Arena, F., Tascini, C., Cannatelli, A., Henrici De Angelis, L., Fortunato, S., Giani, T., Menichetti, F., & Rossolini, G. M. (2016). mcr-1.2, a new mcr variant carried on a transferable plasmid from a colistin-resistant KPC carbapenemase-producing *Klebsiella pneumoniae* strain of sequence type 512. Antimicrobial Agents and Chemotherapy, 60(9), 5612–5615. https://doi.org/10.1128/AAC.01075-16/ASSET/0D702DB8-CDDB-4981-A26F-988F83A4C8C3

Diancourt, L., Passet, V., Verhoef, J., Grimont, P. A. D., & Brisse, S. (2005). Multilocus Sequence Typing of *Klebsiella pneumoniae* Nosocomial Isolates. Journal of Clinical Microbiology, 43(8), 4178. https://doi.org/10.1128/JCM.43.8.4178-4182.2005

Didelot, X., & Wilson, D. J. (2015). ClonalFrameML: Efficient inference of recombination in whole bacterial genomes. PLoS Computational Biology, 11(2), e1004041. https://doi.org/10.1371/journal.pcbi.1004041

Dingiswayo, L., Adelabu, O. A., Arko-Cobbah, E., Pohl, C., Mokoena, N. Z., Du Plessis, M., & Musoke, J. (2024). Hypervirulent *Klebsiella pneumoniae* in a South African tertiary hospital—Clinical profile, genetic determinants, and virulence in Caenorhabditis elegans. Frontiers in Microbiology, 15, 1385724. https://doi.org/10.3389/FMICB.2024.1385724/FULL

Dong, N., Yang, X., Chan, E. W. C., Zhang, R., & Chen, S. (2022). *Klebsiella* species: Taxonomy, hypervirulence and multidrug resistance. EBioMedicine, 79, 103998. https://doi.org/10.1016/J.EBIOM.2022.103998

Donnenberg, M. S. (2014). Enterobacteriaceae. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 2, 2503–2517. https://doi.org/10.1016/B978-1-4557-4801-3.00220-4

Dutta, A., Islam, M. Z., Barua, H., Rana, E. A., Jalal, M. S., Dhar, P. K., Das, A., Das, T., Sarma, S. M., Biswas, S. K., & Biswas, P. K. (2020). Acquisition of Plasmid-Mediated Colistin Resistance Gene mcr-1 in *Escherichia coli* of Livestock Origin in Bangladesh. Microbial Drug Resistance, 26(9), 1058–1062. https://doi.org/10.1089/MDR.2019.0304,

Eber, M. R., Shardell, M., Schweizer, M. L., Laxminarayan, R., & Perencevich, E. N. (2011). Seasonal and temperature-associated increases in gram-negative bacterial bloodstream infections among hospitalized patients. PloS One, 6(9). https://doi.org/10.1371/JOURNAL.PONE.0025298

ECDC (2024). Emergence of hypervirulent *Klebsiella pneumoniae* ST23 carrying carbapenemase genes in EU/EEA countries, first update. (2024). https://doi.org/10.2900/993023

ECDC. (2023). Sample size guidance for surveillance data. Accessed on 10 February 2025. https://www.ecdc.europa.eu/sites/default/files/documents/Sample_size_guidance_for_surveillance_data.pdf

Eger, E., Heiden, S. E., Becker, K., Rau, A., Geisenhainer, K., Idelevich, E. A., & Schaufler, K. (2021). Hypervirulent *Klebsiella pneumoniae* sequence type 420 with a chromosomally inserted virulence plasmid. International Journal of Molecular Sciences, 22(17), 9196. https://doi.org/10.3390/IJMS22179196/S1

El-Badawy, M. F., Tawakol, W. M., El-Far, S. W., Maghrabi, I. A., Al-Ghamdi, S. A., Mansy, M. S., Ashour, M. S., & Shohayeb, M. M. (2017). Molecular Identification of Aminoglycoside-Modifying Enzymes and Plasmid-Mediated Quinolone Resistance Genes among *Klebsiella pneumoniae* Clinical Isolates Recovered from Egyptian Patients. International Journal of Microbiology. https://doi.org/10.1155/2017/8050432

Elias, R., Spadar, A., Hendrickx, A.P.A., Bonnin, R.A., Dortet, L., Pinto, M., Phelan, J.E., Portugal, I., Campino, S., da Silva, G. J., Clark, T. G., Duarte, A. & Perdigão, J. (2023). Emergence of KPC-3- and OXA-181-producing ST13 and ST17 Klebsiella pneumoniae in Portugal: genomic insights on national and international dissemination. J Antimicrob Chemother, 3;78(5):1300-1308. https://doi.org/10.1093/jac/dkad093. PMID: 36999363.

Ellington, M. J., Ekelund, O., Aarestrup, F. M., Canton, R., Doumith, M., Giske, C., Grundman, H., Hasman, H., Holden, M. T. G., Hopkins, K. L., Iredell, J., Kahlmeter, G., Köser, C. U., MacGowan, A., Mevius, D., Müller, L., Naas, T., Peto, T., Rolain, J. M., … Woodford, N. (2017). The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: Report from the EUCAST Subcommittee. Clinical Microbiology and Infection, 23(1), 2–22. https://doi.org/10.1016/j.cmi.2016.11.012

EUCAST. (2016). Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI–EUCAST Polymyxin Breakpoints Working Group. Clinical Microbiology and Infection, 22(2), 144–149. https://doi.org/10.1016/j.cmi.2015.11.016

EUCAST. 2022. EUCAST V 12.0 Breakpoint Tables | PDF | Urinary Tract Infection | Antimicrobial Resistance. Accessed on 10 August 2024. https://www.scribd.com/document/561411849/v-12-0-Breakpoint-Tables

EMA. (2016). European expert group proposes reduction of use in animals of last resort antibiotic colistin to manage risk of resistance. European Medicines Agency (EMA). Retrieved April 28, 2025, from https://www.ema.europa.eu/en/news/european–expert–group–proposes–reduction–use–animals–last–resort–antibiotic–colistin–manage–risk–resistance

Ewers, C., Stamm, I., Pfeifer, Y., Wieler, L. H., Kopp, P. A., Schønning, K., Prenger-Berninghoff, E., Scheufen, S., Stolle, I., Günther, S., & Bethe, A. (2014). Clonal spread of highly successful ST15-CTX-M-15 *Klebsiella pneumoniae* in companion animals and horses. Journal of Antimicrobial Chemotherapy, 69(10), 2676–2680. https://doi.org/10.1093/JAC/DKU217

FAO, UNEP, WHO, & WOAH. (2022). One Health Joint Plan of Action (2022-2026). Working together for the health of humans, animals, plants and the environment. Rome. https://doi.org/10.4060/cc2289e

FAO. (2016). The FAO action plan on antimicrobial resistance 2016–2020: Supporting the food and agriculture sectors in implementing the global action plan on antimicrobial resistance to minimize the impact of antimicrobial resistance. Accessed on 10 February 2025. https://openknowledge.fao.org/server/api/core/bitstreams/70dd55f7-3348-4895-b651-76308101cb45

FAO. (2023). Guidelines on monitoring antimicrobial use at the farm level. Accessed on 30 August 2025. https://openknowledge.fao.org/server/api/core/bitstreams/452e5497-6776-4e6b-a61b-76da57861b72

Farzana, R., Jones, L. S., Barratt, A., Rahman, M. A., Sands, K., Portal, E., Boostrom, I., Espina, L., Pervin, M., Uddin, A. K. M. N., & Walsh, T. R. (2020). Emergence of Mobile Colistin Resistance (*mcr–8*) in a Highly Successful *Klebsiella pneumoniae* Sequence Type 15 Clone from Clinical Infections in Bangladesh. MSphere, 5(2). https://doi.org/10.1128/MSPHERE.00023–20/SUPPL_FILE/MSPHERE.00023–20SF004.TIF

Farzana, R., Jones, L. S., Rahman, M. A., Andrey, D. O., Sands, K., Portal, E., Watkins, W. J., Pervin, M., Banerjee, M., & Walsh, T. R. (2019). Outbreak of Hypervirulent Multidrug-resistant *Klebsiella variicola* Causing High Mortality in Neonates in Bangladesh. Clinical Infectious Diseases, 68(7), 1225–1227. https://doi.org/10.1093/CID/CIY778

Farzana, R., Jones, L. S., Rahman, M. A., Sands, K., Van Tonder, A. J., Portal, E., Criollo, J. M., Parkhill, J., Guest, M. F., Watkins, W. J., Pervin, M., Boostrom, I., Hassan, B., Mathias, J., Kalam, M. A., & Walsh, T. R. (2023). Genomic Insights Into the Mechanism of Carbapenem Resistance Dissemination in Enterobacterales From a Tertiary Public Heath Setting in South Asia. Clinical Infectious Diseases, 76(1), 119–133. https://doi.org/10.1093/CID/CIAC287

Farzana, R., Jones, L. S., Rahman, M. A., Toleman, M. A., Sands, K., Portal, E., Boostrom, I., Kalam, M. A., Hassan, B., Uddin, A. N., & Walsh, T. R. (2019). Emergence of *mcr-1* mediated colistin resistant *Escherichia coli* from a hospitalized patient in Bangladesh. Journal of Infection in Developing Countries, 13(8), 773–776. https://doi.org/10.3855/JIDC.11541

Farzana R, Shamsuzzaman S, Mamun KZ. (2013). Isolation and molecular characterization of New Delhi metallo-beta-lactamase-1 producing superbug in Bangladesh. J Infect Dev Ctries. 7(3):161-168

Feldman, M. F., Mayer Bridwell, A. E., Scott, N. E., Vinogradov, E., McKee, S. R., Chavez, S. M., Twentyman, J., Stallings, C. L., Rosen, D. A., & Harding, C. M. (2019). A promising bioconjugate vaccine against hypervirulent *Klebsiella pneumoniae*. Proceedings of the National Academy of Sciences, 116(37), 18655–18663. https://doi.org/10.1073/PNAS.1907833116

Feng, Y., Yang, Y., Hu, Y., Xiao, Y., Xie, Y., Wei, L., Wen, H., Zhang, L., McNally, A., & Zong, Z. (2024). Population genomics uncovers global distribution, antimicrobial resistance, and virulence genes of the opportunistic pathogen *Klebsiella* aerogenes. Cell Reports, 43(8), 114602. https://doi.org/10.1016/J.CELREP.2024.114602

Ferdous, J., Hossain, S., Chaity, F. S., Sarkar, R., Bahar, A., Masud, H. M. A. Al, & Islam, S. (2022). Draft Genome Sequence of a Multidrug-Resistant and Novel Sequence Type 6130 *Klebsiella quasipneumoniae* Strain C11S11_BCSIR Isolated from Wastewater of a Tertiary Care Hospital in

Chattogram, Bangladesh. Microbiology Resource Announcements, 11(10). https://doi.org/10.1128/MRA.00654-22

Ferdous, J., Sachi, S., Al Noman, Z., Hussani, S. M. A. K., Sarker, Y. A., & Sikder, M. H. (2019). Assessing farmers' perspective on antibiotic usage and management practices in small-scale layer farms of Mymensingh district, Bangladesh. Veterinary World, 12(9), 1441–1447. https://doi.org/10.14202/VETWORLD.2019.1441-1447

Fernández Salgueiro, M., Cernuda Martínez, J. A., Gan, R. K., & Arcos González, P. (2024). Climate change and antibiotic resistance: A scoping review. Environmental Microbiology Reports, 16(5), e70008. https://doi.org/10.1111/1758-2229.70008

Flatgard, B. M., Williams, A. D., Amin, M. B., Hobman, J. L., Stekel, D. J., Rousham, E. K., & Islam, M. A. (2024). Tracking antimicrobial resistance transmission in urban and rural communities in Bangladesh: a One Health study of genomic diversity of ESBL-producing and carbapenem-resistant *Escherichia coli* . Microbiology Spectrum, 12(6). https://doi.org/10.1128/SPECTRUM.03956-23/SUPPL_FILE/SPECTRUM.03956-23-S0004.TIFF

Fleming, A. (1929). On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. British Journal of Experimental Pathology, 10(3), 226. https://pmc.ncbi.nlm.nih.gov/articles/PMC2048009/

Fontana, L., Bonura, E., Lyski, Z., & Messer, W. (2019). The Brief Case: *Klebsiella variicola*-Identifying the Misidentified. Journal of Clinical Microbiology, 57(1). https://doi.org/10.1128/JCM.00826-18

Formenti, N., Guarneri, F., Bertasio, C., Parisio, G., Romeo, C., Scali, F., Birbes, L., Boniotti, M. B., Diegoli, G., Candela, L., Romeo, G. A., Pasquali, P., & Alborali, G. L. (2022). Wastewater-based surveillance in Italy leading to the first detection of mcr-10-positive *Klebsiella* quasipneumoniae. Antimicrobial Resistance and Infection Control, 11(1), 155. https://doi.org/10.1186/S13756-022-01194-9

Fostervold, A., Raffelsberger, N., Hetland, M. A. K., Bakksjø, R., Bernhoff, E., Samuelsen, Ø., Sundsfjord, A., Afset, J. E., Berntsen, C. F., Bævre-Jensen, R., Ebbesen, M. H., Gammelsrud, K. W., Guleng, A. D., Handal, N., Jakovljev, A., Johal, S. K., Marvik, Å., Natvik, A., Sandnes, R. A., … Löhr, I. H. (2024). Risk of death in *Klebsiella pneumoniae* bloodstream infections is associated with specific phylogenetic lineages. Journal of Infection, 88(5), 106155. https://doi.org/10.1016/J.JINF.2024.106155

Fotedar, R., Banerjee, U., Samantray, J. C., & Shriniwas. (1992). Vector potential of hospital houseflies with special reference to *Klebsiella* species. Epidemiology and Infection, 109(1), 143. https://pmc.ncbi.nlm.nih.gov/articles/PMC2272229

Foysal, M., Imam, T., Das, S. B., Gibson, J. S., Mahmud, R., Gupta, S. D., Fournié, G., Hoque, M. A., & Henning, J. (2024). Association between antimicrobial usage and resistance on commercial broiler and layer farms in Bangladesh. Frontiers in Veterinary Science, 11, 1435111. https://doi.org/10.3389/FVETS.2024.1435111/BIBTEX

Furlan, J. P. R., Sellera, F. P., Lincopan, N., Debone, D., Miraglia, S. G. E. K., & Tavella, R. A. (2024). Catastrophic floods and antimicrobial resistance: Interconnected threats with wide-ranging impacts. One Health, 19, 100891. https://doi.org/10.1016/J.ONEHLT.2024.100891

Gao, H., Wang, B., Li, M., Zhou, P., Wu, C., Wan, C., Shen, L., Fu, J., Han, W., Zhou, Y., Yu, F., Wang, H., Shen, Z., & Liu, G. (2025). Emergence and dissemination of multidrug–resistant *Klebsiella pneumoniae* harboring the novel tmexCD–toprJ RND efflux pump operon. Frontiers in Cellular and Infection Microbiology, 15, 1579880. https://doi.org/10.3389/FCIMB.2025.1579880

García-Cobos, S., Oteo-Iglesias, J., & Pérez-Vázquez, M. (2025). Hypervirulent *Klebsiella pneumoniae*: Epidemiology outside Asian countries, antibiotic resistance association, methods of detection and clinical management. Enfermedades Infecciosas y Microbiología Clínica, 43(2), 102–109. https://doi.org/10.1016/J.EIMC.2024.12.008

Ge, H., Qiao, J., Xu, H., Liu, R., Chen, R., Li, C., Hu, X., Zhou, J., Guo, X., & Zheng, B. (2022). First report of *Klebsiella pneumoniae* co-producing OXA-181, CTX-M-55, and MCR-8 isolated from the patient with bacteremia. Frontiers in Microbiology, 13. https://doi.org/10.3389/FMICB.2022.1020500

Gelmez, G. A., Can, B., Hasdemir, U., & Soyletir, G. (2021). Evaluation of phenotypic tests for detection of carbapenemases: New modifications with new interpretation. Journal of Infection and Chemotherapy, 27(2), 226–231. https://doi.org/10.1016/J.JIAC.2020.09.021

General Assembly of the World Medical Association. (2014). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Am Coll Dent. 81(3):14-18

Giannattasio-Ferraz, S., Ene, A., Johnson, G., Maskeri, L., Oliveira, A. P., Banerjee, S., Barbosa-Stancioli, E. F., & Putonti, C. (2022). Multidrug-Resistant *Klebsiella variicola* Isolated in the Urine of Healthy Bovine Heifers, a Potential Risk as an Emerging Human Pathogen. Applied and Environmental Microbiology, 88(9), e00044-22. https://doi.org/10.1128/AEM.00044-22

GitHub. (2018). GitHub - gtonkinhill/pairsnp: A set of scripts for calculating pairwise SNP distance. Accessed on 10 February 2025. https://github.com/gtonkinhill/pairsnp

Gondal, A. J., Saleem, S., Choudhry, N., Ahmad, F. J., Bukhari, H., Yasmin, N., & Jahan, S. (2023). Emergence of multidrug–resistant ST11 blaKPC–2 producing *Klebsiella pneumoniae* coharboring blaCTX–M and blaSHV in Pakistan. Journal of Infection in Developing Countries, 17(2), 210–217. https://doi.org/10.3855/JIDC.17041

González-Montalvo, M. A., Sorescu, J. M., Baltes, G., Juárez, O., & Tuz, K. (2024). The respiratory chain of *Klebsiella aerogenes* in urine-like conditions: critical roles of NDH-2 and bd-terminal oxidases. Frontiers in Microbiology, 15, 1479714. https://doi.org/10.3389/FMICB.2024.1479714/BIBTEX

Gould, K. (2016). Antibiotics: from prehistory to the present day. Journal of Antimicrobial Chemotherapy, 71(3), 572–575. https://doi.org/10.1093/JAC/DKV484

Gorrie, C. L., Mirceta, M., Wick, R. R., Edwards, D. J., Thomson, N. R., Strugnell, R. A., Pratt, N. F., Garlick, J. S., Watson, K. M., Pilcher, D. V., McGloughlin, S. A., Spelman, D. W., Jenney, A. W. J., & Holt, K. E. (2017). Gastrointestinal Carriage Is a Major Reservoir of Klebsiella pneumoniae Infection in Intensive Care Patients. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 65(2), 208–215. https://doi.org/10.1093/cid/cix270

Gravey, F., Sévin, C., Castagnet, S., Foucher, N., Maillard, K., Tapprest, J., Léon, A., Langlois, B., Le Hello, S., & Petry, S. (2023). Antimicrobial resistance and genetic diversity of *Klebsiella pneumoniae* strains from different clinical sources in horses. Frontiers in Microbiology, 14, 1334555. https://doi.org/10.3389/FMICB.2023.1334555/FULL

Gu, D., Dong, N., Zheng, Z., Lin, D., Huang, M., Wang, L., Chan, E. W. C., Shu, L., Yu, J., Zhang, R., & Chen, S. (2018). A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. The Lancet Infectious Diseases, 18(1), 37–46. https://doi.org/10.1016/S1473-3099(17)30489-9

Guedes, M., Gathara, D., López-Hernández, I., Pérez-Crespo, P. M. M., Pérez-Rodríguez, M. T., Sousa, A., Plata, A., Reguera-Iglesias, J. M., Boix-Palop, L., Dietl, B., Blanco, J. S., Castillo, C. A., Galán-Sánchez, F., Kindelán, C. N., Jover-Saenz, A., Aguirre, J. G., Alemán, A. A., Ciordia, T. M., del Arco Jiménez, A., … Rodríguez-Baño, J. (2024). Differences in clinical outcomes of bloodstream infections caused by *Klebsiella* aerogenes, *Klebsiella pneumoniae* and Enterobacter cloacae: a multicentre cohort study. Annals of Clinical Microbiology and Antimicrobials, 23(1), 1–11. https://doi.org/10.1186/S12941-024-00700-8/TABLES/3

Guo, X., Chen, R., Wang, Q., Li, C., Ge, H., Qiao, J., & Li, Y. (2022). Global prevalence, characteristics, and future prospects of IncX3 plasmids: A review. Frontiers in Microbiology, 13, 979558. https://doi.org/10.3389/FMICB.2022.979558/BIBTEX

Gürbüz, M., Şen, E. T., Şimşek, M., & Demir, C. (2024). Investigation of Plasmid-Mediated Colistin Resistance Genes (mcr-1–8) in Enterobacterales Isolates. Cureus, 16(6), e61538. https://doi.org/10.7759/CUREUS.61538

Gurieva, T., Dautzenberg, M. J. D., Gniadkowski, M., Derde, L. P. G., Bonten, M. J. M., & Bootsma, M. C. J. (2018). The Transmissibility of Antibiotic-Resistant Enterobacteriaceae in Intensive Care Units. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 66(4), 489–493. https://doi.org/10.1093/cid/cix825

Hadjadj, L., Baron, S. A., Olaitan, A. O., Morand, S., & Rolain, J. M. (2019). Co-occurrence of Variants of *mcr-3* and *mcr-8* Genes in a *Klebsiella pneumoniae* Isolate From Laos. Frontiers in Microbiology, 10, 485843

Hameed, F., Khan, M. A., Bilal, H., Muhammad, H., & Tayyab Ur Rehman. (2020). Detection of MCR-1 Gene in Multiple Drug Resistant *Escherichia coli* and *Klebsiella pneumoniae* in Human Clinical Samples from Peshawar, Pakistan. Combinatorial Chemistry & High Throughput Screening, 24(5), 737–742. https://doi.org/10.2174/1386207323666200914100119

Hammad, H. A., Mohamed, I. S., El-Badawy, O., Zakaria, A. M., Shabaan, L., & Aly, S. A. (2023). pKpQIL-like plasmid contributes to the dissemination of blaNDM-1 and plasmid mediated quinolone resistance determinants among multi drug resistant *Klebsiella pneumoniae* in Assiut university hospital, Egypt. Iranian Journal of Microbiology, 15(2), 208. https://doi.org/10.18502/IJM.V15I2.12471

Harun, M. G. D., Anwar, M. M. U., Sumon, S. A., Hassan, M. Z., Haque, T., Mah-E-Muneer, S., Rahman, A., Abdullah, S. A. H. M., Islam, M. S., Styczynski, A. R., & Kaydos-Daniels, S. C. (2022). Infection prevention and control in tertiary care hospitals of Bangladesh: results from WHO infection prevention and control assessment framework (IPCAF). Antimicrobial Resistance and Infection Control, 11(1), 1–13. https://doi.org/10.1186/S13756-022-01161-4/TABLES/4

Harun, M. G. D., Sumon, M. S. A., & Islam, M. S. (2025). P-403. Incidence of Surgical Site Infections in Selected Tertiary Care Hospitals in Bangladesh: Findings From A Pilot Surveillance Project. Open Forum Infectious Diseases, 12(Supplement_1). https://doi.org/10.1093/OFID/OFAE631.604

Haruta, S., Yamaguchi, H., Yamamoto, E. T., Eriguchi, Y., Nukaga, M., O'Hara, K., & Sawai, T. (2000). Functional analysis of the active site of a metallo-β-lactamase proliferating in Japan. Antimicrobial Agents and Chemotherapy, 44(9), 2304–2309. https://doi.org/10.1128/AAC.44.9.2304-2309.2000

Hassan, B., Ijaz, M., Khan, A., Sands, K., Serfas, G. I., Clayfield, L., El-Bouseary, M. M., Lai, G., Portal, E., Khan, A., Watkins, W. J., Parkhill, J., & Walsh, T. R. (2021). A role for arthropods as vectors of multidrug-resistant Enterobacterales in surgical site infections from South Asia. Nature Microbiology, 6(10), 1259–1270. https://doi.org/10.1038/S41564-021-00965-1

Hassan, M. M., Kalam, M. A., Alim, M. A., Shano, S., Nayem, M. R. K., Badsha, M. R., Mamun, M. A. Al, Hoque, A., Tanzin, A. Z., Nath, C., Khanom, H., Khan, S. A., Islam, M. M., Uddin, M. B., & Islam, A. (2021). Knowledge, attitude, and practices on antimicrobial use and antimicrobial resistance among commercial poultry farmers in Bangladesh. Antibiotics, 10(7), 784. https://doi.org/10.3390/ANTIBIOTICS10070784/S1

Hassing, R. J., Alsma, J., Arcilla, M. S., van Genderen, P. J., Stricker, B. H., & Verbon, A. (2015). International travel and acquisition of multidrug-resistant Enterobacteriaceae: a systematic review. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, 20(47), 10.2807/1560-7917.ES.2015.20.47.30074. https://doi.org/10.2807/1560-7917.ES.2015.20.47.30074

Hatrongjit, R., Wongsurawat, T., Jenjaroenpun, P., Chopjitt, P., Boueroy, P., Akeda, Y., Okada, K., Iida, T., Hamada, S., & Kerdsin, A. (2024). Genomic analysis of carbapenem- and colistin-resistant *Klebsiella pneumoniae* complex harbouring mcr-8 and mcr-9 from individuals in Thailand. Scientific Reports, 14(1), 1–13. https://doi.org/10.1038/S41598-024-67838-5

Hayati, M., Indrawati, A., Mayasari, N. L. P. I., Istiyaningsih, I., & Atikah, N. (2019). Molecular detection of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates of chicken origin from East Java, Indonesia. Veterinary World, 12(4), 578. https://doi.org/10.14202/VETWORLD.2019.578-583

Hawkey P. M. (2015). Multidrug-resistant Gram-negative bacteria: a product of globalization. The Journal of hospital infection, 89(4), 241–247. https://doi.org/10.1016/j.jhin.2015.01.008

Heinz, E., Pearse, O., Zuza, A., Bilima, S., Msefula, C., Musicha, P., Siyabu, P., Tewesa, E., Graf, F. E., Lester, R., Lissauer, S., Cornick, J., Lewis, J. M., Kawaza, K., Thomson, N. R., & Feasey, N. A. (2024). Longitudinal analysis within one hospital in sub-Saharan Africa over 20 years reveals repeated replacements of dominant clones of *Klebsiella pneumoniae* and stresses the importance to include temporal patterns for vaccine design considerations. Genome Medicine, 16(1), 1–17. https://doi.org/10.1186/S13073-024-01342-3/FIGURES/5

Heng, H., Yang, X., Ye, L., Tang, Y., Guo, Z., Li, J., Chan, E. W. C., Zhang, R., & Chen, S. (2024). Global genomic profiling of *Klebsiella pneumoniae*: A spatio-temporal population structure analysis. International Journal of Antimicrobial Agents, 63(2), 107055. https://doi.org/10.1016/J.IJANTIMICAG.2023.107055

Hennart, M., Guglielmini, J., Bridel, S., Maiden, M. C. J., Jolley, K. A., Criscuolo, A., & Brisse, S. (2022). A Dual Barcoding Approach to Bacterial Strain Nomenclature: Genomic Taxonomy of *Klebsiella pneumoniae* Strains. Molecular Biology and Evolution, 39(7). https://doi.org/10.1093/MOLBEV/MSAC135

Hennessey, M., Alarcon, P., Samanta, I., Fournié, G., Paleja, H., Papaiyan, K., & Gautham, M. (2025). Formulating antibiotic policy: Analysis of India's ban on colistin use in food-producing animals. Preventive Veterinary Medicine, 240, 106534. https://doi.org/10.1016/J.PREVETMED.2025.106534

Hernández, M., López-Urrutia, L., Abad, D., Serna, M. D. F., Ocampo-Sosa, A. A., & Eiros, J. M. (2021). First Report of an Extensively Drug-Resistant ST23 *Klebsiella pneumoniae* of Capsular Serotype K1 Co-Producing CTX-M-15, OXA-48 and ArmA in Spain. Antibiotics, 10(2), 157. https://doi.org/10.3390/ANTIBIOTICS10020157

Hetland, M. A. K., Winkler, M. A., Kaspersen, H. P., Håkonsholm, F., Bakksjø, R. J., Bernhoff, E., Delgado-Blas, J. F., Brisse, S., Correia, A., Fostervold, A., Lam, M. M. C., Lunestad, B. T., Marathe, N. P., Raffelsberger, N., Samuelsen, Ø., Sunde, M., Sundsfjord, A., Urdahl, A. M., Wick, R. R., … Holt, K. E. (2025). A genome-wide One Health study of *Klebsiella pneumoniae* in Norway reveals overlapping populations but few recent transmission events across reservoirs. Genome Medicine, 17(1), 1–18. https://doi.org/10.1186/S13073-025-01466-0/FIGURES/5

Hetta, H. F., Alanazi, F. E., Ali, M. A. S., Alatawi, A. D., Aljohani, H. M., Ahmed, R., Alansari, N. A., Alkhathami, F. M., Albogmi, A., Alharbi, B. M., Alanzi, H. S., Alaqyli, A. B., & Ramadan, Y. N. (2025). Hypervirulent *Klebsiella pneumoniae*: Insights into Virulence, Antibiotic Resistance, and Fight Strategies Against a Superbug. Pharmaceuticals 2025, Vol. 18, Page 724, 18(5), 724. https://doi.org/10.3390/PH18050724

Hinthong, W., Phelan, J., Hussain, A., Mazumder, R., Azra, Haq, I. U., Ullah, I., Roberts, T., Thawong, N., Billows, N., Campino, S., Khan, T. A., Mondal, D., & Clark, T. G. (2025). Genomic insights into *Klebsiella pneumoniae*: Virulence, resistance, and transmission in South and Southeast Asia.

International Journal of Medical Microbiology, 320, 151666. https://doi.org/10.1016/J.IJMM.2025.151666

Holt, K. E., Wertheim, H., Zadoks, R. N., Baker, S., Whitehouse, C. A., Dance, D., Jenney, A., Connor, T. R., Hsu, L. Y., Severin, J., Brisse, S., Cao, H., Wilksch, J., Gorrie, C., Schultz, M. B., Edwards, D. J., Van Nguyen, K., Nguyen, T. V., Dao, T. T., … Thomson, N. R. (2015). Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proceedings of the National Academy of Sciences of the United States of America, 112(27), E3574–E3581. https://doi.org/10.1073/PNAS.1501049112

Hooper, D. C., & Jacoby, G. A. (2015). Mechanisms of drug resistance: Quinolone resistance. Annals of the New York Academy of Sciences, 1354(1), 12–31. https://doi.org/10.1111/nyas.12830

Hoque, R., Ahmed, S. M., Naher, N., Islam, M. A., Rousham, E. K., Islam, B. Z., & Hassan, S. (2020). Tackling antimicrobial resistance in Bangladesh: A scoping review of policy and practice in human, animal and environment sectors. PLOS ONE, 15(1), e0227947. https://doi.org/10.1371/JOURNAL.PONE.0227947

Hosain, M. Z., Islam, S. S., Kamal, M. M., & Kabir, S. L. (2022). Recent trends and scenario of antibiotic use in veterinary practices for livestock production in Bangladesh: a review. Asian–Australasian Journal of Bioscience and Biotechnology, 7(3), 90–106. https://doi.org/10.3329/AAJBB.V7I3.63361

Hossain, M. S., Ali, S., Hossain, M., Uddin, S. Z., Moniruzzaman, M., Islam, M. R., Shohael, A. M., Islam, M. S., Ananya, T. H., Rahman, M. M., Rahman, M. A., Worth, M., Mondal, D., & Mahmud, Z. H. (2021). ESBL Producing *Escherichia coli* in Faecal Sludge Treatment Plants: An Invisible Threat to Public Health in Rohingya Camps, Cox's Bazar, Bangladesh. Frontiers in Public Health, 9, 783019. https://doi.org/10.3389/FPUBH.2021.783019/FULL

Huang, T. W., Chen, T. L., Chen, Y. T., Lauderdale, T. L., Liao, T. L., Lee, Y. T., Chen, C. P., Liu, Y. M., Lin, A. C., Chang, Y. H., Wu, K. M., Kirby, R., Lai, J. F., Tan, M. C., Siu, L. K., Chang, C. M., Fung, C. P., & Tsai, S. F. (2013). Copy Number Change of the NDM-1 Sequence in a Multidrug-Resistant *Klebsiella pneumoniae* Clinical Isolate. PLOS ONE, 8(4), e62774. https://doi.org/10.1371/JOURNAL.PONE.0062774

Huang, X., Yao, X., Hou, Y., Zhang, D., Xie, R., Shi, C., Shang, Y., Bi, H., Song, W., Hua, L., Li, C., Chen, H., Wu, B., & Peng, Z. (2025). Global trends of antimicrobial resistance and virulence of *Klebsiella pneumoniae* from different host sources. Communications Medicine, 5(1), 1–14. https://doi.org/10.1038/S43856-025-01112-1

Hubbard, A. T. M., Newire, E., Botelho, J., Reiné, J., Wright, E., Murphy, E. A., Hutton, W., & Roberts, A. P. (2020). Isolation of an antimicrobial-resistant, biofilm-forming, *Klebsiella grimontii* isolate from a reusable water bottle. MicrobiologyOpen, 9(6), 1128–1134. https://doi.org/10.1002/MBO3.1023

Humphries, R. M., Abbott, A. N., & Hindler, J. A. (2019). Understanding and addressing CLSI breakpoint revisions: A primer for clinical laboratories. Journal of Clinical Microbiology, 57(6), e00203-19. https://doi.org/10.1128/JCM.00203-19

Hussain, A., Mazumder, R., Ahmed, A., Saima, U., Phelan, J. E., Campino, S., Ahmed, D., Asadulghani, M., Clark, T. G., & Mondal, D. (2023). Genome dynamics of high-risk resistant and hypervirulent *Klebsiella pneumoniae* clones in Dhaka, Bangladesh. Frontiers in Microbiology, 14, 1184196. https://doi.org/10.3389/FMICB.2023.1184196

Hutchings, M., Truman, A., & Wilkinson, B. (2019). Antibiotics: past, present and future. Current Opinion in Microbiology, 51, 72–80. https://doi.org/10.1016/J.MIB.2019.10.008

IEDCR. (2024). The National Antimicrobial Resistance (AMR) Surveillance Report, Bangladesh, 2024. Accessed on 10 February 2025.

https://iedcr.portal.gov.bd/sites/default/files/files/iedcr.portal.gov.bd/page/a595993f_f73f_401e_96f9_a8ce489add2c/2025-06-22-18-27-e49dacc8d1401c6fde43a3bed2acee60.pdf

Ikhimiukor, O. O., Zac Soligno, N. I., Akintayo, I. J., Marcovici, M. M., Souza, S. S. R., Workman, A., Martin, I. W., & Andam, C. P. (2024). Clonal background and routes of plasmid transmission underlie antimicrobial resistance features of bloodstream *Klebsiella pneumoniae*. Nature Communications 2024 15:1, 15(1), 1–13. https://doi.org/10.1038/s41467-024-51374-x

Imam, T., Gibson, J. S., Gupta, S. Das, Hoque, M. A., Fournié, G., & Henning, J. (2021). Association between farm biosecurity practices and antimicrobial usage on commercial chicken farms in Chattogram, Bangladesh. Preventive Veterinary Medicine, 196, 105500. https://doi.org/10.1016/J.PREVETMED.2021.105500

Imtiaz, W., Dasti, J. I., & Andrews, S. C. (2021). Draft genome sequence of a carbapenemase-producing (NDM-1) and multidrug-resistant, hypervirulent *Klebsiella pneumoniae* ST11 isolate from Pakistan, with a non-hypermucoviscous phenotype associated with rmpA2 mutation. Journal of Global Antimicrobial Resistance, 25, 359–362. https://doi.org/10.1016/J.JGAR.2021.04.017

Institut Pasteur MLST database (2025). *Klebsiella* locus/sequence definitions. BIGSdb-Pasteur. Accessed on 20 December 2025. https://bigsdb.pasteur.fr/cgi-bin/bigsdb.pl?db=pubmlst_klebsiella_seqdef&page=schemeInfo&scheme_id=1

Islam, M. A., Islam, M. R., Khan, R., Amin, M. B., Rahman, M., Hossain, M. I., Ahmed, D., Asaduzzaman, M., & Riley, L. W. (2022). Prevalence, etiology and antibiotic resistance patterns of community-acquired urinary tract infections in Dhaka, Bangladesh. PLoS ONE, 17(9), e0274423. https://doi.org/10.1371/JOURNAL.PONE.0274423

Islam, M., Singha, S., Belgrad, J. P., Hasib, F. M. Y., Sayeed, M. A., Haque, M. E., Ahad, A., & Hoque, M. A. (2021). Common Chicken Diseases in Kishoreganj, Bangladesh: Estimation Through the Veterinary Hospital–Based Passive Surveillance System. Advances in Animal and Veterinary Sciences, 9(11), 1951–1958. https://doi.org/10.17582/JOURNAL.AAVS/2021/9.11.1951.1958

Islam, S., Urmi, U. L., Rana, M., Sultana, F., Jahan, N., Hossain, B., Iqbal, S., Hossain, M. M., Mosaddek, A. S. M., & Nahar, S. (2020). High abundance of the colistin resistance gene mcr-1 in chicken gut-bacteria in Bangladesh. Scientific Reports, 10(1), 1–11. https://doi.org/10.1038/S41598-020-74402-4

Islam MA, Huq M, Nabi A, et al. (2013). Occurrence and characterization of multidrug-resistant New Delhi metallo-β-lactamase-1-producing bacteria isolated between 2003 and 2010 in Bangladesh. J Med Microbiol. 62(Pt 1):62-68

Jaccard, P. (1901). Étude comparative de la distribution florale dans une portion des Alpes et des Jura. Bulletin de la Société Vaudoise des Sciences Naturelles, 37, 547–579.

Jacob, J. J., Aravind, V., Beresford-Jones, B. S., Lal, Y. B., Shankar, C., Yesudoss, M., Abdullah, F., Priya, T. M., Kulkarni, S., Baker, S., Veeraraghavan, B., & Walia, K. (2024). Limited Evidence of Spillover of Antimicrobial-Resistant *Klebsiella pneumoniae* from Animal/Environmental Reservoirs to Humans in Vellore, India. Journal of Epidemiology and Global Health, 14(4), 1668. https://doi.org/10.1007/S44197-024-00323-4

Jacoby, G. A. (2009). AmpC B-Lactamases. Clinical Microbiology Reviews, 22(1), 161–182. https://doi.org/10.1128/CMR.00036-08

Jacoby, G. A., Strahilevitz, J., & Hooper, D. C. (2014). Plasmid-mediated quinolone resistance. Microbiology Spectrum, 2(5), 1–24. https://doi.org/10.1128/microbiolspec.PLAS-0006-2013

Jati, A. P., Sola-Campoy, P. J., Bosch, T., Schouls, L. M., Hendrickx, A. P. A., Bautista, V., Lara, N., Raangs, E., Aracil, B., Rossen, J. W. A., Friedrich, A. W., Navarro Riaza, A. M., Cañada-García, J. E., Ramírez de Arellano, E., Oteo-Iglesias, J., Pérez-Vázquez, M., García-Cobos, S., Maijer-Reuwer, A., Hall, M. A. L., … Rodríguez-Polo, J. A. (2023). Widespread Detection of Yersiniabactin Gene Cluster and Its Encoding Integrative Conjugative Elements (ICE Kp) among Nonoutbreak OXA-48-Producing *Klebsiella pneumoniae* Clinical Isolates from Spain and the Netherlands. Microbiology Spectrum, 11(4). https://doi.org/10.1128/SPECTRUM.04716-22

Jayol, A., Poirel, L., Brink, A., Villegas, M. V., Yilmaz, M., & Nordmann, P. (2014). Resistance to colistin associated with a single amino acid change in protein PmrB among *Klebsiella pneumoniae* isolates of worldwide origin. Antimicrobial Agents and Chemotherapy, 58(8), 4762–4766. https://doi.org/10.1128/AAC.00084-14

Jenkins, C., Rentenaar, R. J., Landraud, L., & Brisse, S. (2017). Enterobacteriaceae. Infectious Diseases, 2-Volume Set, 1565-1578.e2. https://doi.org/10.1016/B978-0-7020-6285-8.00180-5

Jiang, J., Wang, L., Hu, Y., Chen, X., Li, P., Zhang, J., Zhang, Y., Su, J., Xu, X., Xiao, Y., Liu, Z., Yu, Y., Gao, H., Doi, Y., van Duin, D., Fowler, V. G., Chen, L., & Wang, M. (2025). Global emergence of Carbapenem-resistant Hypervirulent *Klebsiella pneumoniae* driven by an IncFIIK34 KPC-2 plasmid. EBioMedicine, 113, 105627. https://doi.org/10.1016/J.EBIOM.2025.105627

Jiang, Y. L., Lyu, Y. Y., Liu, L. L., Li, Z. P., Liu, D., Tai, J. H., Hu, X. Q., Zhang, W. H., Chu, W. W., Zhao, X., Huang, W., & Wu, Y. Le. (2024). Carbapenem-resistant *Klebsiella oxytoca* transmission linked to preoperative shaving in emergency neurosurgery, tracked by rapid detection via chromogenic medium and whole genome sequencing. Frontiers in Cellular and Infection Microbiology, 14, 1464411. https://doi.org/10.3389/FCIMB.2024.1464411/BIBTEX

Jin, X., Chen, Q., Shen, F., Jiang, Y., Wu, X., Hua, X., Fu, Y., & Yu, Y. (2021). Resistance evolution of hypervirulent carbapenem–resistant *Klebsiella pneumoniae* ST11 during treatment with tigecycline and polymyxin. Emerging Microbes and Infections, 10(1), 1129–1136. https://doi.org/10.1080/22221751.2021.1937327

Johura, F. T., Tasnim, J., Barman, I., Biswas, S. R., Jubyda, F. T., Sultana, M., George, C. M., Camilli, A., Seed, K. D., Ahmed, N., & Alam, M. (2020). Colistin-resistant *Escherichia coli* carrying *mcr-1* in food, water, hand rinse, and healthy human gut in Bangladesh. Gut Pathogens, 12(1), 1–8. https://doi.org/10.1186/S13099-020-0345-2/FIGURES/1

Jonas, O. B., Irwin, A., Berthe, F. C. J., Le Gall, F. G., & Marquez, P. V. (2017). Drug-resistant infections: A threat to our economic future (Vol. 2): Final report. HNP/Agriculture Global Antimicrobial Resistance Initiative. Washington, DC: World Bank Group. Accessed on 10 February 2025. http://documents.worldbank.org/curated/en/323311493396993758

Kalla, H., & Paila, M. (2023). A study of *Klebsiella pneumoniae* isolated from water samples in and around Tirupati. ~ 413 ~ The Pharma Innovation Journal, 7, 413–417. www.thepharmajournal.com

Kang, E., Crouse, A., Chevallier, L., Pontier, S. M., Alzahrani, A., Silué, N., Campbell-Valois, F. X., Montagutelli, X., Gruenheid, S., & Malo, D. (2018). Enterobacteria and host resistance to infection. Mammalian Genome 2018 29:7, 29(7), 558–576. https://doi.org/10.1007/S00335-018-9749-4

Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. Journal of Anaesthesiology, Clinical Pharmacology, 33(3), 300. https://doi.org/10.4103/JOACP.JOACP_349_15

Kar, S., Kawser, Z., Sridhar, S., Mukta, S. A., Hasan, N., Siddik, A. B., Habib, M. T., Slater, D. M., Earl, A. M., Worby, C. J., Azad, K., Shamsuzzaman, S. M., Tanni, N. N., Khan, R. T., Moonmoon, M., Qadri, F., Harris, J. B., & Larocque, R. C. (2024). High Prevalence of Carbapenem-resistant *Klebsiella pneumoniae* in Fecal and Water Samples in Dhaka, Bangladesh. Open Forum Infectious Diseases, 11(11). https://doi.org/10.1093/OFID/OFAE612

Karampatakis, T., Tsergouli, K., & Behzadi, P. (2023). Carbapenem-Resistant *Klebsiella pneumoniae*: Virulence Factors, Molecular Epidemiology and Latest Updates in Treatment Options. Antibiotics, 12(2). https://doi.org/10.3390/ANTIBIOTICS12020234

Karlsson, M., Stanton, R. A., Ansari, U., McAllister, G., Chan, M. Y., Sula, E., Grass, J. E., Duffy, N., Anacker, M. L., Witwer, M. L., Kamile Rasheed, J., Elkins, C. A., & Halpin, A. L. (2019). Identification of a carbapenemase-producing hypervirulent *Klebsiella pneumoniae* isolate in the United States. Antimicrobial Agents and Chemotherapy, 63(7). https://doi.org/10.1128/AAC.00519-19

Kawser, Z., & Shamsuzzaman, S. M. (2022). Association of Virulence with Antimicrobial Resistance among *Klebsiella pneumoniae* Isolated from Hospital Settings in Bangladesh. International Journal of Applied & Basic Medical Research, 12(2), 123–129. https://doi.org/10.4103/IJABMR.IJABMR_747_21

Kawser, Z., Sridhar, S., Kar, S., Habib, T., Mukta, S. A., Azad, K., Hasan, N., Kulsum, U., Siddik, A. B., Rahman, S., Tanni, N. N., Nesa, M., Earl, A. M., Worby, C. J., Turbett, S. E., Shamsuzzaman, S. M., Harris, J. B., Qadri, F., & LaRocque, R. C. (2025). Clinical and genomic characterization of *Klebsiella pneumoniae* infections in Dhaka, Bangladesh. Journal of Global Antimicrobial Resistance, 41, 52–58. https://doi.org/10.1016/J.JGAR.2024.12.016

Kawser, Z., Sridhar, S., Kar, S., Habib, T., Mukta, S. A., Azad, K., Hasan, N., Kulsum, U., Siddik, A. B., Rahman, S., Tanni, N. N., Nesa, M., Earl, A. M., Worby, C. J., Turbett, S. E., Shamsuzzaman, S. M., Harris, J. B., Qadri, F., & LaRocque, R. C. (2025). Clinical and genomic characterization of *Klebsiella pneumoniae* infections in Dhaka, Bangladesh. Journal of Global Antimicrobial Resistance, 41, 52–58. https://doi.org/10.1016/J.JGAR.2024.12.016

Kaye, K. S., Gupta, V., Mulgirigama, A., Joshi, A. V., Ye, G., Scangarella-Oman, N. E., Yu, K., & Mitrani-Gold, F. S. (2024). Prevalence, regional distribution, and trends of antimicrobial resistance among female outpatients with urine *Klebsiella* spp. isolates: a multicenter evaluation in the United States between 2011 and 2019. Antimicrobial Resistance and Infection Control, 13(1), 1–9. https://doi.org/10.1186/S13756-024-01372-X/TABLES/1

Kerro Dego, O., & Vidlund, J. (2024). Staphylococcal mastitis in dairy cows. Frontiers in Veterinary Science, 11, 1356259. https://doi.org/10.3389/FVETS.2024.1356259/XML

Khan, E. R., Aung, M. S., Paul, S. K., Ahmed, S., Haque, N., Ahamed, F., Sarkar, S. R., Roy, S., Rahman, M. M., Mahmud, M. C., Hossain, M. A., Urushibara, N., Kawaguchiya, M., Sumi, A., & Kobayashi, N. (2018). Prevalence and Molecular Epidemiology of Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae* Harboring Extended-Spectrum Beta-Lactamase and Carbapenemase Genes in Bangladesh. Microbial Drug Resistance (Larchmont, N.Y.), 24(10), 1568–1579. https://doi.org/10.1089/MDR.2018.0063

Khan, S. A., Imtiaz, M. A., Sayeed, M. A., Shaikat, A. H., & Hassan, M. M. (2020). Antimicrobial resistance pattern in domestic animal - Wildlife - environmental niche via the food chain to humans with a Bangladesh perspective; A systematic review. BMC Veterinary Research, 16(1), 1–13. https://doi.org/10.1186/S12917-020-02519-9/TABLES/2

Khan, S. I., Khan, S., Hossain, M. M., Talukder Iqbal Hossain, Ali Mohammad, & Biswas Swapan Kumar. (2022). Incidence and etiology of abdominal surgical site infections among emergency postoperative patients in a tertiary medical college hospital, Faridpur, Bangladesh. Journal of Surgery and Allied Sciences, 4(2), 49–55. https://pdf.ipinnovative.com/pdf/16626

Kito, Y., Kuwabara, K., Ono, K., Kato, K., Yokoi, T., Horiguchi, K., Kato, K., Hirose, M., Ohara, T., Goto, K., Nakamura, Y., Koike, Y., & Horiguchi, T. (2021). Seasonal variation in the prevalence of Gram-negative bacilli in sputum and urine specimens from outpatients and inpatients. Fujita Medical Journal, 8(2), 46. https://doi.org/10.20407/FMJ.2021-003

Klaper, K., Hammerl, J. A., Rau, J., Pfeifer, Y., & Werner, G. (2021). Genome-based analysis of *Klebsiella* spp. Isolates from animals and food products in Germany, 2013–2017. Pathogens, 10(5), 573. https://doi.org/10.3390/PATHOGENS10050573/S1

Kliebe, C., Nies, B. A., Meyer, J. F., Tolxdorff-Neutzling, R. M., & Wiedemann, B. (1985). Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrobial Agents and Chemotherapy, 28(2), 302–307. https://doi.org/10.1128/AAC.28.2.302

Kochan, T. J., Nozick, S. H., Valdes, A., Mitra, S. D., Cheung, B. H., Lebrun-Corbin, M., Medernach, R. L., Vessely, M. B., Mills, J. O., Axline, C. M. R., Nelson, J. A., VanGosen, E. M., Ward, T. J., Ozer, E. A., van Duin, D., Chen, L., Kreiswirth, B. N., Long, S. W., Musser, J. M., … Hauser, A. R. (2023). *Klebsiella pneumoniae* clinical isolates with features of both multidrug-resistance and hypervirulence have unexpectedly low virulence. Nature Communications 2023 14:1, 14(1), 1–13. https://doi.org/10.1038/s41467-023-43802-1

Krause, K. M., Serio, A. W., Kane, T. R., & Connolly, L. E. (2016). Aminoglycosides: An overview. Cold Spring Harbor Perspectives in Medicine, 6(6). https://doi.org/10.1101/CSHPERSPECT.A027029

Kumarasamy KK, Toleman MA, Walsh TR, et al. (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 10(9):597-602

Kuralayanapalya, S. P., Patil, S. S., Hamsapriya, S., Shinduja, R., Roy, P., & Amachawadi, R. G. (2019). Prevalence of extended-spectrum beta-lactamase producing bacteria from animal origin: A systematic review and meta-analysis report from India. PLoS ONE, 14(9), e0221771. https://doi.org/10.1371/JOURNAL.PONE.0221771

Kuzina, E. S., Kislichkina, A. A., Sizova, A. A., Skryabin, Y. P., Novikova, T. S., Ershova, O. N., Savin, I. A., Khokhlova, O. E., Bogun, A. G., & Fursova, N. K. (2023). High-Molecular-Weight Plasmids Carrying Carbapenemase Genes blaNDM-1, blaKPC-2, and blaOXA-48 Coexisting in Clinical *Klebsiella pneumoniae* Strains of ST39. Microorganisms, 11(2), 459. https://doi.org/10.3390/MICROORGANISMS11020459

Kyany’a, C., & Musila, L. (2020). Colistin Resistance Gene mcr-8 in a High-Risk Sequence Type 15 *Klebsiella pneumoniae* Isolate from Kenya. Microbiology Resource Announcements, 9(39), e00783-20. https://doi.org/10.1128/MRA.00783-20

Lam, M. M. C., Wick, R. R., Judd, L. M., Holt, K. E., & Wyres, K. L. (2022). Kaptive 2.0: updated capsule and lipopolysaccharide locus typing for the *Klebsiella pneumoniae* species complex. Microbial Genomics, 8(3), 000800. https://doi.org/10.1099/MGEN.0.000800

Lam, M. M. C., Wick, R. R., Watts, S. C., Cerdeira, L. T., Wyres, K. L., & Holt, K. E. (2021). A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex. Nature Communications 2021 12:1, 12(1), 1–16. https://doi.org/10.1038/s41467-021-24448-3

Lam, M. M. C., Wick, R. R., Wyres, K. L., Gorrie, C. L., Judd, L. M., Jenney, A. W. J., Brisse, S., & Holt, K. E. (2018). Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICEKp in *Klebsiella pneumoniae* populations. Microbial Genomics, 4(9). https://doi.org/10.1099/MGEN.0.000196

Lam, M. M. C., Wyres, K. L., Judd, L. M., Wick, R. R., Jenney, A., Brisse, S., & Holt, K. E. (2018). Tracking key virulence loci encoding aerobactin and salmochelin siderophore synthesis in *Klebsiella pneumoniae*. Genome Medicine, 10(1). https://doi.org/10.1186/S13073-018-0587-5

Lam, M. M. C., Wyres, K. L., Wick, R. R., Judd, L. M., Fostervold, A., Holt, K. E., & Löhr, I. H. (2019). Convergence of virulence and MDR in a single plasmid vector in MDR *Klebsiella pneumoniae* ST15. Journal of Antimicrobial Chemotherapy, 74(5), 1218–1222. https://doi.org/10.1093/JAC/DKZ028

Lan, P., Jiang, Y., Zhou, J., & Yu, Y. (2021). A global perspective on the convergence of hypervirulence and carbapenem resistance in *Klebsiella pneumoniae*. Journal of Global Antimicrobial Resistance, 25, 26–34. https://doi.org/10.1016/j.jgar.2021.02.020

Langford, B. J., So, M., Raybardhan, S., Leung, V., Westwood, D., MacFadden, D. R., Soucy, J. P. R., & Daneman, N. (2020). Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clinical Microbiology and Infection, 26(12), 1622–1629. https://doi.org/10.1016/j.cmi.2020.07.016

Larsen, A. L., Pedersen, T., Sundsfjord, A., Ross, T. A., Guleng, A. D., Haug, J. B., Pöntinen, A. K., & Samuelsen, Ø. (2025). Hospital toilets and drainage systems as a reservoir for a long-term polyclonal outbreak of clinical infections with multidrug-resistant *Klebsiella oxytoca* species complex. Infection Prevention in Practice, 7(1), 100430. https://doi.org/10.1016/J.INFPIP.2024.100430

Larsson, D. G. J., & Flach, C. F. (2022). Antibiotic resistance in the environment. Nature Reviews Microbiology, 20(5), 257–269. https://doi.org/10.1038/S41579-021-00649-X;SUBJMETA

Lee, C. R., Lee, J. H., Park, K. S., Jeon, J. H., Kim, Y. B., Cha, C. J., Jeong, B. C., & Lee, S. H. (2017). Antimicrobial resistance of hypervirulent *Klebsiella pneumoniae*: Epidemiology, hypervirulence-associated determinants, and resistance mechanisms. Frontiers in Cellular and Infection Microbiology, 7(NOV). https://doi.org/10.3389/FCIMB.2017.00483

Legendre, P., & Legendre, L. (2012). Numerical ecology (3rd ed.). Elsevier.

Lei, T. yu, Liao, B. bin, Yang, L. R., Wang, Y., & Chen, X. bing. (2024). Hypervirulent and carbapenem-resistant *Klebsiella pneumoniae*: A global public health threat. Microbiological Research, 288, 127839. https://doi.org/10.1016/J.MICRES.2024.127839

Lekota, K. E., Mabeo, R. O., Ramatla, T., Van Wyk, D. A. B., Thekisoe, O., Molale-Tom, L. G., & Bezuidenhout, C. C. (2024). Genomic insight on *Klebsiella variicola* isolated from wastewater treatment plant has uncovered a novel bacteriophage. BMC Genomics, 25(1), 1–15. https://doi.org/10.1186/S12864-024-10906-X/FIGURES/4

Lerner, A., Romano, J., Chmelnitsky, I., Navon-Venezia, S., Edgar, R., & Carmeli, Y. (2013). Rectal Swabs Are Suitable for Quantifying the Carriage Load of KPC-Producing Carbapenem-Resistant Enterobacteriaceae. Antimicrobial Agents and Chemotherapy, 57(3), 1474. https://doi.org/10.1128/AAC.01275-12

Li, J., Shi, Y., Song, X., Yin, X., & Liu, H. (2025). Mechanisms of Antimicrobial Resistance in Klebsiella: Advances in Detection Methods and Clinical Implications. Infection and drug resistance, 18, 1339–1354. https://doi.org/10.2147/IDR.S509016

Li, L., Zhang, Y., Wang, W., Chen, Y., He, F., & Yu, Y. (2024). Global Emergence and Genomic Epidemiology of blaNDM-Carrying *Klebsiella variicola*. Infection and Drug Resistance, 17, 1893. https://doi.org/10.2147/IDR.S460569

Li, Y., Wu, Y., Li, D., Du, L., Zhao, L., Wang, R., Chen, X., Jia, X., Ma, R., Wang, T., Li, J., Zhang, G., Wang, X., Hu, M., Chen, X., Wang, X., Kang, W., Sun, H., Xu, Y., & Liu, Y. (2024). Multicenter comparative genomic study of *Klebsiella oxytoca* complex reveals a highly antibiotic-resistant subspecies of *Klebsiella* michiganensis. Journal of Microbiology, Immunology and Infection, 57(1), 138–147. https://doi.org/10.1016/J.JMII.2023.10.014

Li, Z., Wu, C., Cai, X., Song, Y., Zheng, X., He, Y., & Song, G. (2025). Characterization of OXA232-Producing Carbapenem-Resistant *Klebsiella pneumoniae*: Genomic Analysis and Virulence Assessment. Polish Journal of Microbiology, 74(1), 82. https://doi.org/10.33073/PJM-2025-007

Liao, C.-H., Huang, Y.-L., Ioerger, T., Lim, K. S., Huang, Y.-C., Huang, C.-H., & Hsu, C.-R. (2025). The Journal of Infectious Diseases Genomic and Phenotypic Insights Into Antibiotic Resistance and

Virulence of *Klebsiella pneumoniae* From the Environment in Southern Taiwan. https://doi.org/10.1093/infdis/jiaf396

Liao, W., Liu, Y., & Zhang, W. (2020). Virulence evolution, molecular mechanisms of resistance and prevalence of ST11 carbapenem-resistant *Klebsiella pneumoniae* in China: A review over the last 10 years. Journal of Global Antimicrobial Resistance, 23, 174–180. https://doi.org/10.1016/j.jgar.2020.09.004

LimmaTech. (2024). GSK4429016/LimmaTech Biologics, GSK. Accessed on 30 August 2025. https://delta.larvol.com/Products/?ProductId=70700b39-c783-4ef2-a4de-7a62932a6848

Ling, Z., Yin, W., Shen, Z., Wang, Y., Shen, J., & Walsh, T. R. (2020). Epidemiology of mobile colistin resistance genes mcr-1 to mcr-9. Journal of Antimicrobial Chemotherapy, 75(11), 3087–3095. https://doi.org/10.1093/jac/dkaa205

Liu, C., Wu, Y., Fang, Y., Sang, Z., Huang, L., Dong, N., Zeng, Y., Lu, J., Zhang, R., & Chen, G. (2022). Emergence of an ST1326 (CG258) Multi-Drug Resistant *Klebsiella pneumoniae* Co-harboring *mcr-8.2*, ESBL Genes, and the Resistance-Nodulation-Division Efflux Pump Gene Cluster tmexCD1-toprJ1 in China. Frontiers in Microbiology, 13, 800993. https://doi.org/10.3389/FMICB.2022.800993/BIBTEX

Liu, J. H., Liu, Y. Y., Shen, Y. B., Yang, J., Walsh, T. R., Wang, Y., & Shen, J. (2024). Plasmid-mediated colistin-resistance genes: *mcr*. Trends in Microbiology, 32(4), 365–378. https://doi.org/10.1016/J.TIM.2023.10.006

Liu, L., Feng, Y., Hu, Y., Kang, M., Xie, Y., & Zong, Z. (2018). *Klebsiella grimontii*, a new species acquired carbapenem resistance. Frontiers in Microbiology, 9(SEP), 401574. https://doi.org/10.3389/FMICB.2018.02170/BIBTEX

Liu, M. C., Jian, Z., Liu, W., Li, J., & Pei, N. (2022). One Health Analysis of mcr-Carrying Plasmids and Emergence of *mcr-10.1* in Three Species of *Klebsiella* Recovered from Humans in China. Microbiology Spectrum, 10(6). https://doi.org/10.1128/SPECTRUM.02306-22

Liu, M., Wu, J., Zhao, J., Xi, Y., Jin, Y., Yang, H., Chen, S., Long, J., & Duan, G. (2024). Global epidemiology and genetic diversity of *mcr*-positive *Klebsiella pneumoniae*: A systematic review and genomic analysis. Environmental Research, 259. https://doi.org/10.1016/j.envres.2024.119516

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L. F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J. H., & Shen, J. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. The Lancet Infectious Diseases, 16(2), 161–168. https://doi.org/10.1016/S1473-3099(15)00424-7

Liu, Y.-Y., Zhu, X.-Q., Nang, S. C., Xun, H., Lv, L., Yang, J., & Liu, J.-H. (2023). Greater Invasion and Persistence of mcr-1- Bearing Plasmids in *Escherichia coli* than in *Klebsiella pneumoniae*. Microbiology Spectrum, 11(2). https://doi.org/10.1128/SPECTRUM.03223-22

Lobato, A. R. F., Souza, M. J. S., Pereira, E. S., Cazuza, T. B., Silva, A., Baraúna, R. A., & Brasiliense, D. M. (2025). Emergence of NDM-7-Producing *Klebsiella quasipneumoniae* subs. *simillipneumoniae* ST138 in a Hospital from the Northern Region of Brazil. Microorganisms, 13(2), 314. https://doi.org/10.3390/MICROORGANISMS13020314/S1

Long, J., Chen, J., Yuan, Y., Yang, S., Xie, X., Wu, X., Liu, Y., Guo, J., Chen, Y., Wang, C., & Liu, X. (2024). First Report of Carbapenem-Resistant *Klebsiella michiganensis* Co-Harboring blaKPC-2 and TmexCD2-ToprJ2 Isolated from Wastewater at a Tertiary Hospital in Beijing. Infection and Drug Resistance, 17, 5117–5128. https://doi.org/10.2147/IDR.S448256

Long, S. W., Linson, S. E., Ojeda Saavedra, M., Cantu, C., Davis, J. J., Brettin, T., & Olsen, R. J. (2017). Whole-Genome Sequencing of Human Clinical *Klebsiella pneumoniae* Isolates Reveals

Misidentification and Misunderstandings of *Klebsiella pneumoniae*, *Klebsiella* variicola, and *Klebsiella* quasipneumoniae. MSphere, 2(4). https://doi.org/10.1128/MSPHEREDIRECT.00290-17

Lowe, C., Willey, B., O'Shaughnessy, A., Lee, W., Lum, M., Pike, K., Larocque, C., Dedier, H., Dales, L., Moore, C., & McGeer, A. (2012). Outbreak of Extended-Spectrum β-Lactamase–producing *Klebsiella oxytoca* Infections Associated with Contaminated Handwashing Sinks. Emerging Infectious Diseases, 18(8), 1242. https://doi.org/10.3201/EID1808.111268

Lu, Y., Feng, Y., McNally, A., & Zong, Z. (2018). The occurrence of colistin-resistant hypervirulent *Klebsiella pneumoniae* in China. Frontiers in Microbiology, 9(OCT), 412334. https://doi.org/10.3389/FMICB.2018.02568/BIBTEX

Ludden, C., Moradigaravand, D., Jamrozy, D., Gouliouris, T., Blane, B., Naydenova, P., Hernandez-Garcia, J., Wood, P., Hadjirin, N., Radakovic, M., Crawley, C., Brown, N. M., Holmes, M., Parkhill, J., & Peacock, S. J. (2020). A One Health Study of the Genetic Relatedness of *Klebsiella pneumoniae* and Their Mobile Elements in the East of England. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 70(2), 219–226. https://doi.org/10.1093/CID/CIZ174

Luo, Q., Wang, Y., & Xiao, Y. (2020). Prevalence and transmission of mobilized colistin resistance (*mcr*) gene in bacteria common to animals and humans. Biosafety and Health, 2(2), 71–78. https://doi.org/10.1016/J.BSHEAL.2020.05.001

Luterbach, C. L., Pasquale, D. K., Henderson, H. I., Cober, E., Richter, S. S., Salata, R. A., Kalayjian, R. C., Watkins, R. R., Hujer, A. M., Hujer, K. M., Domitrovic, T. N., Doi, Y., Kaye, K. S., Evans, S., Fowler, V. G., Bonomo, R. A., Kreiswirth, B., Chen, L., Arias, C. A., … Roach, D. (2025). A network analysis of carbapenem-resistant *Klebsiella pneumoniae* among healthcare facilities. Scientific Reports 2025 15:1, 15(1), 1–11. https://doi.org/10.1038/s41598-025-04918-0

Ma, Y., Wu, X., Li, S., Tang, L., Chen, M., & An, Q. (2021). Proposal for reunification of the genus *Raoultella* with the genus *Klebsiella* and reclassification of *Raoultella electrica* as *Klebsiella electrica* comb. nov. Research in Microbiology, 172(6). https://doi.org/10.1016/j.resmic.2021.103851

Maiden, M. C. J., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D. A., Feavers, I. M., Achtman, M., & Spratt, B. G. (1998). Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. Proceedings of the National Academy of Sciences of the United States of America, 95(6), 3140–3145. https://doi.org/10.1073/pnas.95.6.3140

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal. Clinical Microbiology and Infection, 18(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570

Maghembe, R. S., Magulye, M. A. K., Makaranga, A., Moto, E., Sekyanzi, S., Mwesigwa, S., & Katagirya, E. (2024). Comprehensive genomics reveals novel sequence types of multidrug resistant *Klebsiella oxytoca* with uncharacterized capsular polysaccharide K- and lipopolysaccharide O-antigen loci from the National Hospital of Uganda. Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases, 123, 105640. https://doi.org/10.1016/J.MEEGID.2024.105640

Mahbub, H. (2020). Phenotypic and Molecular Characterization of AmpC beta-lactamase Enzyme Producing *Escherichia coli* and *Klebsiella* species Isolated from A Tertiary Care Hospital in Bangladesh. https://doi.org/10.3329/bjmm.v10i2.51924

Mahmud, Z. H., Uddin, S. Z., Moniruzzaman, M., Ali, S., Hossain, M., Islam, M. T., Costa, D. T. D., Islam, M. R., Islam, M. S., Hassan, M. Z., Ong, L. A., Moore, C. E., Charles, K. J., Mondal, D., Lopes, B. S., & Parveen, S. (2022). Healthcare Facilities as Potential Reservoirs of Antimicrobial Resistant

Klebsiella pneumoniae: An Emerging Concern to Public Health in Bangladesh. Pharmaceuticals, 15(9), 1116. https://doi.org/10.3390/PH15091116/S1

Marques, C., Menezes, J., Belas, A., Aboim, C., Cavaco-Silva, P., Trigueiro, G., Gama, L. T., & Pomba, C. (2019). *Klebsiella pneumoniae* causing urinary tract infections in companion animals and humans: Population structure, antimicrobial resistance and virulence genes. Journal of Antimicrobial Chemotherapy, 74(3), 594–602. https://doi.org/10.1093/JAC/DKY499

Martin, G., Tyson, G. H., Guag, J., Strain, E., & Ceric, O. (2025). Genomic snapshot of *Klebsiella* spp. isolates from clinically ill animals reveal diverse lineages with limited relatedness to human isolates. BMC Veterinary Research, 21(1), 458. https://doi.org/10.1186/S12917-025-04686-Z

Martino, F., Petroni, A., Menocal, M. A., Corso, A., Melano, R., & Faccone, D. (2024). New insights on *mcr-1*-harboring plasmids from human clinical *Escherichia coli* isolates. PLOS ONE, 19(2), e0294820. https://doi.org/10.1371/JOURNAL.PONE.0294820

Marsh, J. W., Mustapha, M. M., Griffith, M. P., Evans, D. R., Ezeonwuka, C., Pasculle, A. W., Shutt, K. A., Sundermann, A., Ayres, A. M., Shields, R. K., Babiker, A., Cooper, V. S., Van Tyne, D., & Harrison, L. H. (2019). Evolution of Outbreak-Causing Carbapenem-Resistant Klebsiella pneumoniae ST258 at a Tertiary Care Hospital over 8 Years. mBio, 10(5), e01945-19. https://doi.org/10.1128/mBio.01945-19

Matlock, W., Lipworth, S., Chau, K. K., AbuOun, M., Barker, L., Kavanagh, J., Andersson, M., Oakley, S., Morgan, M., Crook, D. W., Read, D. S., Anjum, M., Shaw, L. P., & Stoesser, N. (2023). Enterobacterales plasmid sharing amongst human bloodstream infections, livestock, wastewater, and waterway niches in Oxfordshire, UK. ELife, 12, e85302. https://doi.org/10.7554/ELIFE.85302

Mazumder, R., Hussain, A., Bhadra, B., Phelan, J., Campino, S., Clark, T. G., & Mondal, D. (2023). Case report: A successfully treated case of community-acquired urinary tract infection due to *Klebsiella aerogenes* in Bangladesh. Frontiers in Medicine, 10, 1206756. https://doi.org/10.3389/FMED.2023.1206756

Mbanga, J., Amoako, D. G., Abia, A. L. K., Fatoba, D., & Essack, S. (n.d.). Whole genome sequencing reveals insights into antibiotic resistant *Klebsiella grimontii* novel sequence type ST350 isolated from a wastewater source in South Africa. Journal of Biotech Research. Retrieved September 1, 2025, from https://cge.cbs.dtu.dk

McAndrew, G., Barbier, E., Rodrigues, C., Piveteau, P., Brisse, S., & Reddington, K. (2025). KpSC-ID: a multiplex real-time PCR assay for the simultaneous detection of the *Klebsiella pneumoniae* species complex and specific identification of *Klebsiella pneumoniae*, *Klebsiella quasipneumoniae* and *Klebsiella variicola*. Microbiology, 171(7), 001587. https://doi.org/10.1099/MIC.0.001587

McElheny, C. L., Iovleva, A., Chen, N., Woods, D., Pradhan, A., Sonnabend, J. L., Matunis, A. R., Raabe, N. J., Lee, J. S., Trevejo-Nuñez, G., Van Tyne, D., & Doi, Y. (2024). Prevalence and features of hypervirulent *Klebsiella pneumoniae* in respiratory specimens at a US hospital system. Infection and Immunity, 93(1), e00486-24. https://doi.org/10.1128/IAI.00486-24

McInnes, R. S., uz-Zaman, M. H., Alam, I. T., Ho, S. F. S., Moran, R. A., Clemens, J. D., Islam, M. S., & van Schaik, W. (2021). Metagenome-Wide Analysis of Rural and Urban Surface Waters and Sediments in Bangladesh Identifies Human Waste as a Driver of Antibiotic Resistance. MSystems, 6(4), e00137-21. https://doi.org/10.1128/MSYSTEMS.00137-21

Merla, C., Kuka, A., Mileto, I., Petazzoni, G., Gaiarsa, S., De Vitis, D., Ardizzone, M., Corbella, M., Baldanti, F., & Cambieri, P. (2024). One-year surveillance for hypervirulent *Klebsiella pneumoniae* detected carbapenem-resistant superbugs. Microbiology Spectrum, 12(3), e03292-23. https://doi.org/10.1128/SPECTRUM.03292-23

Merla, C., Rodrigues, C., Passet, V., Corbella, M., Thorpe, H. A., Kallonen, T. V. S., Zong, Z., Marone, P., Bandi, C., Sassera, D., Corander, J., Feil, E. J., & Brisse, S. (2019). Description of *Klebsiella* spallanzanii sp. nov. and of *Klebsiella* pasteurii sp. nov. Frontiers in Microbiology, 10(OCT). https://doi.org/10.3389/FMICB.2019.02360

Miliotis, G., McDonagh, F., Singh, N. K., O'Connor, L., Tuohy, A., Morris, D., & Venkateswaran, K. (2023). Genomic analysis reveals the presence of emerging pathogenic *Klebsiella* lineages aboard the International Space Station. Microbiology Spectrum, 11(6). https://doi.org/10.1128/SPECTRUM.01897-23

Miller, J. C., Cross, A. S., Tennant, S. M., & Baliban, S. M. (2024). *Klebsiella pneumoniae* Lipopolysaccharide as a Vaccine Target and the Role of Antibodies in Protection from Disease. Vaccines, 12(10), 1177. https://doi.org/10.3390/VACCINES12101177

Mishra, K., Banerjee, T., Yadav, G., Kumar, A., Pratap, A., Chaurasiya, S., & Rakshit, P. (2025). Emergence of drug-resistant *Klebsiella pneumoniae* phylogroups (K. quasipneumoniae and K. variicola) causing human infections. Microbiology Spectrum. https://doi.org/10.1128/SPECTRUM.00198-25

Mixão, V., Pinto, M., Sobral, D., Di Pasquale, A., Gomes, J. P., & Borges, V. (2023). ReporTree: a surveillance-oriented tool to strengthen the linkage between pathogen genetic clusters and epidemiological data. *Genome medicine*, *15*(1), 43. https://doi.org/10.1186/s13073-023-01196-1

Mmatli, M., Mbelle, N. M., & Osei Sekyere, J. (2022). Global epidemiology, genetic environment, risk factors and therapeutic prospects of *mcr* genes: A current and emerging update. Frontiers in Cellular and Infection Microbiology, 12. https://doi.org/10.3389/FCIMB.2022.941358

Mo, X., Zhang, H., Fan, J., Xu, L., Fu, H., Yue, J., Dong, K., Luo, Q., & Wan, F. (2024). Co-existence of two plasmids harboring transferable resistance-nodulation-division pump gene cluster, tmexCD1-toprJ1, and colistin resistance gene *mcr-8* in *Klebsiella pneumoniae*. Annals of Clinical Microbiology and Antimicrobials, 23(1), 1–10. https://doi.org/10.1186/S12941-024-00727-X

Mohanty S, Gajanand M, Gaind R. (2017). Identification of carbapenemase-mediated resistance among Enterobacteriaceae bloodstream isolates: a molecular study from India. Indian J Med Microbiol. 35:421–425

Mohapatra, S., Panigrahy, R., Tak, V., J. V., S., K. C., S., Chaudhuri, S., Pundir, S., Kocher, D., Gautam, H., Sood, S., Das, B. K., Kapil, A., Hari, P., Kumar, A., Kumari, R., Kalaivani, M., R., A., Salve, H. R., Malhotra, S., & Kant, S. (2022). Prevalence and resistance pattern of uropathogens from community settings of different regions: an experience from India. Access Microbiology, 4(2), 000321. https://doi.org/10.1099/ACMI.0.000321

Mondol, S. M., Hossain, M. A., & Haque, F. K. M. (2025). Comprehensive genomic insights into a highly pathogenic clone ST656 of *mcr8.1* containing multidrug-resistant *Klebsiella pneumoniae* from Bangladesh. Scientific Reports, 15(1), 1–14. https://doi.org/10.1038/S41598-025-90414-4

Morgado, S., Fonseca, É., Freitas, F., Caldart, R., & Vicente, A. C. (2024). In-depth analysis of *Klebsiella aerogenes* resistome, virulome and plasmidome worldwide. Scientific Reports, 14(1), 1–11. https://doi.org/10.1038/S41598-024-57245-1

Mourão, J., Magalhães, M., Ribeiro-Almeida, M., Rebelo, A., Novais, C., Peixe, L., Novais, Â., & Antunes, P. (2024). Decoding *Klebsiella pneumoniae* in poultry chain: unveiling genetic landscape, antibiotic resistance, and biocide tolerance in non-clinical reservoirs. Frontiers in Microbiology, 15, 1365011. https://doi.org/10.3389/FMICB.2024.1365011/FULL

Munim, M. A., Tanni, A. A., Hossain, M. M., Chakma, K., Mannan, A., Islam, S. M. R., Tiwari, J. G., & Gupta, S. Das. (2024). Whole genome sequencing of multidrug–resistant *Klebsiella pneumoniae* from poultry in Noakhali, Bangladesh: Assessing risk of transmission to humans in a pilot study. Comparative Immunology, Microbiology and Infectious Diseases, 114. https://doi.org/10.1016/j.cimid.2024.102246

Munny, N. N., Shamsuzzaman, S., & Hossain, T. (2023). Emergence of fosfomycin resistance among isolates of multidrug–resistant Enterobacter species at a tertiary care hospital in Dhaka, Bangladesh. Bangladesh Medical Research Council Bulletin, 48(3), 203–210. https://doi.org/10.3329/BMRCB.V48I3.63812

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet, 399(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Mymensingh District – Banglapedia. Accessed on 10 February 2025.

Naeem, A., Badshah, S. L., Muska, M., Ahmad, N., & Khan, K. (2016). The current case of quinolones: Synthetic approaches and antibacterial activity. Molecules, 21(4). https://doi.org/10.3390/MOLECULES21040268

Nagaraj, G., Shamanna, V., Govindan, V., Rose, S., Sravani, D., Akshata, K. P., Shincy, M. R., Venkatesha, V. T., Abrudan, M., Argimón, S., Kekre, M., Underwood, A., Aanensen, D. M., Ravikumar, K. L., Abudahab, K., Harste, H., Muddyman, D., Taylor, B., Wheeler, N., … Herrera, E. M. (2021). High-Resolution Genomic Profiling of Carbapenem-Resistant *Klebsiella pneumoniae* Isolates: A Multicentric Retrospective Indian Study. Clinical Infectious Diseases, 73(Supplement_4), S300–S307. https://doi.org/10.1093/CID/CIAB767

Naghavi, M., Vollset, S. E., Ikuta, K. S., Swetschinski, L. R., Gray, A. P., Wool, E. E., Robles Aguilar, G., Mestrovic, T., Smith, G., Han, C., Hsu, R. L., Chalek, J., Araki, D. T., Chung, E., Raggi, C., Gershberg Hayoon, A., Davis Weaver, N., Lindstedt, P. A., Smith, A. E., … Murray, C. J. L. (2024). Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. The Lancet, 404(10459), 1199–1226. https://doi.org/10.1016/S0140-6736(24)01867-1

Nahar, A., Islam, A. K. M. A., Islam, M. N., & Alam, M. M. (2022). ESBL-producing *Klebsiella* sp. isolated from raw milk of healthy cow in small holder dairy farms of Mymensingh district in Bangladesh. Bangladesh Journal of Veterinary Medicine, 19(2). https://doi.org/10.33109/BJVMJD2021AMRT1

Nahid F, Zahra R, Sandegren L. (2017). A blaOXA-181-harbouring multi-resistant ST147 Klebsiella pneumoniae isolate from Pakistan that represent an intermediate stage towards pan-drug resistance. PLoS One. 12: e0189438.

Nam, Y. S., Cho, S. Y., Yang, H. Y., Park, K. S., Jang, J. H., Kim, Y. T., Jeong, J. W., Suh, J. T., & Lee, H. J. (2013). Investigation of mutation distribution in DNA gyrase and topoisomerase IV genes in ciprofloxacin-non-susceptible Enterobacteriaceae isolated from blood cultures in a tertiary care university hospital in South Korea, 2005-2010. International Journal of Antimicrobial Agents, 41(2), 126–129. https://doi.org/10.1016/j.ijantimicag.2012.10.004

Nang, S. C., Azad, M. A. K., Velkov, T., Tony Zhou, Q., & Li, J. (2021). Rescuing the last-line polymyxins: Achievements and challenges. Pharmacological Reviews, 73(2), 679–728. https://doi.org/10.1124/PHARMREV.120.000020

Nath, C., Das, T., Islam, M. S., Hasib, F. M. Y., Singha, S., Dutta, A., Barua, H., & Islam, M. Z. (2023). Colistin Resistance in Multidrug-Resistant *Escherichia coli* Isolated from Retail Broiler Meat in Bangladesh. Microbial Drug Resistance, 29(11), 523–532. https://doi.org/10.1089/MDR.2023.0026

Navon-Venezia, S., Kondratyeva, K., & Carattoli, A. (2017). *Klebsiella pneumoniae*: A major worldwide source and shuttle for antibiotic resistance. FEMS Microbiology Reviews, 41(3), 252–275. https://doi.org/10.1093/FEMSRE/FUX013, Nayduch, D., Neupane, S., Pickens, V., Purvis, T., & Olds, C. (2023). House Flies Are Underappreciated Yet Important Reservoirs and Vectors of Microbial Threats to Animal and Human Health. Microorganisms, 11(3), 583. https://doi.org/10.3390/MICROORGANISMS11030583/S1

Negeri, A. A., Mamo, H., Gahlot, D. K., Gurung, J. M., Seyoum, E. T., & Francis, M. S. (2023). Characterization of plasmids carrying bla CTX–M genes among extra–intestinal *Escherichia coli* clinical isolates in Ethiopia. Scientific Reports, 13(1). https://doi.org/10.1038/S41598–023–35402–2

Neog, N., Phukan, U., Puzari, M., Sharma, M., & Chetia, P. (2021). *Klebsiella oxytoca* and Emerging Nosocomial Infections. Current Microbiology, 78(4), 1115–1123. https://doi.org/10.1007/S00284-021-02402-2

Nguyen, Q., Nguyen, Y. T. P., Ha, T. T., Tran, D. T. N., Voong, P. V., Chau, V., Nguyen, P. L. N., Le, N. T. Q., Nguyen, L. P. H., Nguyen, T. T. N., Trinh, T. Van, Carrique-Mas, J. J., Baker, S., Thwaites, G., Rabaa, M. A., Choisy, M., Chung, H. T., & Pham, D. T. (2024). Genomic insights unveil the plasmid transfer mechanism and epidemiology of hypervirulent *Klebsiella pneumoniae* in Vietnam. Nature Communications 2024 15:1, 15(1), 1–15. https://doi.org/10.1038/s41467-024-48206-3

Nijhuis, R. H. T., Oueslati, S., Zhou, K., Bosboom, R. W., Rossen, J. W. A., & Naas, T. (2015). OXY-2-15, a novel variant showing increased ceftazidime hydrolytic activity. Journal of Antimicrobial Chemotherapy, 70(5), 1429–1433. https://doi.org/10.1093/JAC/DKV002

Nisa, T. T., Sugawara, Y., Hamaguchi, S., Takeuchi, D., Abe, R., Kuroda, E., Morita, M., Zuo, H., Ueda, A., Nishi, I., Hossain, N., Hasan, M. M., Siddiqee, M. H., Nakatani, D., Nakata, K., & Akeda, Y. (2024). Genomic characterization of carbapenemase-producing Enterobacterales from Dhaka food markets unveils the spread of high-risk antimicrobial-resistant clones and plasmids co-carrying blaNDM and mcr-1.1. JAC-Antimicrobial Resistance, 6(4). https://doi.org/10.1093/JACAMR/DLAE124

Noman, Z. Al, Anika, T. T., Safa, U. H., Alam, S., Dey, S. S., Bhuiyan, M. N. H., Siddique, M. P., & sikder, M. M. H. (2024). Antibiotic resistance risk assessment in Mymensingh, Bangladesh: Current scenario from human-animal-environmental interfaces viewpoint. Heliyon, 10(15), e35878. https://doi.org/10.1016/J.HELIYON.2024.E35878

Obeng-Nkrumah, N., Kwao, P., Owusu, E., Tawiah-Abrokwa, G. Della, Dzidzornu-Gomez, J., Baidoo, D., Duah, F. N. A., Saidu, S., & Labi, A. K. (2025). Houseflies as Vectors of ESBL-Producing Enterobacterales: Insights from Hospital Settings in a Low- to Middle-Income Country. Foodborne Pathogens and Disease. https://doi.org/10.1089/FPD.2024.0099,

Octavia, S., & Lan, R. (2014). The family enterobacteriaceae. The Prokaryotes: Gammaproteobacteria, 9783642389221, 225–286. https://doi.org/10.1007/978-3-642-38922-1_167/TABLES/24

OECD. (2023). Embracing a One Health framework to fight antimicrobial resistance. OECD Health Policy Studies. OECD Publishing. https://doi.org/10.1787/ce44c755-en

Ohama, Y., Nomura, Y., Mizoguchi, M., Higurashi, Y., Okamoto, K., & Harada, S. (2022). Accurate Identification of *Klebsiella variicola* by MALDI-TOF Mass Spectrometry in Clinical Microbiology Laboratories. Microbiology Spectrum, 10(5). https://doi.org/10.1128/SPECTRUM.02844-22

Ohno, T., Harada, S., Saito, H., Tanii, R., Komori, K., Kurosawa, M., Wakatake, H., Kanazawa, M., Ohki, U., Minoura, A., Yamada, M., Kaneko, S., Anzai, M., Tsutsui, Y., Kuhara, A., Oyanagi, T., Tanaka, Y., Takemura, H., Takeuchi, H., … Martin, R. M. (2025). Molecular epidemiology and clinical features of *Klebsiella variicola* bloodstream infection compared with infection with other *Klebsiella pneumoniae* species complex strains. Microbiology Spectrum, 13(6). https://doi.org/10.1128/SPECTRUM.03017-24

Okanda, T., Haque, A., Koshikawa, T., Islam, A., Huda, Q., Takemura, H., Matsumoto, T., & Nakamura, S. (2021). Characteristics of Carbapenemase-Producing *Klebsiella pneumoniae* Isolated in the Intensive Care Unit of the Largest Tertiary Hospital in Bangladesh. Frontiers in Microbiology, 11, 612020. https://doi.org/10.3389/FMICB.2020.612020/BIBTEX

Olaitan, A. O., Morand, S., & Rolain, J. M. (2014). Mechanisms of polymyxin resistance: Acquired and intrinsic resistance in bacteria. Frontiers in Microbiology, 5, 643. https://doi.org/10.3389/fmicb.2014.00643

Olaitan, A. O., Dandachi, I., Baron, S. A., Daoud, Z., Morand, S., & Rolain, J. M. (2021). Banning colistin in feed additives: a small step in the right direction. The Lancet Infectious Diseases, 21(1), 29–30. https://doi.org/10.1016/S1473-3099(20)30915-4

O'Neill J. (2016). Tackling drug-resistant infections globally: final report and recommendations. Accessed on 30 August 2024. https://amr-review.org.

Onwugamba, F. C., Mellmann, A., Nwaugo, V. O., Süselbeck, B., & Schaumburg, F. (2020). Antimicrobial resistant and enteropathogenic bacteria in 'filth flies': a cross-sectional study from Nigeria. Scientific Reports, 10(1), 1–9. https://doi.org/10.1038/S41598-020-74112

Oren, A., & Garrity, G. M. (2021). Valid publication of the names of forty-two phyla of prokaryotes. International Journal of Systematic and Evolutionary Microbiology, 71(10). https://doi.org/10.1099/IJSEM.0.005056

Osbelt, L., Almási, É. d. H., Wende, M., Kienesberger, S., Voltz, A., Lesker, T. R., Muthukumarasamy, U., Knischewski, N., Nordmann, E., Bielecka, A. A., Giralt-Zúñiga, M., Kaganovitch, E., Kühne, C., Baier, C., Pietsch, M., Müsken, M., Greweling-Pils, M. C., Breinbauer, R., Flieger, A., … Strowig, T. (2024). *Klebsiella oxytoca* inhibits Salmonella infection through multiple microbiota-context-dependent mechanisms. Nature Microbiology 2024 9:7, 9(7), 1792–1811. https://doi.org/10.1038/s41564-024-01710-0

Osei Sekyere, J., Govinden, U., Bester, L. A., & Essack, S. Y. (2016). Colistin and tigecycline resistance in carbapenemase-producing Gram-negative bacteria: emerging resistance mechanisms and detection methods. Journal of Applied Microbiology, 121(3), 601–617. https://doi.org/10.1111/JAM.13169

Paczosa, M. K., & Mecsas, J. (2016). *Klebsiella pneumoniae*: Going on the Offense with a Strong Defense. Microbiology and Molecular Biology Reviews: MMBR, 80(3), 629. https://doi.org/10.1128/MMBR.00078-15

Padilla, E., Llobet, E., Doménech-Sánchez, A., Martínez-Martínez, L., Bengoechea, J. A., & Albertí, S. (2010). *Klebsiella pneumoniae* AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrobial Agents and Chemotherapy, 54(1), 177–183. https://doi.org/10.1128/AAC.00715-09

Palmieri, M., Wyres, K. L., Mirande, C., Qiang, Z., Liyan, Y., Gang, C., Goossens, H., van Belkum, A., & Ping, L. Y. (2021). Genomic evolution and local epidemiology of *Klebsiella pneumoniae* from a major hospital in Beijing, China, over a 15-year period: Dissemination of known and novel high-risk clones. Microbial Genomics, 7(6), 000520. https://doi.org/10.1099/MGEN.0.000520/CITE/REFWORKS

Pan, T., & Li, Q. (2025). Mobile genetic elements in *Klebsiella pneumoniae*. Journal of Bacteriology, 207(5). https://doi.org/10.1128/JB.00012-25/ASSET/98ABDFF0-360B-42C8-8D9E-9668E644630C

Pan, Y. J., Lin, T. L., Chen, C. T., Chen, Y. Y., Hsieh, P. F., Hsu, C. R., Wu, M. C., & Wang, J. T. (2015). Genetic analysis of capsular polysaccharide synthesis gene clusters in 79 capsular types of *Klebsiella* spp. Scientific Reports, 5. https://doi.org/10.1038/SREP15573

Pankok, F., Taudien, S., Dekker, D., Thye, T., Oppong, K., Akenten, C. W., Lamshöft, M., Jaeger, A., Kaase, M., Scheithauer, S., Tanida, K., Frickmann, H., May, J., & Loderstädt, U. (2022). Epidemiology of Plasmids in *Escherichia coli* and *Klebsiella pneumoniae* with Acquired Extended Spectrum Beta–Lactamase Genes Isolated from Chronic Wounds in Ghana. Antibiotics, 11(5), 689. https://doi.org/10.3390/ANTIBIOTICS11050689

Parte, A. C. (2014). LPSN - List of prokaryotic names with standing in nomenclature. Nucleic Acids Research, 42(D1). https://doi.org/10.1093/NAR/GKT1111

Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. Clinical Microbiology Reviews, 31(4), e00088-17. https://doi.org/10.1128/CMR.00088-17

Peirano, G., *et al.* (2020). Genomic epidemiology of global *Klebsiella pneumoniae* carbapenemase-producing K. pneumoniae strains. Emerging Infectious Diseases, 26(7), 1341-1351

Pendleton, J. N., Gorman, S. P., & Gilmore, B. F. (2013). Clinical relevance of the ESKAPE pathogens. Expert Review of Anti-Infective Therapy, 11(3), 297–308. https://doi.org/10.1586/ERI.13.12

Peters, A. C., Larsson, D. G. J., Laxminarayan, R., & Munthe, C. (2024). Barriers and pathways to environmental surveillance of antibiotic resistance in middle- and low-income settings: a qualitative exploratory key expert study. Global Health Action, 17(1). https://doi.org/10.1080/16549716.2024.2343318

Phetburom, N., Boueroy, P., Chopjitt, P., Hatrongjit, R., Akeda, Y., Hamada, S., Nuanualsuwan, S., & Kerdsin, A. (2021). *Klebsiella pneumoniae* Complex Harboring *mcr-1*, *mcr-7*, and *mcr-8* Isolates from Slaughtered Pigs in Thailand. Microorganisms, 9(12), 2436. https://doi.org/10.3390/MICROORGANISMS9122436

Piñeiro, C., & Pichel, J. C. (2024). Efficient phylogenetic tree inference for massive taxonomic datasets: harnessing the power of a server to analyze 1 million taxa. *GigaScience*, *13*. https://doi.org/10.1093/GIGASCIENCE/GIAE055

Piperaki, E. T., Syrogiannopoulos, G. A., Tzouvelekis, L. S., & Daikos, G. L. (2017). *Klebsiella pneumoniae*: Virulence, Biofilm and Antimicrobial Resistance. Pediatric Infectious Disease Journal, 36(10), 1002–1005. https://doi.org/10.1097/INF.0000000000001675

Pirnay, J. P., Djebara, S., Steurs, G., Griselain, J., Cochez, C., De Soir, S., Glonti, T., Spiessens, A., Vanden Berghe, E., Green, S., Wagemans, J., Lood, C., Schrevens, E., Chanishvili, N., Kutateladze, M., de Jode, M., Ceyssens, P. J., Draye, J. P., Verbeken, G., … Kilcher, S. (2024). Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective observational study. Nature Microbiology, 9(6), 1434–1453. https://doi.org/10.1038/S41564-024-01705-X;TECHMETA

Podder, M. P., Rogers, L., Daley, P. K., Keefe, G. P., Whitney, H. G., & Tahlan, K. (2014). *Klebsiella* species associated with bovine mastitis in Newfoundland. PloS One, 9(9). https://doi.org/10.1371/JOURNAL.PONE.0106518

Podschun, R., & Ullmann, U. (1998). *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical Microbiology Reviews, 11(4), 589–603. https://doi.org/10.1128/CMR.11.4.589

Poirel, L., Héritier, C., Tolün, V., & Nordmann, P. (2004). Emergence of Oxacillinase-Mediated Resistance to Imipenem in *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy, 48(1), 15–22. https://doi.org/10.1128/AAC.48.1.15-22.2004

Poirel, L., Jayol, A., & Nordmanna, P. (2017). Polymyxins: Antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clinical Microbiology Reviews, 30(2), 557–596. https://doi.org/10.1128/CMR.00064-16

Poirel, L., Jayol, A., Bontron, S., Villegas, M. V., Ozdamar, M., Türkoglu, S., & Nordmann, P. (2015). The *mgrB* gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*. Journal of Antimicrobial Chemotherapy, 70(1), 75–80. https://doi.org/10.1093/JAC/DKU323

Potron, A., Nordmann, P., Lafeuille, E., Al Maskari, Z., Al Rashdi, F., & Poirel, L. (2011). Characterization of OXA-181, a Carbapenem-Hydrolyzing Class D β-Lactamase from *Klebsiella* *pneumoniae*. Antimicrobial Agents and Chemotherapy, 55(10), 4896. https://doi.org/10.1128/AAC.00481-11

Poudel, A., Hathcock, T., Butaye, P., Kang, Y., Price, S., Macklin, K., Walz, P., Cattley, R., Kalalah, A., Adekanmbi, F., & Wang, C. (2019). Multidrug-Resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus* spp. in Houseflies and Blowflies from Farms and Their Environmental Settings. International Journal of Environmental Research and Public Health 2019, Vol. 16, Page 3583, 16(19), 3583. https://doi.org/10.3390/IJERPH16193583

Prah, I., Nukui, Y., Yamaoka, S., & Saito, R. (2022). Emergence of a High-Risk *Klebsiella michiganensis* Clone Disseminating Carbapenemase Genes. Frontiers in Microbiology, 13, 880248. https://doi.org/10.3389/FMICB.2022.880248/FULL

Prifti, K., Chi, K. Y. K., Eraly, E., Joh, H. S., Sujan, M. J., Poudyal, N., Marks, F., & Holm, M. (2023). Collecting Multi-country Retrospective Antimicrobial Consumption and Use Data: Challenges and Experience. Clinical Infectious Diseases, 77(Supplement_7), S528–S535. https://doi.org/10.1093/CID/CIAD667

Prinzi, A. M., & Moore, N. M. (2023). Change of Plans: Overview of Bacterial Taxonomy, Recent Changes of Medical Importance, and Potential Areas of Impact. Open Forum Infectious Diseases, 10(7). https://doi.org/10.1093/OFID/OFAD269

Pu, D., Zhao, J., Chang, K., Zhuo, X., & Cao, B. (2023). “Superbugs” with hypervirulence and carbapenem resistance in *Klebsiella pneumoniae*: the rise of such emerging nosocomial pathogens in China. Science Bulletin, 68(21), 2658–2670. https://doi.org/10.1016/j.scib.2023.09.040

Qu, Y., Wang, W., Lu, Q., Qiu, J., Wang, D., & Ma, L. (2023). Occurrence and characterization of plasmids carrying tmexCD1-toprJ1, bla DHA-1, and bla CTX-M-127, in clinical *Klebsiella pneumoniae* strains. Frontiers in Cellular and Infection Microbiology, 13, 1260066. https://doi.org/10.3389/FCIMB.2023.1260066/FULL

Quansah, E., Barnie, P. A., Acheampong, D. O., Obiri-Yeboah, D., Mills, R. O., Asmah, E., Cudjoe, O., & Dadzie, I. (2019). Geographical Distribution of β-Lactam Resistance among *Klebsiella* spp. from Selected Health Facilities in Ghana. Tropical Medicine and Infectious Disease, 4(3), 117. https://doi.org/10.3390/TROPICALMED4030117

Quintelas, M., Silva, V., Araújo, S., Tejedor-Junco, M. T., Pereira, J. E., Igrejas, G., & Poeta, P. (2024). *Klebsiella* in Wildlife: Clonal Dynamics and Antibiotic Resistance Profiles, a Systematic Review. Pathogens 2024, Vol. 13, Page 945, 13(11), 945. https://doi.org/10.3390/PATHOGENS13110945

Rahman, H., Happy, F., Efan, A., & Hera, M. (2020). The Small-Scale Dairy Value Chain Analysis: Challenges And Opportunities For Dairy Development In Mymensingh District Of Bangladesh. SAARC Journal of Agriculture, 17(2), 213–226. https://doi.org/10.3329/SJA.V17I2.45307

Rahman, M. M., Chowdhury, O. A., Hoque, M. M., Hoque, S. A., Chowdhury, S. M. R., & Rahman, M. A. (2018). Antimicrobial Resistance Pattern of Uropathogenic *Escherichia coli* and *Klebsiella* species Isolated in a Tertiary Care Hospital of Sylhet. Medicine Today, 30(2), 61–66. https://doi.org/10.3329/medtoday.v30i2.3781

Rahman, Md. A., Begum, Mst. D., Afroz, F., & Rahman, Md. K. (2023). Isolation and Molecular Detection of *Klebsiella pneumoniae* from Children Affected by Pneumonia in Dinajpur District, Bangladesh. South Asian Journal of Research in Microbiology, 16(4), 14–26. https://doi.org/10.9734/SAJRM/2023/V16I4314

Raihan, M. A., Islam, M. S., Islam, S., Islam, A. F. M. M., Ahmed, K. T., Ahmed, T., Islam, M. N., Ahmed, S., Chowdhury, M. S., Sarker, D. K., & Lamisa, A. B. (2024). Knowledge, attitudes, and practices regarding antibiotic use in Bangladesh: Findings from a cross-sectional study. PLOS ONE, 19(2), e0297653. https://doi.org/10.1371/JOURNAL.PONE.0297653

Ramirez, M. S., Traglia, G. M., Lin, D. L., Tran, T., & Tolmasky, M. E. (2014). Plasmid-mediated antibiotic resistance and virulence in Gram-negatives: The Klebsiella pneumoniae paradigm. Microbiology Spectrum, 2(5), 1–15. https://doi.org/10.1128/microbiolspec.PLAS-0016-2013

Ramirez, M. S., Iriarte, A., Reyes-Lamothe, R., Sherratt, D. J., & Tolmasky, M. E. (2019). Small *Klebsiella pneumoniae* Plasmids: Neglected Contributors to Antibiotic Resistance. Frontiers in Microbiology, 10, 2182. https://doi.org/10.3389/FMICB.2019.02182/FULL

Ranjbar, R., Izadi, M., Hafshejani, T. T., & Khamesipour, F. (2016). Molecular detection and antimicrobial resistance of *Klebsiella pneumoniae* from house flies (Musca domestica) in kitchens, farms, hospitals and slaughterhouses. Journal of Infection and Public Health, 9(4), 499–505. https://doi.org/10.1016/J.JIPH.2015.12.012

Rashid, M. M., Akhtar, Z., Chowdhury, S., Islam, M. A., Parveen, S., Ghosh, P. K., Rahman, A., Khan, Z. H., Islam, K., Debnath, N., Rahman, M., & Chowdhury, F. (2022). Pattern of Antibiotic Use among Hospitalized Patients according to WHO Access, Watch, Reserve (AWaRe) Classification: Findings from a Point Prevalence Survey in Bangladesh. Antibiotics (Basel, Switzerland), 11(6). https://doi.org/10.3390/ANTIBIOTICS11060810

Redgrave, L. S., Sutton, S. B., Webber, M. A., & Piddock, L. J. V. (2014). Fluoroquinolone resistance: Mechanisms, impact on bacteria, and role in evolutionary success. Trends in Microbiology, 22(8), 438–445. https://doi.org/10.1016/j.tim.2014.04.007

Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiology, 4(3), 482. https://doi.org/10.3934/MICROBIOL.2018.3.482

Ristuccia, P. A., & Cunha, B. A. (1984). *Klebsiella*. Infection Control, 5(7), 343–347. https://doi.org/10.1017/S0195941700060549

Rodrigues, C., Passet, V., Rakotondrasoa, A., & Brisse, S. (2018). Identification of *Klebsiella pneumoniae*, *Klebsiella quasipneumoniae*, *Klebsiella variicola* and related phylogroups by MALDI-TOF mass spectrometry. Frontiers in Microbiology, 9(DEC). https://doi.org/10.3389/FMICB.2018.03000

Rodrigues, S. H., Nunes, G. D., Soares, G. G., Ferreira, R. L., Damas, M. S. F., Laprega, P. M., Shilling, R. E., Campos, L. C., da Costa, A. S., Malavazi, I., da Cunha, A. F., & Pranchevicius, M. C. da S. (2024). First report of coexistence of blaKPC-2 and blaNDM-1 in carbapenem-resistant clinical isolates of *Klebsiella aerogenes* in Brazil. Frontiers in Microbiology, 15, 1352851. https://doi.org/10.3389/FMICB.2024.1352851/FULL

Rodríguez-Medina, N., Barrios-Camacho, H., Duran-Bedolla, J., & Garza-Ramos, U. (2019). *Klebsiella variicola*: an emerging pathogen in humans. Emerging Microbes & Infections, 8(1), 973. https://doi.org/10.1080/22221751.2019.1634981

Rodríguez-Medina, N., Rodríguez-Santiago, J., Alvarado-Delgado, A., Sagal-Prado, A., Silva-Sánchez, J., De la Cruz, M. A., Ares, M. A., Sánchez-Arias, M., Morfín-Otero, R., Hernández-Castro, R., Cornejo-Juárez, P., Jiménez-Villanueva, E., Sánchez-Francia, D., & Garza-Ramos, U. (2024). Comprehensive study reveals phenotypic heterogeneity in *Klebsiella pneumoniae* species complex isolates. Scientific Reports, 14(1), 1–12. https://doi.org/10.1038/S41598-024-55546-Z

Roisin, S., Gaudin, C., De Mendonça, R., Bellon, J., Van Vaerenbergh, K., De Bruyne, K., Byl, B., Pouseele, H., Denis, O., & Supply, P. (2016). Pan-genome multilocus sequence typing and outbreak-specific reference-based single nucleotide polymorphism analysis to resolve two concurrent Staphylococcus aureus outbreaks in neonatal services. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 22(6), 520–526. https://doi.org/10.1016/j.cmi.2016.01.024

Roulston, K. J., Bharucha, T., Turton, J. F., Hopkins, K. L., & Mack, D. J. F. (2018). A case of NDM-carbapenemase-producing hypervirulent *Klebsiella pneumoniae* sequence type 23 from the UK. JMM Case Reports, 5(9), e005130. https://doi.org/10.1099/JMMCR.0.005130

Rozwandowicz, M., Brouwer, M. S. M., Fischer, J., Wagenaar, J. A., Gonzalez-Zorn, B., Guerra, B., Mevius, D. J., & Hordijk, J. (2018). Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. The Journal of antimicrobial chemotherapy, 73(5), 1121–1137. https://doi.org/10.1093/jac/dkx488

Ruiz, E., Sáenz, Y., Zarazaga, M., Rocha-Gracia, R., Martínez-Martínez, L., Arlet, G., & Torres, C. (2012). Qnr, *aac(6')-ib-cr* and *qepA* genes in *Escherichia coli* and *Klebsiella* spp.: Genetic environments and plasmid and chromosomal location. Journal of Antimicrobial Chemotherapy, 67(4), 886–897. https://doi.org/10.1093/JAC/DKR548

Russo, T. A., & MacDonald, U. (2020). The Galleria mellonella Infection Model Does Not Accurately Differentiate between Hypervirulent and Classical *Klebsiella pneumoniae*. MSphere, 5(1). https://doi.org/10.1128/MSPHERE.00850-19

Russo, T. A., & Marr, C. M. (2019). Hypervirulent *Klebsiella pneumoniae*. Clinical Microbiology Reviews, 32(3), e00001-19. https://doi.org/10.1128/CMR.00001-19

Russo, T. A., Alvarado, C. L., Davies, C. J., Drayer, Z. J., Carlino-MacDonald, U., Hutson, A., Luo, T. L., Martin, M. J., Corey, B. W., Moser, K. A., Kamile Rasheed, J., Halpin, A. L., McGann, P. T., & Lebreton, F. (2024). Differentiation of hypervirulent and classical *Klebsiella pneumoniae* with acquired drug resistance. MBio, 15(2). https://doi.org/10.1128/MBIO.02867-23/SUPPL_FILE/MBIO.02867-23-S0004.XLSX

Russo, T. A., Carlino-MacDonald, U., Drayer, Z. J., Davies, C. J., Alvarado, C. L., Hutson, A., Luo, T. L., Martin, M. J., McGann, P. T., & Lebreton, F. (2024). Deciphering the relative importance of genetic elements in hypervirulent *Klebsiella pneumoniae* to guide countermeasure development. EBioMedicine, 107, 105302. https://doi.org/10.1016/J.EBIOM.2024.105302

Russo, T. A., Olson, R., Fang, C. T., Stoesser, N., Miller, M., MacDonald, U., Hutson, A., Barker, J. H., La Hoz, R. M., Johnson, J. R., Backer, M., Bajwa, R., Catanzaro, A. T., Crook, D., De Almeida, K., Fierer, J., Greenberg, D. E., Klevay, M., Patel, P., … Zola, J. (2018). Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from classical K. pneumoniae. Journal of Clinical Microbiology, 56(9). https://doi.org/10.1128/JCM.00776-18

Sabnis, A., Hagart, K. L. H., Klöckner, A., Becce, M., Evans, L. E., Furniss, R. C. D., Mavridou, D. A. I., Murphy, R., Stevens, M. M., Davies, J. C., Larrouy-Maumus, G. J., Clarke, T. B., & Edwards, A. M. (2021). Colistin kills bacteria by targeting lipopolysaccharide in the cytoplasmic membrane. ELife, 10. https://doi.org/10.7554/ELIFE.65836

Saeed, M. A., Khan, A. U., Ehtisham-ul-Haque, S., Waheed, U., Qamar, M. F., Rehman, A. ur, Nasir, A., Zaman, M. A., Kashif, M., Gonzalez, J. P., & El-Adawy, H. (2023). Detection and Phylogenetic Analysis of Extended-Spectrum β-Lactamase (ESBL)-Genetic Determinants in Gram-Negative Faecal-Microbiota of Wild Birds and Chicken Originated at Trimmu Barrage. Antibiotics 2023, Vol. 12, Page 1376, 12(9), 1376. https://doi.org/10.3390/ANTIBIOTICS12091376

Saha, O., Basri, R., Hossain, M. A., & Sultana, M. (2023). Characterization of multidrug and heavy metal resistance of carbapenemases producing *Klebsiella pneumoniae* from poultry samples in Bangladesh. Veterinary Medicine and Science, 9(4), 1685. https://doi.org/10.1002/VMS3.1168

Sahoo, S., Nath Mohanty, J., Routray, S. P., Sarangi, A., Swapnesh, D., Nayak, K., Shah, S., Das, J., Subudhi, E., & Swarnkar, T. (2025). Prevalence of multidrug-resistant *Klebsiella pneumoniae* in urinary tract infections: A retrospective observational study in eastern India. Microbes and Infectious Diseases, 6(1), 299–307. https://doi.org/10.21608/MID.2024.276619.1844

Sahoo, S., Routray, S. P., Mohanty, J. N., Pattnaik, A., Nayak, D. S. K., Shah, S., Das, J., Subudhi, E., & Swarnkar, T. (2024). Genomic characterization and distribution of XDR *Klebsiella pneumoniae* ST15 carrying blaOXA-181 on ColKP3 plasmid from an ICH patient in eastern India: A global comparative analysis. Gene Reports, 36, 101978. https://doi.org/10.1016/J.GENREP.2024.101978

Sahoo, S., Sahoo, R. K., Gaur, M., Behera, D. U., Sahu, A., Das, A., Dey, S., Dixit, S., & Subudhi, E. (2023). Urban wastewater contributes to the emergence of carbapenem–resistant *Klebsiella pneumoniae* (CRKP) in an urban receiving river in eastern India. Letters in Applied Microbiology, 76(1), 1–11. https://doi.org/10.1093/LAMBIO/OVAC005

Sakamoto, N., Akeda, Y., Sugawara, Y., Takeuchi, D., Motooka, D., Yamamoto, N., Laolerd, W., Santanirand, P., & Hamada, S. (2018). Genomic Characterization of Carbapenemase-Producing *Klebsiella pneumoniae* with Chromosomally Carried blaNDM-1. Antimicrobial Agents and Chemotherapy, 62(12), e01520-18. https://doi.org/10.1128/AAC.01520-18

Salloum, T., Panossian, B., Bitar, I., Bitar, I., Hrabak, J., Hrabak, J., Araj, G. F., & Tokajian, S. (2020). First report of plasmid-mediated colistin resistance *mcr-8.1* gene from a clinical *Klebsiella pneumoniae* isolate from Lebanon. Antimicrobial Resistance and Infection Control, 9(1), 94. https://doi.org/10.1186/S13756-020-00759-W

San Millan, A. (2018). Evolution of plasmid-mediated antibiotic resistance. Trends in Microbiology, 26(12), 978–990. https://doi.org/10.1016/j.tim.2018.06.007

Saperston, K. N., Shapiro, D. J., Hersh, A. L., & Copp, H. L. (2014). A comparison of inpatient versus outpatient resistance patterns of pediatric urinary tract infection. The Journal of Urology, 191(5 Suppl), 1608–1613. https://doi.org/10.1016/J.JURO.2013.10.064

Sarjana Safain, K., Bhuyan, G. S., Hassan Hasib, S., Islam, M. S., Mahmud–Un–Nabi, M. Al, Sultana, R., Tasnim, S., Noor, F. A., Sarker, S. K., Islam, M. T., Rahat, A., Leung, D. T., Domman, D., Manzoor, F., Anwar, S., Majid Bhuiyan, M. A., Chowdhury, E. K., Qadri, S. S., Qadri, F., & Mannoor, K. (2021). Genotypic and phenotypic profiles of antibiotic–resistant bacteria isolated from hospitalised patients in Bangladesh. Tropical Medicine and International Health, 26(7), 720–729. https://doi.org/10.1111/TMI.13584

Sarker, S., Neeloy, R. M., Habib, M. B., Urmi, U. L., Al Asad, M., Mosaddek, A. S. M., Khan, M. R. K., Nahar, S., Godman, B., & Islam, S. (2024). Mobile Colistin-Resistant Genes *mcr-1*, *mcr-2*, and *mcr-3* Identified in Diarrheal Pathogens among Infants, Children, and Adults in Bangladesh: Implications for the Future. Antibiotics, 13(6), 534. https://doi.org/10.3390/ANTIBIOTICS13060534/S1

Sati, H., Carrara, E., Savoldi, A., Hansen, P., Garlasco, J., Campagnaro, E., Boccia, S., Castillo-Polo, J. A., Magrini, E., Garcia-Vello, P., Wool, E., Gigante, V., Duffy, E., Cassini, A., Huttner, B., Pardo, P. R., Naghavi, M., Mirzayev, F., Zignol, M., Umubyeyi Nyaruhirira, A. (2025). The WHO Bacterial Priority Pathogens List 2024: a prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. The Lancet Infectious Diseases, 25(9), 1033–1043. https://doi.org/10.1016/S1473-3099(25)00118-5

Sawa, T., Moriyama, K., & Kinoshita, M. (2024). Current status of bacteriophage therapy for severe bacterial infections. Journal of Intensive Care, 12(1), 44. https://doi.org/10.1186/S40560-024-00759-7

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. Emerging Infectious Diseases, 17(1), 7–15. https://doi.org/10.3201/EID1701.P11101

Schedlbauer, A., Kaminishi, T., Ochoa-Lizarralde, B., Dhimole, N., Zhou, S., López-Alonso, J. P., Connell, S. R., & Fucini, P. (2015). Structural characterization of an alternative mode of tigecycline binding to the bacterial ribosome. Antimicrobial Agents and Chemotherapy, 59(5), 2849–2854. https://doi.org/10.1128/AAC.04895-14

Scholtz, M., Guthrie, A. J., Newton, R., & Schulman, M. L. (2024). Review of Pseudomonas aeruginosa and *Klebsiella pneumoniae* as venereal pathogens in horses. Equine Veterinary Journal, 57(3), 587–597. https://doi.org/10.1111/EVJ.14201

Sekizuka, T., Yatsu, K., Inamine, Y., Segawa, T., Nishio, M., Kishi, N., & Kuroda, M. (2018). Complete Genome Sequence of a bla KPC-2 -Positive *Klebsiella pneumoniae* Strain Isolated from the Effluent of an Urban Sewage Treatment Plant in Japan . MSphere, 3(5). https://doi.org/10.1128/MSPHERE.00314-18

Sękowska, A., Konechnyi, Y., & Carrazco-Montalvo, A. (2025). Whole-Genome Sequencing of *Klebsiella quasipneumoniae* subsp. similipneumoniae Isolated from a Patient with Pneumonia. Current Microbiology, 82(9), 1–7. https://doi.org/10.1007/S00284-025-04414-8/FIGURES/1

Shahida, S., Islam, A. & Dey, B. (2016). Hospital Acquired Infections in Low- and Middle-Income Countries: Root Cause Analysis and the Development of Infection Control Practices in Bangladesh. Open Journal of Obstetrics and Gynecology. 6:28-39.

Shamanna, V., Srinivas, S., Couto, N., Nagaraj, G., Sajankila, S. P., Krishnappa, H. G., Kumar, K. A., Aanensen, D. M., Lingegowda, R. K., David, S., Abrudan, M., Caballero, J. D., Kumaran, E., Lewis-Woodhouse, G., Abudahab, K., Pascoe, B., Donado-Godoy, P., Shreedhanya, D. M., Shincy, M. R., … Vashishtha, R. (2024). Geographical distribution, disease association and diversity of *Klebsiella pneumoniae* K/L and O antigens in India: roadmap for vaccine development. Microbial Genomics, 10(7), 001271. https://doi.org/10.1099/MGEN.0.001271/CITE/REFWORKS

Shankar, C., Basu, S., Lal, B., Shanmugam, S., Vasudevan, K., Mathur, P., Ramaiah, S., Anbarasu, A., & Veeraraghavan, B. (2021). Aerobactin Seems To Be a Promising Marker Compared With Unstable RmpA2 for the Identification of Hypervirulent Carbapenem-Resistant *Klebsiella pneumoniae*: In Silico and In Vitro Evidence. Frontiers in Cellular and Infection Microbiology, 11, 709681. https://doi.org/10.3389/FCIMB.2021.709681/BIBTEX

Shankar, C., *et al.* (2019). Biofilm formation and associated multi-drug resistance in clinical isolates of *Klebsiella pneumoniae*. Indian Journal of Medical Microbiology, 37(4), 512-516

Shankar, C., Jacob, J. J., Sugumar, S. G., Natarajan, L., Rodrigues, C., Mathur, P., Mukherjee, D. N., Sharma, A., Chitnis, D. S., Bharagava, A., Manesh, A., Gunasekaran, K., & Veeraraghavan, B. (2021). Distinctive Mobile Genetic Elements Observed in the Clonal Expansion of Carbapenem–Resistant *Klebsiella pneumoniae* in India. Microbial Drug Resistance, 27(8), 1096–1104. https://doi.org/10.1089/MDR.2020.0316

Shankar, C., Jacob, J. J., Vasudevan, K., Biswas, R., Manesh, A., Sethuvel, D. P. M., Varughese, S., Biswas, I., & Veeraraghavan, B. (2020). Emergence of Multidrug Resistant Hypervirulent ST23 *Klebsiella pneumoniae*: Multidrug Resistant Plasmid Acquisition Drives Evolution. Frontiers in Cellular and Infection Microbiology, 2020, 10, 1. https://doi.org/10.3389/FCIMB.2020.575289/FULL

Shankar, C., Vasudevan, K., Jacob, J. J., Baker, S., Isaac, B. J., Neeravi, A. R., Sethuvel, D. P. M., George, B., & Veeraraghavan, B. (2022). Hybrid Plasmids Encoding Antimicrobial Resistance and Virulence Traits Among Hypervirulent *Klebsiella pneumoniae* ST2096 in India. Frontiers in Cellular and Infection Microbiology, 12. https://doi.org/10.3389/FCIMB.2022.875116/PDF

Shapovalova, V. V., Chulkova, P. S., Ageevets, V. A., Nurmukanova, V., Verentsova, I. V., Girina, A. A., Protasova, I. N., Bezbido, V. S., Sergevnin, V. I., Feldblum, I. V., Kudryavtseva, L. G., Sharafan, S. N., Semerikov, V. V., Babushkina, M. L., Valiullina, I. R., Chumarev, N. S., Isaeva, G. S., Belyanina, N. A., Shirokova, I. U., … Sidorenko, S. V. (2024). High-Risk Lineages of Hybrid Plasmids Carrying Virulence and Carbapenemase Genes. Antibiotics, 13(12), 1224. https://doi.org/10.3390/ANTIBIOTICS13121224/S1

Shen, D., Seco, B. M. S., Teixeira Alves, L. G., Yao, L., Bräutigam, M., Opitz, B., Witzenrath, M., Fries, B. C., & Seeberger, P. H. (2024). Semisynthetic Glycoconjugate Vaccine Lead against *Klebsiella pneumoniae* Serotype O2afg Induces Functional Antibodies and Reduces the Burden of Acute Pneumonia. Journal of the American Chemical Society, 146(51), 35356–35366. https://doi.org/10.1021/JACS.4C13972

Sheng, J., Cave, R., Ter-Stepanyan, M. M., Lu, S., Wang, Y., Liu, T., & Mkrtchyan, H. V. (2025). Emergence of *mcr-8.1*-bearing MDR-hypervirulent *Klebsiella pneumoniae* ST307. Microbiology Spectrum, 13(2). https://doi.org/10.1128/SPECTRUM.01910-24/SUPPL_FILE/SPECTRUM.01910-24-S0005.XLSX

Shibu, P., McCuaig, F., McCartney, A. L., Kujawska, M., Hall, L. J., & Hoyles, L. (2020). The kleboxymycin biosynthetic gene cluster is encoded by several species belonging to the *Klebsiella oxytoca* complex. BioRxiv, 2020.07.24.215400. https://doi.org/10.1101/2020.07.24.215400

Shibu, P., McCuaig, F., McCartney, A. L., Kujawska, M., Hall, L. J., & Hoyles, L. (2021). Improved molecular characterization of the *Klebsiella oxytoca* complex reveals the prevalence of the kleboxymycin biosynthetic gene cluster. Microbial Genomics, 7(6), 000592. https://doi.org/10.1099/MGEN.0.000592

Siddique, A. B., Munni, A., Hasan, M., Raj, R., Mutalib, Md. A., Sikder, Md. T., Okino, T., Ahmed, A., & Hossain, Md. S. (2024). Investigation and detection of multiple antibiotic-resistant pathogenic bacteria in municipal wastewater of Dhaka city. Discover Water 2024 4:1, 4(1), 1–14. https://doi.org/10.1007/S43832-024-00114-9

Siddiqui, Md. S. I., Akter, S., & Islam, Mohd. R. (2023). A Survey of Antibiotics Use and the Awareness of Antibiotics Resistance Among Farmers of Broiler Farms at Sylhet Sadar Upazilla in Bangladesh. Veterinary Sciences: Research and Reviews, 9(1). https://doi.org/10.17582/JOURNAL.VSRR/2023/9.1.57.64

Silva, K. P. T., & Khare, A. (2024). Antibiotic resistance mediated by gene amplifications. Antimicrobials and Resistance, 2(1), 1–10. https://doi.org/10.1038/S44259-024-00052-5

Silvester, R., Madhavan, A., Kokkat, A., Parolla, A., B.M., A., M., H., & Abdulla, M. H. (2022). Global surveillance of antimicrobial resistance and hypervirulence in *Klebsiella pneumoniae* from LMICs: An in-silico approach. Science of The Total Environment, 802, 149859. https://doi.org/10.1016/J.SCITOTENV.2021.149859

Singh, S., Pathak, A., Rahman, M., Singh, A., Nag, S., Sahu, C., & Prasad, K. N. (2021). Genetic Characterisation of Colistin Resistant *Klebsiella pneumoniae* Clinical Isolates From North India. Frontiers in Cellular and Infection Microbiology, 11. https://doi.org/10.3389/FCIMB.2021.666030/PDF

Sirot, D., Sirot, J., Labia, R., Morand, A., Courvalin, P., Darfeuille-michaud, A., Perroux, R., & Cluzel, R. (1987). Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: Identification of CTX-1, a novel β-lactamase. Journal of Antimicrobial Chemotherapy, 20(3), 323–334. https://doi.org/10.1093/JAC/20.3.323

Sobur, M. A., Ievy, S., Haque, Z. F., Nahar, A., Zaman, S. B., & Rahman, M. T. (2019). Emergence of colistin–resistant *Escherichia coli* in poultry, house flies, and pond water in Mymensingh, Bangladesh. Journal of Advanced Veterinary and Animal Research, 6(1), 50. https://doi.org/10.5455/JAVAR.2019.F311

Soliman, E. A., Saad, A., Abd El Tawab, A. A., Elhofy, F. I., Rizk, A. M., Elkhayat, M., Kozytska, T., Ilyas, M., Bassiouny, M., Brangsch, H., Pletz, M. W., Neubauer, H., Sprague, L. D., & Wareth, G. (2024). Exploring AMR and virulence in *Klebsiella pneumoniae* isolated from humans and pet animals: A complement of phenotype by WGS-derived profiles in a One Health study in Egypt. One Health, 19, 100904. https://doi.org/10.1016/J.ONEHLT.2024.100904

Song, J., Xiang, W., Wang, Q., Yin, J., Tian, T., Yang, Q., Zhang, M., Ge, G., Li, J., Diao, N., Liu, F., Shi, K., Cai, R., Du, R., & Gong, Q. (2023). Prevalence and risk factors of *Klebsiella* spp. in milk samples from dairy cows with mastitis—A global systematic review. Frontiers in Veterinary Science, 10, 1143257. https://doi.org/10.3389

Sonia, S. J., Uddin, K. H., & Shamsuzzaman, S. M. (2022). Prevalence of Colistin Resistance in *Klebsiella pneumoniae* Isolated from a Tertiary Care Hospital in Bangladesh and Molecular Characterization of Colistin Resistance Genes among Them by Polymerase Chain Reaction and Sequencing. Mymensingh medical journal: MMJ, 31(3), 733–740.

Spadar, A., Perdigão, J., Campino, S., & Clark, T. G. (2022). Genomic analysis of hypervirulent *Klebsiella pneumoniae* reveals potential genetic markers for differentiation from classical strains. Scientific Reports, 12(1), 1–8. https://doi.org/10.1038/S41598-022-17995-2

Spadar, A., Perdigão, J., Campino, S., & Clark, T. G. (2023). Large-scale genomic analysis of global *Klebsiella pneumoniae* plasmids reveals multiple simultaneous clusters of carbapenem-resistant hypervirulent strains. Genome Medicine, 15(1), 1–12. https://doi.org/10.1186/S13073-023-01153-Y/FIGURES/3

Spadar, A., Phelan, J., Elias, R., Modesto, A., Caneiras, C., Marques, C., Lito, L., Pinto, M., Cavaco-Silva, P., Ferreira, H., Pomba, C., Da Silva, G. J., Saavedra, M. J., Melo-Cristino, J., Duarte, A., Campino, S., Perdigão, J., & Clark, T. G. (2022). Genomic epidemiological analysis of *Klebsiella pneumoniae* from Portuguese hospitals reveals insights into circulating antimicrobial resistance. Scientific Reports, 12(1), 1–13. https://doi.org/10.1038/S41598-022-17996-1

Srinivasan, V. B., & Rajamohan, G. (2013). KpnEF, a new member of the *Klebsiella pneumoniae* cell envelope stress response regulon, is an SMR-type efflux pump involved in broad-spectrum antimicrobial resistance. Antimicrobial Agents and Chemotherapy, 57(9), 4449–4462. https://doi.org/10.1128/AAC.02284-12

Stanton, T. D., & Wyres, K. L. (2024). What defines hypervirulent *Klebsiella pneumoniae*? EBioMedicine, 108, 105331. https://doi.org/10.1016/J.EBIOM.2024.105331

Stanton, T. D., Hetland, M. A. K., Löhr, I. H., Holt, K. E., & Wyres, K. L. (2025). Fast and accurate in silico antigen typing with Kaptive 3. Microbial Genomics, 11(6), 001428. https://doi.org/10.1099/MGEN.0.001428

Stennett, H. L., Back, C. R., & Race, P. R. (2022). Derivation of a Precise and Consistent Timeline for Antibiotic Development. Antibiotics (Basel, Switzerland), 11(9). https://doi.org/10.3390/ANTIBIOTICS11091237

Stewart, J., Judd, L. M., Jenney, A., Holt, K. E., Wyres, K. L., & Hawkey, J. (2022). Epidemiology and genomic analysis of *Klebsiella oxytoca* from a single hospital network in Australia. BMC Infectious Diseases, 22(1), 704. https://doi.org/10.1186/S12879-022-07687-7

Stimson, J., Gardy, J., Mathema, B., Crudu, V., Cohen, T., & Colijn, C. (2019). Beyond the SNP Threshold: Identifying Outbreak Clusters Using Inferred Transmissions. Molecular biology and evolution, 36(3), 587–603. https://doi.org/10.1093/molbev/msy242

Stojowska-swędrzyńska, K., Łupkowska, A., Kuczyńska-wiśnik, D., & Laskowska, E. (2021). Antibiotic Heteroresistance in *Klebsiella pneumoniae*. International Journal of Molecular Sciences 2022, Vol. 23, Page 449, 23(1), 449. https://doi.org/10.3390/IJMS23010449

Strahilevitz, J., Jacoby, G. A., Hooper, D. C., & Robicsek, A. (2009). Plasmid-mediated quinolone resistance: A multifaceted threat. Clinical Microbiology Reviews, 22(4), 664–689. https://doi.org/10.1128/CMR.00016-09

Strunk, T., Molloy, E. J., Mishra, A., & Bhutta, Z. A. (2024). Neonatal bacterial sepsis. The Lancet, 404(10449), 277–293. https://doi.org/10.1016/S0140-6736(24)00495-1

Sujan, M. J., Habib, Z. H., Rahman, A., Shahriar Rizvi, S. M., Barua, H. T., Aboushady, A. T., Hasnat, M. A., Rasul, S. B. G., Joh, H. S., Prifti, K., Chi, K. Y. K., Kwon, S. Y., Clark, A., Gautam, S., Holm, M., Marks, F., Stelling, J., Shaw, A., & Poudyal, N. (2023). Antimicrobial Resistance Surveillance Methods in Bangladesh: Present and Way Forward. Clinical Infectious Diseases, 77(Supplement_7), S549–S559. https://doi.org/10.1093/CID/CIAD561

Supty, S. M., Saadat, T. M. F., Islam, K. T., Rubaiyat, R. N., Kubra, K., & Islam, S. M. B. U. (2023). Multidrug Resistance and Molecular Characterization of *Klebsiella* spp. Isolated from the Cloacal Samples of Broiler Chickens in Bangladesh. Microbial Bioactives, 6(1), 1–11. https://doi.org/10.25163/MICROBBIOACTS.619419

Surleac, M., Barbu, I. C., Paraschiv, S., Popa, L. I., Gheorghe, I., Marutescu, L., Popa, M., Sarbu, I., Talapan, D., Nita, M., Iancu, A. V., Arbune, M., Manole, A., Nicolescu, S., Sandulescu, O., Streinu-Cercel, A., Otelea, D., & Chifiriuc, M. C. (2020). Whole genome sequencing snapshot of multidrug resistant *Klebsiella pneumoniae* strains from hospitals and receiving wastewater treatment plants in Southern Romania. PLoS ONE, 15(1). https://doi.org/10.1371/JOURNAL.PONE.0228079

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E. M., Houchens, C. R., Grayson, M. L., Hansen, P., Singh, N., … Zorzet, A. (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet Infectious Diseases, 18(3), 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3

Takei, S., Lu, Y. J., Tohya, M., Watanabe, S., Misawa, S., Tabe, Y., Miida, T., Mya, S., Tin, H. H., Tada, T., & Kirikae, T. (2022). Spread of Carbapenem-Resistant *Klebsiella pneumoniae* Clinical Isolates Producing NDM-Type Metallo-β-Lactamase in Myanmar. Microbiology Spectrum, 10(4), e00673-22. https://doi.org/10.1128/SPECTRUM.00673-22

Tamma, P. D., Doi, Y., Bonomo, R. A., Johnson, J. K., & Simner, P. J. (2019). A Primer on AmpC β-Lactamases: Necessary Knowledge for an Increasingly Multidrug-resistant World. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 69(8), 1446. https://doi.org/10.1093/CID/CIZ173

Tan, Y. H., Arros, P., Berríos-Pastén, C., Wijaya, I., Chu, W. H. W., Chen, Y., Cheam, G., Naim, A. N. M., Marcoleta, A. E., Ravikrishnan, A., Nagarajan, N., Lagos, R., & Gan, Y. H. (2024). Hypervirulent *Klebsiella pneumoniae* employs genomic island encoded toxins against bacterial competitors in the gut. The ISME Journal, 18(1), wrae054. https://doi.org/10.1093/ISMEJO/WRAE054

Tang, Y., Du, P., Du, C., Yang, P., Shen, N., Russo, T. A., & Liu, C. (2025). Genomically defined hypervirulent *Klebsiella pneumoniae* contributed to early-onset increased mortality. Nature Communications 2025 16:1, 16(1), 1–11. https://doi.org/10.1038/s41467-025-57379-4

Tanni, A. A., Hasan, M. M., Sultana, N., Ahmed, W., & Mannan, A. (2021). Prevalence and molecular characterization of antibiotic resistance and associated genes in *Klebsiella pneumoniae* isolates: A clinical observational study in different hospitals in Chattogram, Bangladesh. PLoS ONE, 16(9), e0257419. https://doi.org/10.1371/JOURNAL.PONE.0257419

Tanni, A. A., Sultana, N., Ahmed, W., Hasan, M. M., Hossain, M. S., Noyon, S. H., Hossain, M. M., & Mannan, A. (2022). Investigating Antimicrobial Resistance and ESBL Producing Gene in *Klebsiella* Isolates among Neonates and Adolescents in Southern Bangladesh. Canadian Journal of Infectious Diseases and Medical Microbiology, 2022. https://doi.org/10.1155/2022/7071009

Tanni, F. Y., Rahman Chowdhury, M. S., Hossain, H., Faysal, M. A., Rahman, M. A., Al Emon, A., Asha, M. N., Hossain, M. M., & Rahman, M. M. (2025). Prevalence and antimicrobial resistance of extended spectrum beta-lactamase (ESBL) producing *Klebsiella* spp. in poultry meat. Heliyon, 11(1). https://doi.org/10.1016/j.heliyon.2025.e41748

Tesfa, T., Mitiku, H., Edae, M., & Assefa, N. (2022). Prevalence and incidence of carbapenem-resistant K. pneumoniae colonization: systematic review and meta-analysis. Systematic Reviews, 11(1), 240. https://doi.org/10.1186/S13643-022-02110-3

The World Bank. (2016). By 2050, drug-resistant infections could cause global economic damage on par with 2008 financial crisis. Accessed on 30 August 2024. https://www.worldbank.org/en/news/press-release/2016/09/18/by-2050-drug-resistant-infections-could-cause-global-economic-damage-on-par-with-2008- financial-crisis.

Thorpe, H. A., Booton, R., Kallonen, T., Gibbon, M. J., Couto, N., Passet, V., López–Fernández, S., Rodrigues, C., Matthews, L., Mitchell, S., Reeve, R., David, S., Merla, C., Corbella, M., Ferrari, C., Comandatore, F., Marone, P., Brisse, S., Sassera, D., … Feil, E. J. (2022). A large–scale genomic snapshot of *Klebsiella* spp. isolates in Northern Italy reveals limited transmission between clinical and non–clinical settings. Nature Microbiology, 7(12), 2054–2067.https://doi.org/10.1038/S41564–022–01263–0

Tian, C., Zhao, Y., Dong, S., You, Z., Chen, J., Xu, H., Fang, Y., & Zhang, Y. (2025). Emergence and characterization of a ST852 *Klebsiella quasipneumoniae* clinical isolate coharboring bla NDM-1 and bla KPC-2 in China. Frontiers in Cellular and Infection Microbiology, 15, 1564277. https://doi.org/10.3389/FCIMB.2025.1564277/BIBTEX

Tsitsos, A., Damianos, A., Kiskinis, K., Tsiouris, V., Tirodimos, I., Soultos, N., Papa, A., & Economou, V. (2025). Prevalence, Characterization, and Proteomic Relatedness Among β-Lactam-Resistant Bacteria Throughout the Poultry Production Chain in Greece. Foods, 14(2), 224. https://doi.org/10.3390/FOODS14020224/S1

Tzouvelekis, L. S., Markogiannakis, A., Psichogiou, M., Tassios, P. T., & Daikos, G. L. (2012). Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: An evolving crisis of global dimensions. Clinical Microbiology Reviews, 25(4), 682–707. https://doi.org/10.1128/CMR.05035-11

Umair, M., Hassan, B., Farzana, R., Ali, Q., Sands, K., Mathias, J., Afegbua, S., Haque, M. N., Walsh, T. R., & Mohsin, M. (2023). International manufacturing and trade in colistin, its implications in colistin resistance and One Health global policies: a microbiological, economic, and anthropological study. The Lancet Microbe, 4(4), e264–e276. https://doi.org/10.1016/S2666-5247(22)00387-1

UN. (2015). Global sustainable development report 2015 (Advance unedited version). Accessed on 10 February 2025. https://sdgs.un.org/publications/global-sustainable-development-report-2015-advance-unedited-version-gsdr-2015-17874

UNEP. (2023). Bracing for superbugs: Strengthening environmental action in the One Health response to antimicrobial resistance. Accessed on 10 February 2025. https://www.unep.org/resources/superbugs/environmental-action

UNICEF & WaterAid. (2025). 230 tons of faecal waste end up in open water bodies in Dhaka daily - UNICEF and WaterAid call for stronger sanitation management. Accessed on 10 August 2025. https://www.unicef.org/bangladesh/en/press-releases/230-tons-fecal-waste-end-open-water-bodies-bangladesh-daily-unicef-and-wateraid-call

UNICEF. (2024). Bangladesh Situation Report on Cyclone Remal and Floods in Bangladesh No. 1 11 July 2024. Accessed on 15 August 2025. https://www.unicef.org/media/159221/file/Bangladesh-Humanitarian-SitRep-Cyclone-REMAL-Floods-11-July-2024.pdf

UNICEF & WHO. (2021). The measurement and monitoring of water supply, sanitation and hygiene (WASH) affordability: a missing element of monitoring of Sustainable Development Goal (SDG) Targets 6.1 and 6.2. New York: United Nations Children's Fund (UNICEF) and the World Health Organization, 2021. Accessed on 15 August 2025. https://washdata.org/sites/default/files/2021-05/unicef-who-2021-affordability-of-wash-services-full.pdf

UN. (2024). UNData app. Accessed on 10 February 2025. https://data.un.org/en/iso/bd

Verburg, I., Hernández Leal, L., Waar, K., Rossen, J. W. A., Schmitt, H., & García-Cobos, S. (2024). *Klebsiella pneumoniae* species complex: From wastewater to the environment. One Health, 19, 100880. https://doi.org/10.1016/J.ONEHLT.2024.100880

Von Wintersdorff, C. J. H., Penders, J., Van Niekerk, J. M., Mills, N. D., Majumder, S., Van Alphen, L. B., Savelkoul, P. H. M., & Wolffs, P. F. G. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Frontiers in Microbiology, 7(FEB), 174871. https://doi.org/10.3389/FMICB.2016.00173/FULL

Waksman, S. A. (1947). What is an antibiotic or an antibiotic substance? Mycologia, 39(5), 565–569. https://doi.org/10.2307/3755219

Waksman, S. A., & Flynn, J. E. (1973). History of the Word 'Antibiotic.' Journal of the History of Medicine and Allied Sciences, XXVIII(3), 284–286. https://doi.org/10.1093/JHMAS/XXVIII.3.284

Walker, K. A., & Miller, V. L. (2020). The intersection of capsule gene expression, hypermucoviscosity and hypervirulence in *Klebsiella pneumoniae*. Current Opinion in Microbiology, 54, 95. https://doi.org/10.1016/J.MIB.2020.01.006

Walker, K. A., Miner, T. A., Palacios, M., Trzilova, D., Frederick, D. R., Broberg, C. A., Sepúlveda, V. E., Quinn, J. D., & Miller, V. L. (2019). A *Klebsiella pneumoniae* regulatory mutant has reduced capsule expression but retains hypermucoviscosity. MBio, 10(2), 1–16. https://doi.org/10.1128/MBIO.00089-19

Walsh, T. R. (2018). A one-health approach to antimicrobial resistance. Nature Microbiology, 3(8), 854–855. https://doi.org/10.1038/S41564-018-0208-5

Wang, C. Z., Gao, X., Yang, Q. W., Lv, L. C., Wan, M., Yang, J., Cai, Z. P., & Liu, J. H. (2021). A novel transferable resistance-nodulation-division pump Gene Cluster, tmexCD2-toprJ2, Confers Tigecycline Resistance in *Raoultella ornithinolytica*. Antimicrobial Agents and Chemotherapy, 65(4). https://doi.org/10.1128/AAC.02229-20

Wang, C., Feng, Y., Liu, L., Wei, L., Kang, M., & Zong, Z. (2020). Identification of novel mobile colistin resistance gene *mcr-10*. Emerging Microbes & Infections, 9(1), 508. https://doi.org/10.1080/22221751.2020.1732231

Wang, G., Zhao, G., Chao, X., Xie, L., & Wang, H. (2020). The Characteristic of Virulence, Biofilm and Antibiotic Resistance of *Klebsiella pneumoniae*. International Journal of Environmental Research and Public Health, 17(17), 6278. https://doi.org/10.3390/IJERPH17176278

Wang, N., Zhan, M., Liu, J., Wang, Y., Hou, Y., Li, C., Li, J., Han, X., Liu, J., Chen, Y., Fan, J., Tang, J., Lu, W., Zhong, X., Zhang, Z., & Zhang, W. (2022). Prevalence of Carbapenem–Resistant *Klebsiella pneumoniae* Infection in a Northern Province in China: Clinical Characteristics, Drug Resistance, and Geographic Distribution. Infection and Drug Resistance, 15, 569–579. https://doi.org/10.2147/IDR.S347343

Wang, X., Wang, Y., Zhou, Y., Li, J., Yin, W., Wang, S., Zhang, S., Shen, J., Shen, Z., & Wang, Y. (2018). Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. Emerging Microbes & Infections, 7(1), 122. https://doi.org/10.1038/S41426-018-0124-Z

Wang, Y., Xu, C., Zhang, R., Chen, Y., Shen, Y., Hu, F., Liu, D., Lu, J., Guo, Y., Xia, X., Jiang, J., Wang, X., Fu, Y., Yang, L., Wang, J., Li, J., Cai, C., Yin, D., Che, J., … Shen, J. (2020). Changes in colistin resistance and *mcr-1* abundance in *Escherichia coli* of animal and human origins following the ban of colistin-positive additives in China: an epidemiological comparative study. The Lancet Infectious Diseases, 20(10), 1161–1171. https://doi.org/10.1016/S1473-3099(20)30149-3

Wang, Y., Zhou, J., Liu, H., Wang, Q., Zhang, P., Zhu, J., Zhao, D., Wu, X., Yu, Y., & Jiang, Y. (2023). Emergence of high-level colistin resistance mediated by multiple determinants, including *mcr-1.1*, *mcr-*

8.2 and *crrB* mutations, combined with tigecycline resistance in an ST656 *Klebsiella pneumoniae*. Frontiers in Cellular and Infection Microbiology, 13. https://doi.org/10.3389/FCIMB.2023.1122532

Wantuch, P. L., Knoot, C. J., Robinson, L. S., Vinogradov, E., Scott, N. E., Harding, C. M., & Rosen, D. A. (2024). Heptavalent O-Antigen Bioconjugate Vaccine Exhibiting Differential Functional Antibody Responses Against Diverse *Klebsiella pneumoniae* Isolates. The Journal of Infectious Diseases, 230(3), 578–589. https://doi.org/10.1093/INFDIS/JIAE097

Wesevich, A., Sutton, G., Ruffin, F., Park, L. P., Fouts, D. E., Fowler, V. G., & Thaden, J. T. (2020). Newly Named *Klebsiella aerogenes* (formerly Enterobacter aerogenes) Is Associated with Poor Clinical Outcomes Relative to Other Enterobacter Species in Patients with Bloodstream Infection. Journal of Clinical Microbiology, 58(9), e00582-20. https://doi.org/10.1128/JCM.00582-20

WHO. (2024). Antimicrobial Resistance, Hypervirulent *Klebsiella pneumoniae* - Global situation. Accessed on 20 August 2025.

WHO. (2016). Guidelines on core components of infection prevention and control programmes at the national and acute health care facility level. Accessed on 30 August 2025. https://www.who.int/publications/i/item/9789241549929

WHO. (2017). Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, Acinetobacter baumannii and Pseudomonas aeruginosa in health care facilities. Accessed on 30 August 2025. https://www.who.int/publications/i/item/guidelines-for-the-prevention-and-control-of-carbapenem-resistant-enterobacteriaceae-acinetobacter-baumannii-and-pseudomonas-aeruginosa-in-health-care-facilities

WHO. (2018). Global guidelines for the prevention of surgical site infection. Accessed on 30 August 2025. https://www.who.int/publications/i/item/9789241550475

WHO. (2019). Antimicrobial stewardship programmes in health-care facilities in low - and middle-income countries. Accessed on 30 August 2025. https://www.who.int/publications/i/item/9789241515481

WHO. (2022). Antimicrobial resistance TrACSS: Bangladesh 2022 country profile. Accessed on 10 February 2025. https://www.who.int/publications/m/item/antimicrobial-resistance-tracss-bgd-2022-country-profile

WHO. (2022). TrACSS report: Bangladesh 2022. Accessed on 10 February 2025. https://cdn.who.int/media/docs/default-source/antimicrobial-resistance/amr-spc-npm/tracss/2022/tracss-2022-bangladesh.pdf

WHO. (2023). Infection prevention and control. Accessed on 30 August 2025. https://www.who.int/teams/integrated-health-services/infection-prevention-control/hand-hygiene

WHO. (2023). Antimicrobial resistance. Accessed on 10 February 2025. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

WHO. (2024). Antimicrobial Resistance, Hypervirulent *Klebsiella pneumoniae* - Global situation. Accessed on 10 February 2025. https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON527

WHO. (2024). Guidance on wastewater and solid waste management for manufacturing of antibiotics. Accessed on 30 August 2025. https://www.who.int/publications/i/item/9789240097254

WHO. (2024). WHO releases guidance on gender inequalities and antimicrobial resistance. Accessed on 10 February 2025. https://www.who.int/news/item/16-09-2024-who-releases-guidance-on-gender-inequalities-and-antimicrobial-resistance

WHO. (2025). Global Antimicrobial Resistance and Use Surveillance System (GLASS) report: Antibiotic use data for 2022. Accessed on 10 February 2025. https://www.who.int/publications/i/item/9789240108127

WHO. (2017). Integrated surveillance of antimicrobial resistance in foodborne bacteria: application of a one health approach: guidance from the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR). Accessed on 10 February 2025. https://www.who.int/publications/i/item/9789241512411

WHO. (2023). WASH in health care facilities 2023 data update: special focus on primary health care. Accessed on 15 August 2025. https://cdn.who.int/media/docs/default-source/wash-documents/wash-coverage/jmp/jmp-2023_hcf_launch.pdf

Wick, R. R., Judd, L. M., Gorrie, C. L., & Holt, K. E. (2017). Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS computational biology, 13(6), e1005595. https://doi.org/10.1371/journal.pcbi.1005595

Wick, R. R., Heinz, E., Holt, K. E., & Wyres, K. L. (2018). Kaptive web: User-Friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* genomes. Journal of Clinical Microbiology, 56(6). https://doi.org/10.1128/JCM.00197-18,

Wick, R., Holt, K., marlam89, kelwyres, Maranga, M., III, R. A. P., Silva, A. G. da, Deveaud, E., Lampa, S., & Stanton, T. (2024). klebgenomics/Kleborate: v3.0.0. https://doi.org/10.5281/ZENODO.13683348

Wong, M. H. Y., Chan, E. W. C., & Chen, S. (2015). Evolution and dissemination of OqxAB-like efflux pumps, an emerging quinolone resistance determinant among members of Enterobacteriaceae. Antimicrobial Agents and Chemotherapy, 59(6), 3290–3297. https://doi.org/10.1128/AAC.00310-15,

Wu, B., Wang, Y., Ling, Z., Yu, Z., Shen, Z., Zhang, S., & Wang, X. (2021). Heterogeneity and diversity of mcr-8 genetic context in chicken-associated *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy, 65(1). https://doi.org/10.1128/AAC.01872-20/SUPPL_FILE/AAC.01872-20-S0001.PDF

Wyres, K. L., & Holt, K. E. (2016). *Klebsiella pneumoniae* Population Genomics and Antimicrobial-Resistant Clones. Trends in Microbiology, 24(12), 944–956. https://doi.org/10.1016/J.TIM.2016.09.007

Wyres, K. L., & Holt, K. E. (2018). *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Current Opinion in Microbiology, 45, 131–139. https://doi.org/10.1016/J.MIB.2018.04.004

Wyres, K. L., Lam, M. M. C., & Holt, K. E. (2020). Population genomics of *Klebsiella pneumoniae*. Nature Reviews Microbiology, 18(6), 344–359. https://doi.org/10.1038/S41579-019-0315-1

Wyres, K. L., Nguyen, T. N. T., Lam, M. M. C., Judd, L. M., Van Vinh Chau, N., Dance, D. A. B., Ip, M., Karkey, A., Ling, C. L., Miliya, T., Newton, P. N., Lan, N. P. H., Sengduangphachanh, A., Turner, P., Veeraraghavan, B., Vinh, P. V., Vongsouvath, M., Thomson, N. R., Baker, S., & Holt, K. E. (2020). Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. Genome Medicine, 12(1), 1–16. https://doi.org/10.1186/S13073-019-0706-Y/TABLES/5

Wyres, K. L., Wick, R. R., Judd, L. M., Froumine, R., Tokolyi, A., Gorrie, C. L., Lam, M. M. C., Duchêne, S., Jenney, A., & Holt, K. E. (2019). Distinct evolutionary dynamics of horizontal gene transfer in drug resistant and virulent clones of *Klebsiella pneumoniae*. PLoS Genetics, 15(4). https://doi.org/10.1371/JOURNAL.PGEN.1008114

Xu, L., Li, J., Wu, W., Wu, X., & Ren, J. (2024). *Klebsiella pneumoniae* capsular polysaccharide: Mechanism in regulation of synthesis, virulence, and pathogenicity. Virulence, 15(1), 2439509. https://doi.org/10.1080/21505594.2024.2439509

Xu, Y., Chen, Q., Zhao, C., Ma, X., Wang, L., Qian, F., Guan, X., Du, H., & Zhang, H. (2025). A novel mutation of *ramR* involved in tigecycline resistance in *Klebsiella pneumoniae*. Microbiology Spectrum, 13(6), e03204-24. https://doi.org/10.1128/SPECTRUM.03204-24

Yang, F., Liu, F. Y., & Zhong, Y. M. (2024). Comparative Genomics Revealing the Genomic Characteristics of *Klebsiella variicola* Clinical Isolates in China. Tropical Medicine and Infectious Disease, 9(8), 180. https://doi.org/10.3390/TROPICALMED9080180/S1

Yang, J., Long, H., Hu, Y., Feng, Y., McNally, A., & Zong, Z. (2021). *Klebsiella oxytoca* Complex: Update on Taxonomy, Antimicrobial Resistance, and Virulence. Clinical Microbiology Reviews, 35(1), e00006-21. https://doi.org/10.1128/CMR.00006-21

Yang, X., Liu, L., Wang, Z., Bai, L., & Li, R. (2019). Emergence of *mcr-8.2*-bearing *Klebsiella quasipneumoniae* of animal origin. Journal of Antimicrobial Chemotherapy, 74(9), 2814–2817. https://doi.org/10.1093/JAC/DKZ213

Yang, X., Liu, X., Xu, Y., Yang, C., Chan, E. W. C., Shum, H. P., & Chen, S. (2022). Genetic and Functional Characterization of a Conjugative KpVP-2-Type Virulence Plasmid From a Clinical *Klebsiella pneumoniae* Strain. Frontiers in Microbiology, 13, 914884. https://doi.org/10.3389/FMICB.2022.914884/BIBTEX

Yang, Y. Q., Li, Y. X., Lei, C. W., Zhang, A. Y., & Wang, H. N. (2018). Novel plasmid-mediated colistin resistance gene mcr–7.1 in *Klebsiella pneumoniae*. Journal of Antimicrobial Chemotherapy, 73(7), 1791–1795. https://doi.org/10.1093/JAC/DKY111

Yao, H., Qin, S., Chen, S., Shen, J., & Du, X. D. (2018). Emergence of carbapenem-resistant hypervirulent *Klebsiella pneumoniae*. The Lancet Infectious Diseases, 18(1), 25. https://doi.org/10.1016/S1473-3099(17)30628-X

Yasmeen, N., Aslam, B., Fang, L. X., Baloch, Z., & Liu, Y. (2023). Occurrence of extended–spectrum β-lactamase harbouring *K. pneumoniae* in various sources: a one health perspective. Frontiers in Cellular and Infection Microbiology, 13, 1103319. https://doi.org/10.3389/FCIMB.2023.1103319/FULL

Yigit, H., Queenan, A. M., Anderson, G. J., Domenech-Sanchez, A., Biddle, J. W., Steward, C. D., Alberti, S., Bush, K., & Tenover, F. C. (2001). Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy, 45(4), 1151–1161. https://doi.org/10.1128/AAC.45.4.1151-1161.2001

Yin, J. H., Kelly, P. J., & Wang, C. (2022). Flies as Vectors and Potential Sentinels for Bacterial Pathogens and Antimicrobial Resistance: A Review. Veterinary Sciences, 9(6), 300. https://doi.org/10.3390/VETSCI9060300

Yin, Q., da Silva, A. C., Zorrilla, F., Almeida, A. S., Patil, K. R., & Almeida, A. (2025). Ecological dynamics of Enterobacteriaceae in the human gut microbiome across global populations. Nature Microbiology, 10(2), 541–553. https://doi.org/10.1038/S41564-024-01912-6

Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., & Walsh, T. R. (2009). Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrobial Agents and Chemotherapy, 53(12), 5046–5054. https://doi.org/10.1128/AAC.00774-09

Yu, K., Huang, Z., Xiao, Y., Gao, H., Bai, X., & Wang, D. (2024). Global spread characteristics of CTX-M-type extended-spectrum β-lactamases: A genomic epidemiology analysis. Drug Resistance Updates, 73, 101036. https://doi.org/10.1016/J.DRUP.2023.101036

Yu, Y., Andrey, D. O., Yang, R. S., Sands, K., Tansawai, U., Li, M., Portal, E., Gales, A. C., Niumsup, P. R., Sun, J., Liao, X., Liu, Y. H., & Walsh, T. R. (2020). A *Klebsiella pneumoniae* strain co-harbouring *mcr-1* and *mcr-3* from a human in Thailand. Journal of Antimicrobial Chemotherapy, 75(8), 2372–2374. https://doi.org/10.1093/JAC/DKAA133

Zamudio, R., Yap, M., Bloomfield, S. J., Palau, R., Janecko, N., & Mather, A. E. (2026). Genomics uncover resistant and virulent *Klebsiella* on foods: a potential risk to human health. Food Microbiology, 133, 104899. https://doi.org/10.1016/J.FM.2025.104899

Zhai, W., Tian, Y., Lu, M., Zhang, M., Song, H., Fu, Y., Ma, T., Sun, C., Bai, L., Wang, Y., Liu, D., & Zhang, Y. (2022). Presence of Mobile Tigecycline Resistance Gene *tet(X4)* in Clinical *Klebsiella pneumoniae*. Microbiology Spectrum, 10(1), e01081-21. https://doi.org/10.1128/SPECTRUM.01081-21

Zhang, P., *et al.* (2023). Environmental dissemination of carbapenem-resistant *Klebsiella pneumoniae*: A systematic review. Science of the Total Environment, 867, 161437.

Zhang, X. W., Huang, X. Y., Zhou, Z. Y., Li, B. L., Lu, J. H., Song, J. J., & Li, X. Y. (2025). Genetic framework and evolutionary dynamics of *mcr*-positive *Klebsiella pneumoniae* from 2000 to 2023. International Journal of Antimicrobial Agents, 66(3). https://doi.org/10.1016/j.ijantimicag.2025.107533

Zhang, Y., Zhao, C., Wang, Q., Wang, X., Chen, H., Li, H., Zhang, F., Li, S., Wang, R., & Wang, H. (2016). High Prevalence of Hypervirulent *Klebsiella pneumoniae* Infection in China: Geographic Distribution, Clinical Characteristics, and Antimicrobial Resistance. Antimicrobial Agents and Chemotherapy, 60(10), 6115. https://doi.org/10.1128/AAC.01127-16

Zhao, J., Li, Z., Zhang, Y., Liu, X., Lu, B., & Cao, B. (2022). Convergence of MCR-8.2 and Chromosome-Mediated Resistance to Colistin and Tigecycline in an NDM-5-Producing ST656 *Klebsiella pneumoniae* Isolate From a Lung Transplant Patient in China. Frontiers in Cellular and Infection Microbiology, 12, 922031. https://doi.org/10.3389

Zhao, Q., Guo, L., Wang, L. F., Zhao, Q., & Shen, D. X. (2020). Prevalence and characteristics of surgical site hypervirulent *Klebsiella pneumoniae* isolates. Journal of Clinical Laboratory Analysis, 34(9), e23364. https://doi.org/10.1002/JCLA.23364

Zhao, Y., Zhang, X., Torres, V. V. L., Liu, H., Rocker, A., Zhang, Y., Wang, J., Chen, L., Bi, W., Lin, J., Strugnell, R. A., Zhang, S., Lithgow, T., Zhou, T., & Cao, J. (2019). An Outbreak of Carbapenem-Resistant and Hypervirulent *Klebsiella pneumoniae* in an Intensive Care Unit of a Major Teaching Hospital in Wenzhou, China. Frontiers in Public Health, 7, 229. https://doi.org/10.3389/FPUBH.2019.00229/FULL

Zheng, Z., Gorden, P. J., Xia, X., Zheng, Y., & Li, G. (2022). Whole-genome analysis of *Klebsiella pneumoniae* from bovine mastitis milk in the U.S. Environmental Microbiology, 24(3), 1183–1199. https://doi.org/10.1111/1462-2920.15721

Zhou, X., Wagh, K., Lv, G., Sharma, D., & Lei, W. (2024). Genome drafting of nosocomial infection CRE *Klebsiella pneumoniae* confirming resistance to colistin and eravacycline, carrying blaNDM-1, mcr-1, and blaKPC-2, in neonatology from November to December 2023. Frontiers in Cellular and Infection Microbiology, 14. https://doi.org/10.3389/FCIMB.2024.1528017/PDF

Zhou, Y., Farzana, R., Sihalath, S., Rattanavong, S., Vongsouvath, M., Mayxay, M., Sands, K., Newton, P. N., Dance, D. A. B., Hassan, B., & Walsh, T. R. (2022). A One-Health Sampling Strategy to Explore the Dissemination and Relationship Between Colistin Resistance in Human, Animal, and Environmental Sectors in Laos. Engineering, 15, 45–56. https://doi.org/10.1016/J.ENG.2022.01.013

Zhou, Z., Alikhan, N. F., Sergeant, M. J., Luhmann, N., Vaz, C., Francisco, A. P., Carriço, J. A., & Achtman, M. (2018). Grapetree: Visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Research, 28(9), 1395–1404. https://doi.org/10.1101/GR.232397.117

Zhu, J., Jiang, X., Zhao, L., & Li, M. (2022). An Outbreak of ST859-K19 Carbapenem-Resistant Hypervirulent *Klebsiella pneumoniae* in a Chinese Teaching Hospital. MSystems, 7(3). https://doi.org/10.1128/MSYSTEMS.01297-21

Zhu, J., Wang, T., Chen, L., & Du, H. (2021). Virulence Factors in Hypervirulent *Klebsiella pneumoniae*. Frontiers in Microbiology, 12, 642484. https://doi.org/10.3389/FMICB.2021.642484

Zhu, W., Wang, X., Qin, J., Liang, W., Shen, Z., & Gales, A. C. (2020). Dissemination and Stability of the blaNDM-5-Carrying IncX3-Type Plasmid among Multiclonal *Klebsiella pneumoniae* Isolates. MSphere, 5(6). https://doi.org/10.1128/MSPHERE.00917-20

Zong, Z., *et al.* (2021). Foodborne transmission of antimicrobial-resistant *Klebsiella pneumoniae*: A neglected route. Frontiers in Microbiology, 12, 673460.

Appendices

Appendix A

Summary of studies on *Klebsiella* spp. in Bangladesh identified in the scoping review (2015–present).

Article attributes	**Study location in Bangladesh**	**Species and sequence type (ST)**	**Sample source**	**Study year(s)**	**Methods (AST/PCR/WGS)**	**Key resistance/virulence findings**
Toleman *et al*., 2015; PMID:25989320	Dhaka	*K. pneumoniae*	Environmental waters (rivers, lakes, drains, ponds, etc.)	2012	AST, PCR, genotyping by pulsed-field gel electrophoresis	Multiple GNB, including *K. pneumoniae,* carried $bla_{\text{NDM-1}}$.
Islam *et al*., 2017; PMID:28526792	Dhaka	*K. pneumoniae*	Environmental water near hospitals vs community areas	2016	AST, PCR	*K. pneumoniae* (44% of $bla_{\text{NDM-1}}$ positive isolates); frequent ESBLs (CTX-M-1/15, TEM, SHV).
Mahmudunnabi *et al*., 2018; PMID:30647708	Sylhet	*K. pneumoniae*	Urinary tract infection (women)	2018	PCR, RFLP	MDR *K. pneumoniae* identified from UTI cases.
Khan *et al*., 2018; PMID:29958064	Mymensingh	*K. pneumoniae*	Clinical isolates	2015-2016	PCR	ESBL/carbapenemase genes in *K. pneumoniae* and *E. coli*; multiple ESBLs and OXA-48-like documented.

Article attributes	**Study location in Bangladesh**	**Species and sequence type (ST)**	**Sample source**	**Study year(s)**	**Methods (AST/PCR/WGS)**	**Key resistance/virulence findings**
Farzana *et al*., 2020; PMID:32161143	Dhaka	*K. pneumoniae* (ST15)	Clinical infections	2017	AST, WGS, plasmid analysis	First Bangladesh report of transferable colistin resistance *mcr-8.1* on IncFIB(pQil) plasmid.
Satter *et al*., 2020; PMID:33116093	Dhaka	*K. oxytoca*	Clinical (urine, wound swab, pus, sputum, blood)	2015	AST, PCR, sequencing	High proportion of AmpC producers among cefoxitin-resistant isolates; dominant AmpC genotypes: CIT and DHA.
Urmi *et al*., 2020; PMID:32903880	Dhaka	*K. pneumoniae*	UTI patients	2016 and 2018	AST, PCR, sequencing	Among 58 UTI isolates: *bla*$_{\text{KPC}}$ 15.5%, *bla*$_{\text{IMP}}$ 10.3%, *bla*$_{\text{NDM-1}}$ 22.4%, *bla*$_{\text{VIM}}$ 19%; genotype-phenotype discrepancies observed.
Okanda *et al*., 2021; PMID: 33519767	Dhaka	*K. pneumoniae* (multiple STs: ST147, ST11, ST14, etc.)	ICU clinical isolates (blood, respiratory, urine, pus)	2015 and 2017	AST, PCR, sequencing	30% carbapenemase-producing *K. pneumoniae*; genes: *bla*$_{\text{NDM-1/5}}$, *bla*$_{\text{OXA-181/232}}$.
Tanni *et al*., 2021; PMID:34506611	Chattogram	*K. pneumoniae*	Multiple clinical specimen types	2019	AST, PCR, sequencing	High MDR; 64% *bla*$_{\text{NDM-1}}$ producers; frequent ESBL genes incl. *bla*$_{\text{SHV-11}}$.

Article attributes	**Study location in Bangladesh**	**Species and sequence type (ST)**	**Sample source**	**Study year(s)**	**Methods (AST/PCR/WGS)**	**Key resistance/virulence findings**
Ferdous *et al*., 2022; PMID: 36036605	Chattogram	*K. quasipneumoniae* (ST6130)	Hospital wastewater	2019	WGS; genome annotation; AMR gene profiling	MDR *K. pneumoniae* detected in wastewater isolate.
Kawser Z & Shamsuzzaman SM, 2022; PMID:35754671	Dhaka	*K. pneumoniae*	Hospital settings	2018-2019	String test for hypermucoidy; AST; PCR	47.7% string-test positive; MDR 34.1%, colistin resistance 31.8%; hypervirulent genes enriched in string-positive isolates.
Hussain *et al*., 2023; PMID: 37303793	Dhaka	*K. pneumoniae* (multiple STs: ST11, ST14, ST15, ST147, ST231, ST307)	Clinical isolates	2019	WGS; resistome and virulome profiling	High-risk MDR and hypervirulent clones circulating, carbapenemases included NDM-5 with OXA-232 or OXA-181.
Mazumder *et al*., 2023; PMID:37435536	Dhaka	*K. aerogenes*	UTI case	2022	Culture and AST; WGS	Extensively drug-resistant profile but susceptible to carbapenems and polymyxins; successful targeted therapy with meropenem.
Tanni *et al*., 2024; PMID: 38940528	Chattogram	*K. pneumoniae*	Clinical isolates	2022	WGS (draft genomes)	MDR *K. pneumoniae* detected.

Article attributes	**Study location in Bangladesh**	**Species and sequence type (ST)**	**Sample source**	**Study year(s)**	**Methods (AST/PCR/WGS)**	**Key resistance/virulence findings**
Kar *et al*., 2024; PMID:39494455	Dhaka	*K. pneumoniae*	Community stool/rectal swabs (adults and children) and household/standing water	2022-2023	AST	High gut carriage (61% children, 81% adults); prevalent in household (64%) and standing water (85%); around 12-14% carbapenem resistance.
Kawser *et al*., 2025; PMID:39725322	Dhaka	*K. pneumoniae*	Clinical infections	2022	AST, WGS	High AMR burden; isolates with both MDR and hypervirulence features.
Tanni *et al*., 2025; PMID:39866402	Sylhet	*K. oxytoca*	Food chain (retail poultry meat)	2023	AST, multiplex PCR	Detected in 4.56% (13/285) poultry meat samples; high phenotypic resistance to multiple agents; bla_{SHV} common (in 77% of *K. oxytoca*).

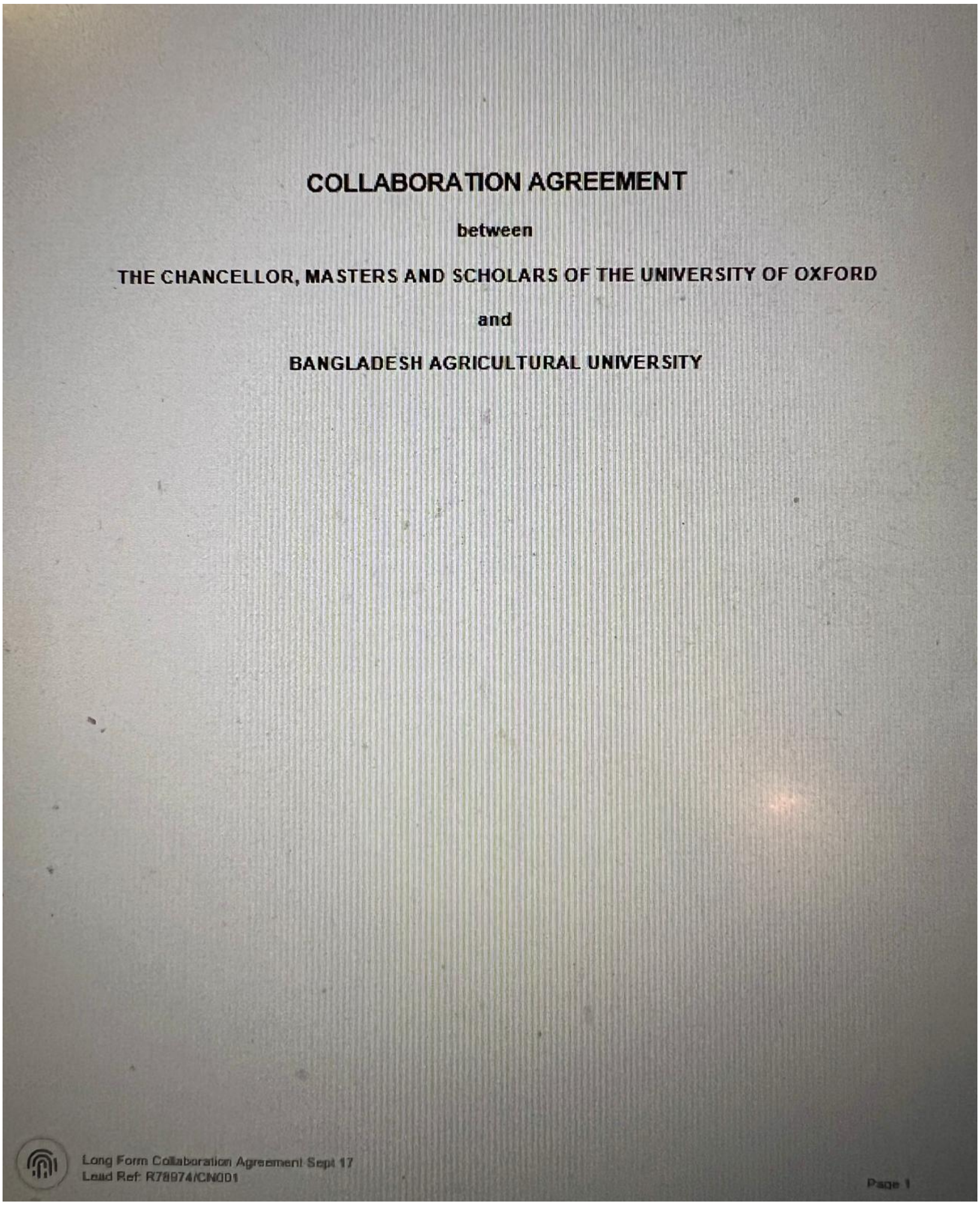
Article attributes	**Study location in Bangladesh**	**Species and sequence type (ST)**	**Sample source**	**Study year(s)**	**Methods (AST/PCR/WGS)**	**Key resistance/virulence findings**
Fahim *et al*., 2025; PMID:40892486	Sylhet	*K. aerogenes*	Food chain (raw milk from dairy farms/milk selling points)	Not stated	AST, PCR	36.4% *Klebsiella*-positive raw milk; isolates closely related to *K. aerogenes*; 100% carried $bla_{\text{TEM,}}$ high resistance to multiple drugs; no *mcr* detected.
Mondol et. al., 2025; PMID: 39966674	Dhaka	*K. pneumoniae* (ST656)	Canal water	Not stated	AST, WGS	MDR *K. pneumoniae* with key resistance genes such as *mcr8.1*, $bla_{\text{LAP-2}}$, $bla_{\text{TEM-1}}$, $bla_{\text{SHV-11}}$ and $bla_{\text{OXA-1}}$.
Kabir *et al*., 2025; PMID:40221642	Dhaka	*K. aerogenes*	Food chain (street food isolate)	Not stated	WGS; virulence gene profiling; infection model (Bombyx mori larvae)	WGS revealed multiple virulence and AMR genes; strain lethal to larvae within 72 h; in vitro ampicillin resistance noted.

PMID, PubMed ID number; AST, antimicrobial susceptibility testing; PCR, polymerase chain reaction; WGS, whole genome sequencing; GNB, Gram-negative bacteria; RFLP, restriction fragment length polymorphism.

Search database: PubMed; Search field: Title/Abstract; Keywords: ‘*Klebsiella*’, ‘*Klebsiella* spp.’, ‘*Klebsiella pneumoniae*’, ‘*Klebsiella quasipneumoniae*’, ‘*Klebsiella variicola*’, ‘*Klebsiella oxytoca*’, ‘*Klebsiella aerogenes*’, ‘Bangladesh’. Reviews and meta-analyses were excluded.

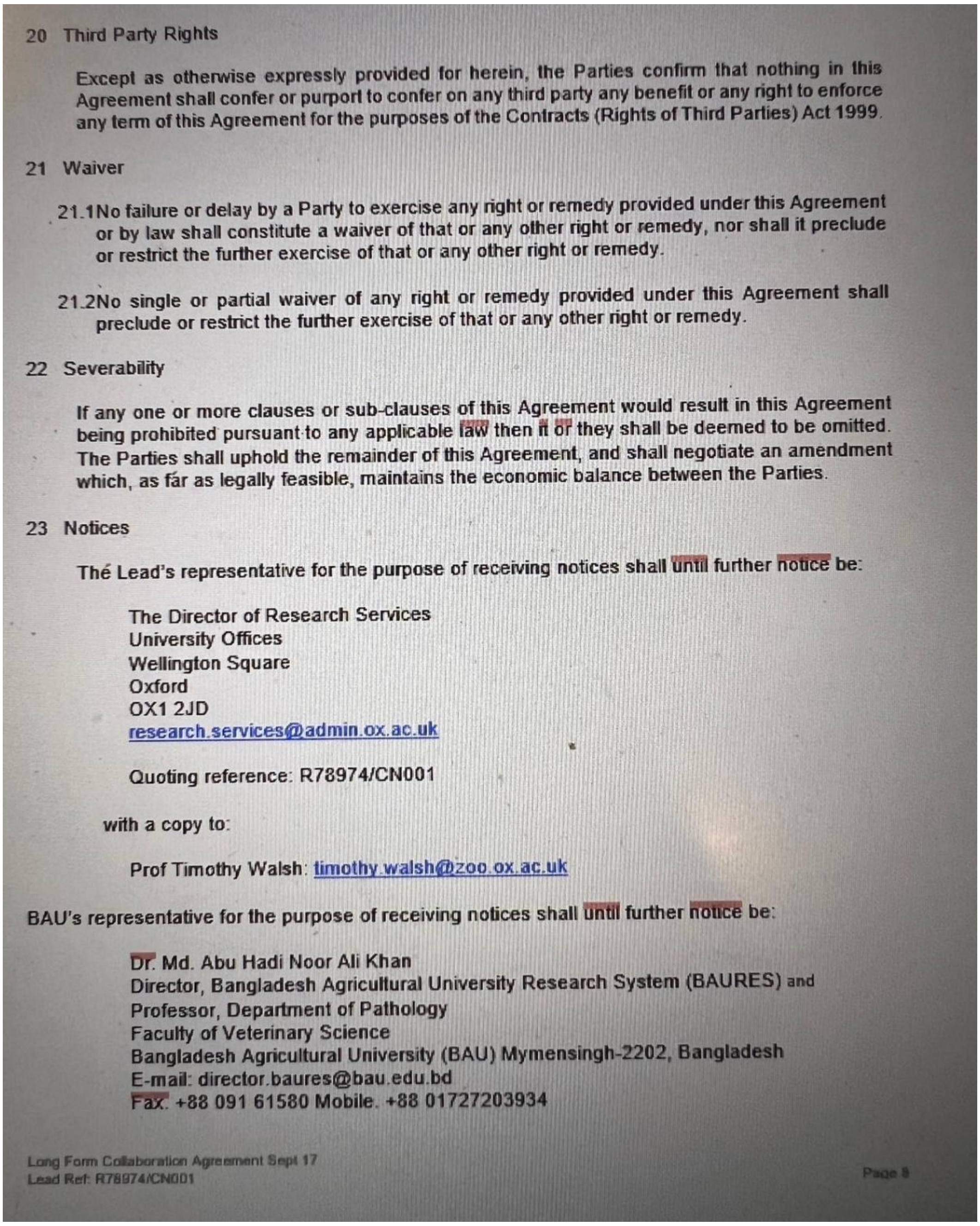
Appendix B

Collaboration agreement between the Chancellor, Masters, and Scholars of the University of Oxford (UOX), UK, and Bangladesh Agricultural University (BAU), Bangladesh [Lead Ref: R78974/CN001]



COLLABORATION AGREEMENT
between
THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF OXFORD
and
BANGLADESH AGRICULTURAL UNIVERSITY

Long Form Collaboration Agreement Sept 17
Lead Ref: R78974/CN001
Page 1



20 Third Party Rights

Except as otherwise expressly provided for herein, the Parties confirm that nothing in this Agreement shall confer or purport to confer on any third party any benefit or any right to enforce any term of this Agreement for the purposes of the Contracts (Rights of Third Parties) Act 1999.

21 Waiver

21.1 No failure or delay by a Party to exercise any right or remedy provided under this Agreement or by law shall constitute a waiver of that or any other right or remedy, nor shall it preclude or restrict the further exercise of that or any other right or remedy.

21.2 No single or partial waiver of any right or remedy provided under this Agreement shall preclude or restrict the further exercise of that or any other right or remedy.

22 Severability

If any one or more clauses or sub-clauses of this Agreement would result in this Agreement being prohibited pursuant to any applicable law then it or they shall be deemed to be omitted. The Parties shall uphold the remainder of this Agreement, and shall negotiate an amendment which, as far as legally feasible, maintains the economic balance between the Parties.

23 Notices

The Lead's representative for the purpose of receiving notices shall until further notice be:

The Director of Research Services
University Offices
Wellington Square
Oxford
OX1 2JD
research.services@admin.ox.ac.uk

Quoting reference: R78974/CN001

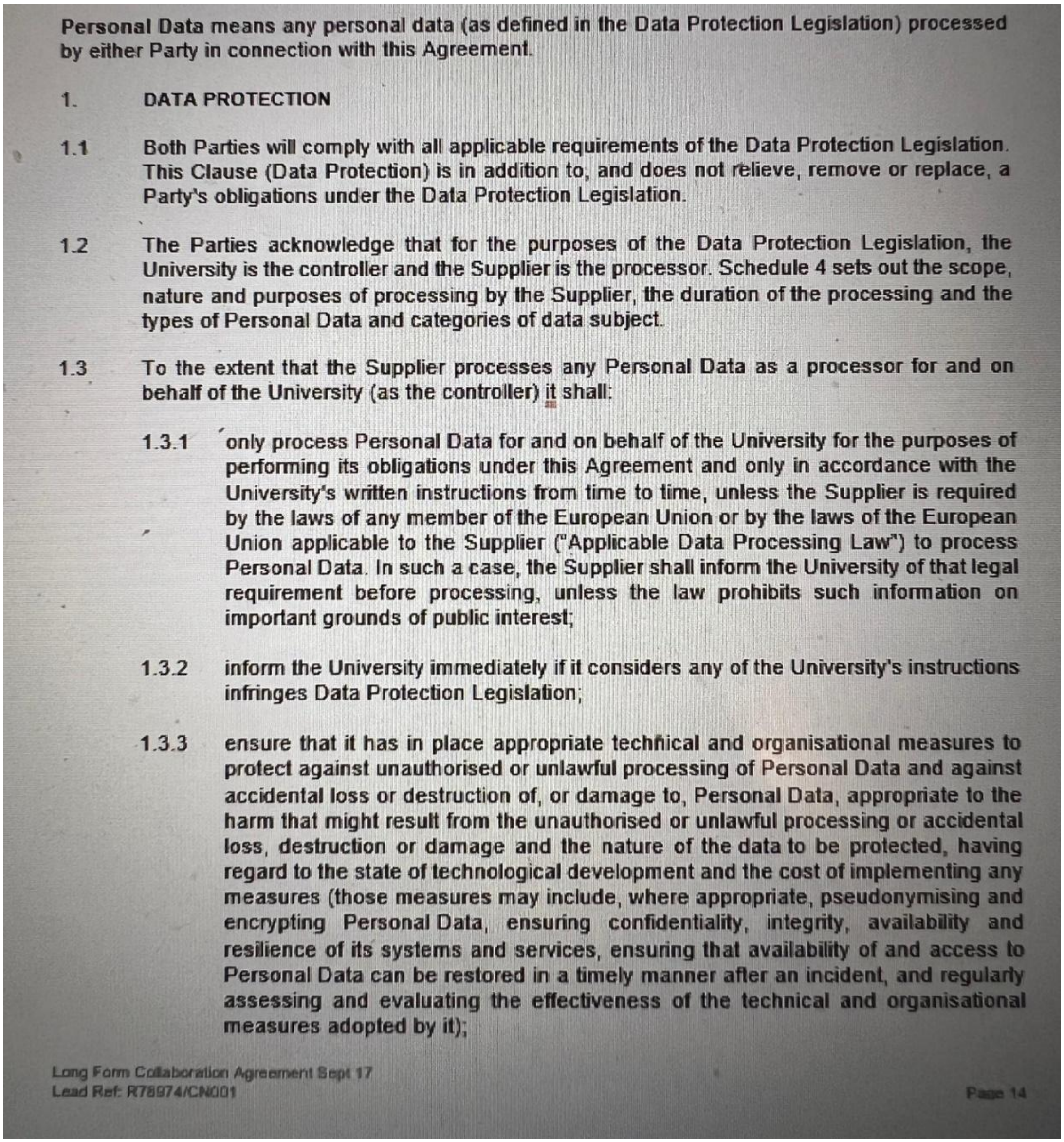
with a copy to:

Prof Timothy Walsh: timothy.walsh@zoo.ox.ac.uk

BAU's representative for the purpose of receiving notices shall until further notice be:

Dr. Md. Abu Hadi Noor Ali Khan
Director, Bangladesh Agricultural University Research System (BAURES) and
Professor, Department of Pathology
Faculty of Veterinary Science
Bangladesh Agricultural University (BAU) Mymensingh-2202, Bangladesh
E-mail: director.baures@bau.edu.bd
Fax: +88 091 61580 Mobile: +88 01727203934

Lead Ref: R78974/CN001



Personal Data means any personal data (as defined in the Data Protection Legislation) processed by either Party in connection with this Agreement.

1. **DATA PROTECTION**

1.1 Both Parties will comply with all applicable requirements of the Data Protection Legislation. This Clause (Data Protection) is in addition to, and does not relieve, remove or replace, a Party's obligations under the Data Protection Legislation.

1.2 The Parties acknowledge that for the purposes of the Data Protection Legislation, the University is the controller and the Supplier is the processor. Schedule 4 sets out the scope, nature and purposes of processing by the Supplier, the duration of the processing and the types of Personal Data and categories of data subject.

1.3 To the extent that the Supplier processes any Personal Data as a processor for and on behalf of the University (as the controller) it shall:

1.3.1 only process Personal Data for and on behalf of the University for the purposes of performing its obligations under this Agreement and only in accordance with the University's written instructions from time to time, unless the Supplier is required by the laws of any member of the European Union or by the laws of the European Union applicable to the Supplier ("Applicable Data Processing Law") to process Personal Data. In such a case, the Supplier shall inform the University of that legal requirement before processing, unless the law prohibits such information on important grounds of public interest;

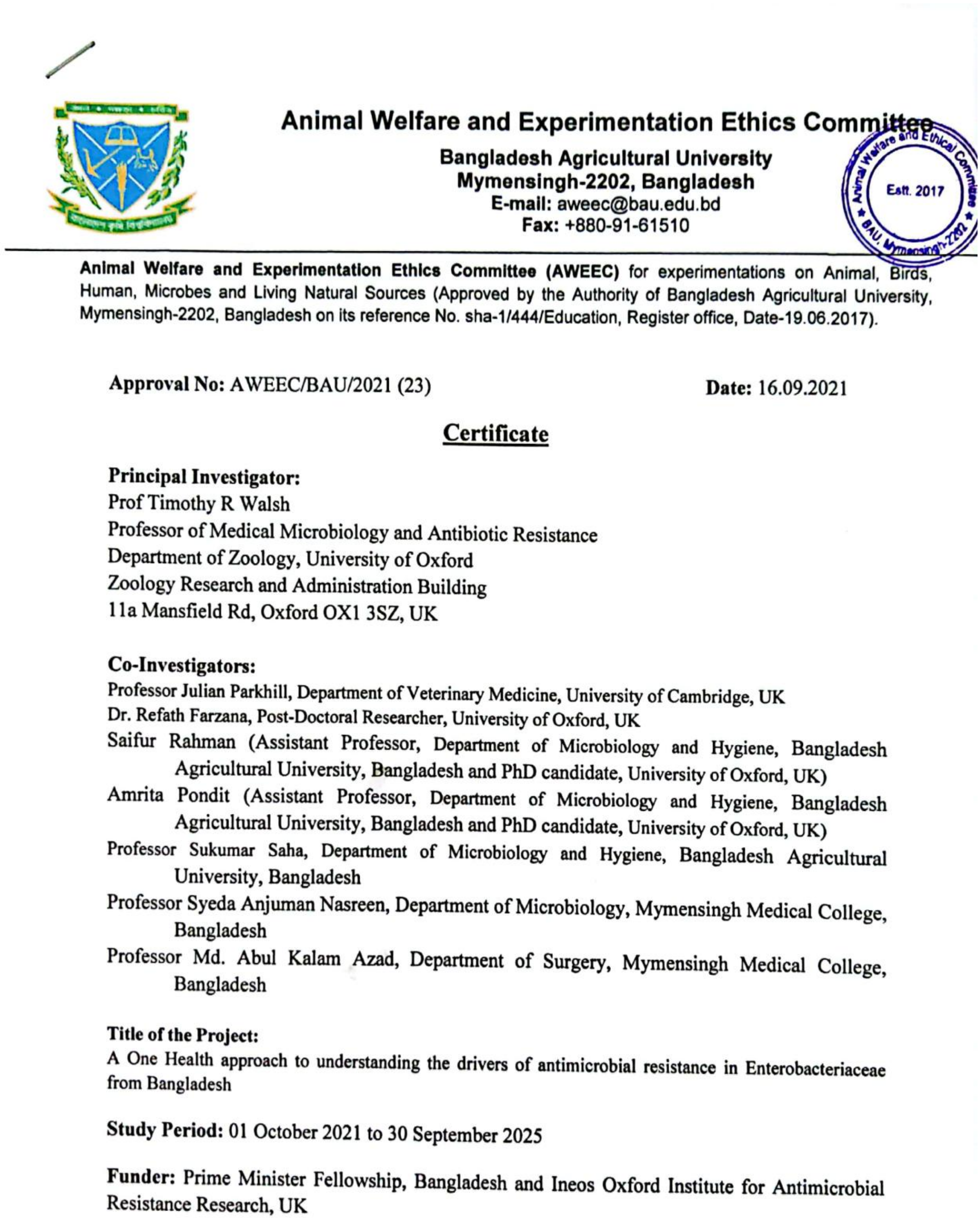
1.3.2 inform the University immediately if it considers any of the University's instructions infringes Data Protection Legislation;

1.3.3 ensure that it has in place appropriate technical and organisational measures to protect against unauthorised or unlawful processing of Personal Data and against accidental loss or destruction of, or damage to, Personal Data, appropriate to the harm that might result from the unauthorised or unlawful processing or accidental loss, destruction or damage and the nature of the data to be protected, having regard to the state of technological development and the cost of implementing any measures (those measures may include, where appropriate, pseudonymising and encrypting Personal Data, ensuring confidentiality, integrity, availability and resilience of its systems and services, ensuring that availability of and access to Personal Data can be restored in a timely manner after an incident, and regularly assessing and evaluating the effectiveness of the technical and organisational measures adopted by it);

Long Form Collaboration Agreement Sept 17
Lead Ref: R78974/CN001 Page 14

Appendix C

Ethical approvals



Animal Welfare and Experimentation Ethics Committee

Bangladesh Agricultural University
Mymensingh-2202, Bangladesh
E-mail: aweec@bau.edu.bd
Fax: +880-91-61510

Estt. 2017

Animal Welfare and Experimentation Ethics Committee (AWEEC) for experimentations on Animal, Birds, Human, Microbes and Living Natural Sources (Approved by the Authority of Bangladesh Agricultural University, Mymensingh-2202, Bangladesh on its reference No. sha-1/444/Education, Register office, Date-19.06.2017).

Approval No: AWEEC/BAU/2021 (23) **Date:** 16.09.2021

Certificate

Principal Investigator:
Prof Timothy R Walsh
Professor of Medical Microbiology and Antibiotic Resistance
Department of Zoology, University of Oxford
Zoology Research and Administration Building
11a Mansfield Rd, Oxford OX1 3SZ, UK

Co-Investigators:

Professor Julian Parkhill, Department of Veterinary Medicine, University of Cambridge, UK

Dr. Refath Farzana, Post-Doctoral Researcher, University of Oxford, UK

Saifur Rahman (Assistant Professor, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Bangladesh and PhD candidate, University of Oxford, UK)

Amrita Pondit (Assistant Professor, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Bangladesh and PhD candidate, University of Oxford, UK)

Professor Sukumar Saha, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Bangladesh

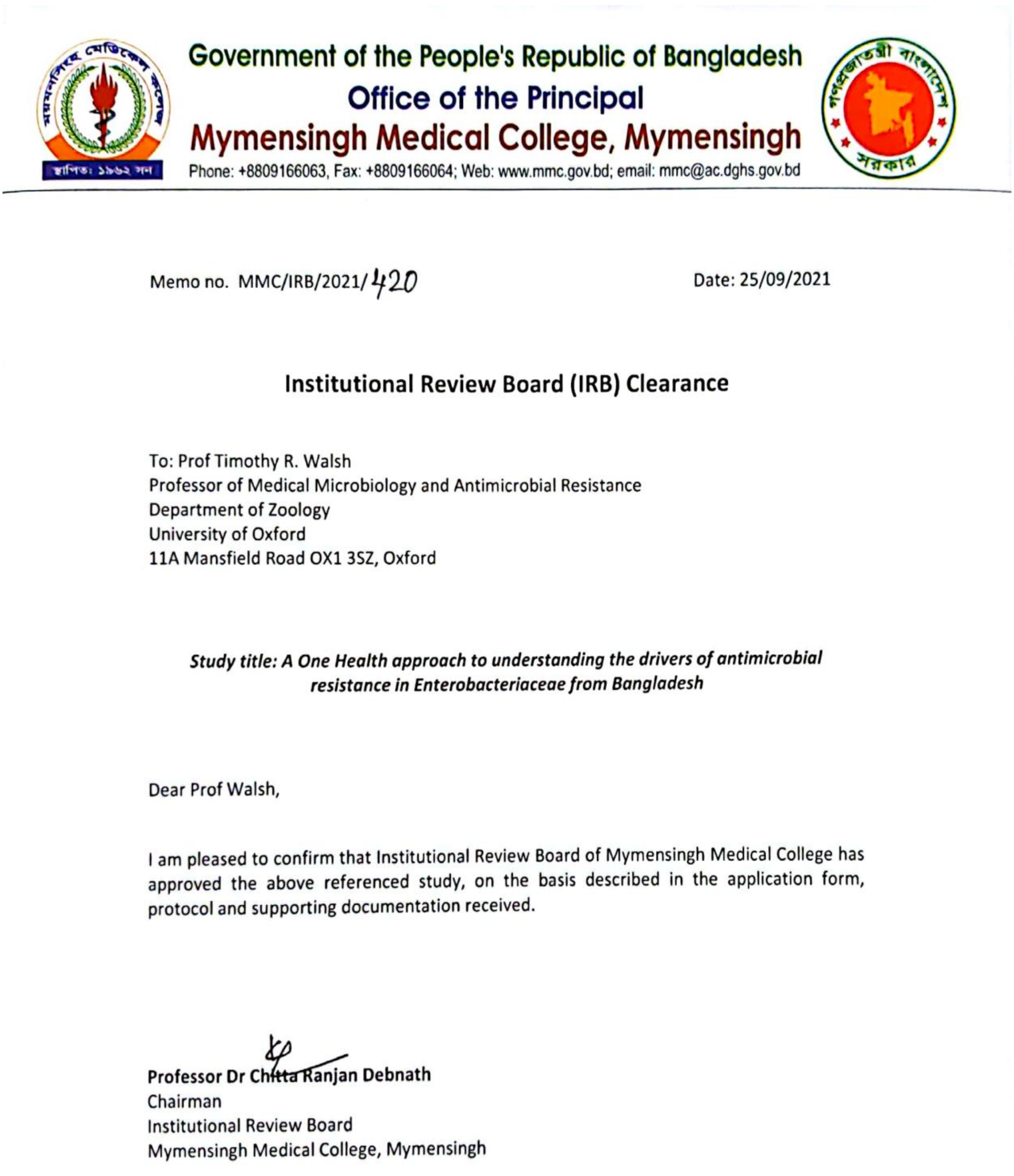
Professor Syeda Anjuman Nasreen, Department of Microbiology, Mymensingh Medical College, Bangladesh

Professor Md. Abul Kalam Azad, Department of Surgery, Mymensingh Medical College, Bangladesh

Title of the Project:
A One Health approach to understanding the drivers of antimicrobial resistance in Enterobacteriaceae from Bangladesh

Study Period: 01 October 2021 to 30 September 2025

Funder: Prime Minister Fellowship, Bangladesh and Ineos Oxford Institute for Antimicrobial Resistance Research, UK



Government of the People's Republic of Bangladesh
Office of the Principal
Mymensingh Medical College, Mymensingh
Phone: +8809166063, Fax: +8809166064; Web: www.mmc.gov.bd; email: mmc@ac.dghs.gov.bd

Memo no. MMC/IRB/2021/420 Date: 25/09/2021

Institutional Review Board (IRB) Clearance

To: Prof Timothy R. Walsh
Professor of Medical Microbiology and Antimicrobial Resistance
Department of Zoology
University of Oxford
11A Mansfield Road OX1 3SZ, Oxford

Study title: A One Health approach to understanding the drivers of antimicrobial resistance in Enterobacteriaceae from Bangladesh

Dear Prof Walsh,

I am pleased to confirm that Institutional Review Board of Mymensingh Medical College has approved the above referenced study, on the basis described in the application form, protocol and supporting documentation received.

Professor Dr Chitta Ranjan Debnath
Chairman
Institutional Review Board
Mymensingh Medical College, Mymensingh

Oxford Tropical Research Ethics Committee

University of Oxford
Research Services, Research Governance Ethics & Assurance
Boundary Brook House, Churchill Drive, Oxford OX3 7GB
Tel. +44 (0) 1865 (2)82106
E-mail: oxtrec@admin.ox.ac.uk

Professor Timothy Rutland Walsh
Professor of Medical Microbiology and Antimicrobial Resistance
University of Oxford Department of Zoology
Zoology Research and Administration Building
11a Mansfield Rd, Oxford OX1 3SZ

8 March 2022

Dear Professor Walsh

Full Title of Study: A One Health approach to understanding the drivers of antimicrobial resistance in Enterobacteriaceae from Bangladesh

OxTREC Reference: 30-21

Thank you for the letter of 07 March 2022 in which Dr Farzana has responded to the Committee's request for further clarification.
I am pleased to confirm that approval has now been granted for this study. This is valid for the planned duration of the study set out in the application form and is subject to receiving the local ethical approval (if this approval has not yet been received).

The documents approved for this study are as follows:

Documents:	**Version:**	**Date:**
Protocol - OHB	2.0	17 Feb 2022
PIS - OHB	2.0	17 Feb 2022
OHB CRF		

Any subsequent changes to the application must be submitted to the Committee as an Amendment. This should include a letter to give the reasons for the proposed modifications and all revised documents with changes tracked.
Please ensure that you submit a completed Annual Report form on every anniversary of this approval and a final End of Study Report. The relevant forms can be found on the OxTREC website.
Finally, please note the following **important information**:

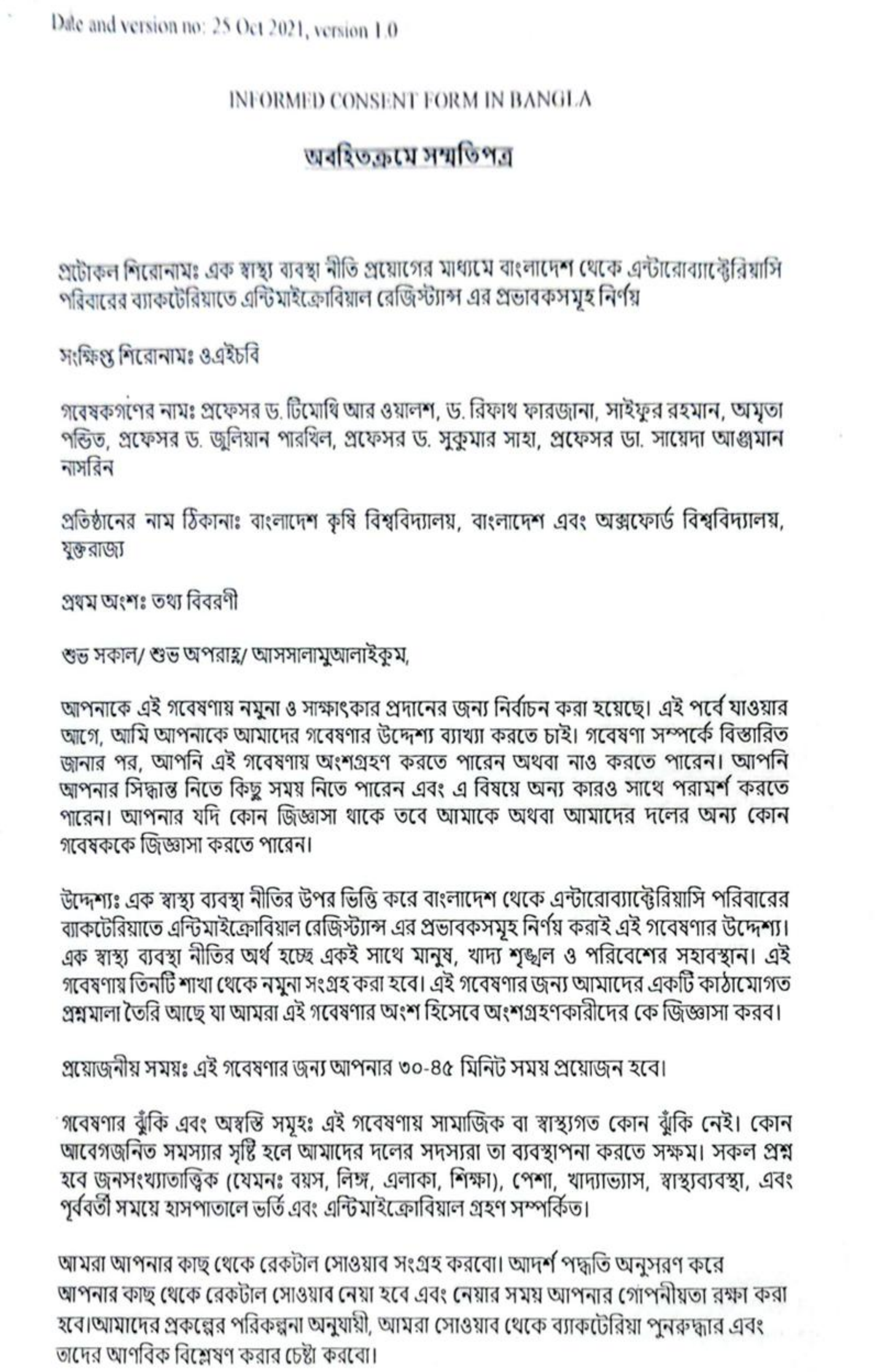
Data safety—all studies
It is the responsibility of the PI to ensure that all data collected during the course of the study is stored and transferred safely and securely. Further guidance and advice is available from the Research Data Team.
Only studies that will involve storing human tissue samples in Oxford
If you are planning to import the samples into England, you will need to make arrangements before the samples are transferred to store them under the governance of a Human Tissue Authority (HTA) licence. It is a legal requirement that any tissue or fluid made up of or containing human cells to be used for the purpose of research is stored on premises licensed by

the HTA unless covered by an exemption. OxTREC approval is not a recognised exemption. Further information may be found on the University's human tissue governance web pages.

Yours sincerely
DocuSigned by:
Karen Melham
BA168DF4624B463...
Dr Karen Melham
Sponsorship and Ethics Lead
for
Research Ethics Manager, OxTREC

Appendix D

Consent form for collecting data from human participants (Written in Bengali)



Date and version no: 25 Oct 2021, version 1.0

INFORMED CONSENT FORM IN BANGLA

অবহিতক্রমে সম্মতিপত্র

প্রটোকল শিরোনামঃ এক স্বাস্থ্য ব্যবস্থা নীতি প্রয়োগের মাধ্যমে বাংলাদেশ থেকে এন্টারোব্যাক্টেরিয়াসি পরিবারের ব্যাকটেরিয়াতে এন্টিমাইক্রোবিয়াল রেজিস্ট্যান্স এর প্রভাবকসমূহ নির্ণয়

সংক্ষিপ্ত শিরোনামঃ ওএইচবি

গবেষকগণের নামঃ প্রফেসর ড. টিমোথি আর ওয়ালশ, ড. রিফাথ ফারজানা, সাইফুর রহমান, অমৃতা পন্ডিত, প্রফেসর ড. জুলিয়ান পারখিল, প্রফেসর ড. সুকুমার সাহা, প্রফেসর ডা. সায়েদা আঞ্জুমান নাসরিন

প্রতিষ্ঠানের নাম ঠিকানাঃ বাংলাদেশ কৃষি বিশ্ববিদ্যালয়, বাংলাদেশ এবং অক্সফোর্ড বিশ্ববিদ্যালয়, যুক্তরাজ্য

প্রথম অংশঃ তথ্য বিবরণী

শুভ সকাল/ শুভ অপরাহ্ন/ আসসালামুআলাইকুম,

আপনাকে এই গবেষণায় নমুনা ও সাক্ষাৎকার প্রদানের জন্য নির্বাচন করা হয়েছে। এই পর্বে যাওয়ার আগে, আমি আপনাকে আমাদের গবেষণার উদ্দেশ্য ব্যাখ্যা করতে চাই। গবেষণা সম্পর্কে বিস্তারিত জানার পর, আপনি এই গবেষণায় অংশগ্রহণ করতে পারেন অথবা নাও করতে পারেন। আপনি আপনার সিদ্ধান্ত নিতে কিছু সময় নিতে পারেন এবং এ বিষয়ে অন্য কারও সাথে পরামর্শ করতে পারেন। আপনার যদি কোন জিজ্ঞাসা থাকে তবে আমাকে অথবা আমাদের দলের অন্য কোন গবেষককে জিজ্ঞাসা করতে পারেন।

উদ্দেশ্যঃ এক স্বাস্থ্য ব্যবস্থা নীতির উপর ভিত্তি করে বাংলাদেশ থেকে এন্টারোব্যাক্টেরিয়াসি পরিবারের ব্যাকটেরিয়াতে এন্টিমাইক্রোবিয়াল রেজিস্ট্যান্স এর প্রভাবকসমূহ নির্ণয় করাই এই গবেষণার উদ্দেশ্য। এক স্বাস্থ্য ব্যবস্থা নীতির অর্থ হচ্ছে একই সাথে মানুষ, খাদ্য শৃঙ্খল ও পরিবেশের সহাবস্থান। এই গবেষণায় তিনটি শাখা থেকে নমুনা সংগ্রহ করা হবে। এই গবেষণার জন্য আমাদের একটি কাঠামোগত প্রশ্নমালা তৈরি আছে যা আমরা এই গবেষণার অংশ হিসেবে অংশগ্রহণকারীদের কে জিজ্ঞাসা করব।

প্রয়োজনীয় সময়ঃ এই গবেষণার জন্য আপনার ৩০-৪৫ মিনিট সময় প্রয়োজন হবে।

গবেষণার ঝুঁকি এবং অস্বস্তি সমূহঃ এই গবেষণায় সামাজিক বা স্বাস্থ্যগত কোন ঝুঁকি নেই। কোন আবেগজনিত সমস্যার সৃষ্টি হলে আমাদের দলের সদস্যরা তা ব্যবস্থাপনা করতে সক্ষম। সকল প্রশ্ন হবে জনসংখ্যাতাত্ত্বিক (যেমনঃ বয়স, লিঙ্গ, এলাকা, শিক্ষা), পেশা, খাদ্যাভ্যাস, স্বাস্থ্যব্যবস্থা, এবং পূর্ববর্তী সময়ে হাসপাতালে ভর্তি এবং এন্টিমাইক্রোবিয়াল গ্রহণ সম্পর্কিত।

আমরা আপনার কাছ থেকে রেকটাল সোওয়াব সংগ্রহ করবো। আদর্শ পদ্ধতি অনুসরণ করে আপনার কাছ থেকে রেকটাল সোওয়াব নেয়া হবে এবং নেয়ার সময় আপনার গোপনীয়তা রক্ষা করা হবে।আমাদের প্রকল্পের পরিকল্পনা অনুযায়ী, আমরা সোওয়াব থেকে ব্যাকটেরিয়া পুনরুদ্ধার এবং তাদের আণবিক বিশ্লেষণ করার চেষ্টা করবো।

আপনি অবশ্যই জানবেন যে, এই সকল প্রশ্নের উত্তর না দেয়ার সম্পূর্ণ স্বাধীনতা আপনার আছে অথবা আপনি আপনার কাছ থেকে নমুনা সংগ্রহ করায় অস্বীকৃতি জানাতে পারেন।

কোন প্রশ্নের উত্তর না দেয়া অথবা নমুনা দিতে অস্বীকৃতি জানানোর জন্য আপনার আমাদের কাছে কোন প্রকার ব্যাখ্যা দেয়ার প্রয়োজন নেই।

গবেষণায় অংশগ্রহণের সুবিধাদি: এই গবেষণার ফলাফল বাংলাদেশে এন্টিমাইক্রোবিয়াল রেজিস্ট্যান্স সমস্যা প্রতিরোধে সুনির্দিষ্ট নীতি প্রণয়নে সহায়তা করবে।

অংশগ্রহণকারীর সম্মানীঃ এই গবেষণায় অংশগ্রহনের জন্য আপনাকে কোন সম্মানী প্রদান করা হবে না।

গোপনীয়তাঃ আপনার সম্পর্কিত কোন তথ্য এই গবেষণা দলের বাইরে আদান-প্রদান করা হবে না। আপনার থেকে সংগৃহীত তথ্য গোপন রাখা হবে, এজন্য আপনার নামের পরিবর্তে একটি সুনির্দিষ্ট সংখ্যা ব্যবহার করা হবে। একটি গোপন পাসওয়ার্ডের মাধ্যমে তথ্য সুরক্ষিত রাখা হবে, এই গবেষণাদলের সদস্য ব্যতীত কারও সেখানে প্রবেশাধিকার থাকবে না।

গবেষণার তথ্য সরবরাহঃ গবেষণা শেষে বৈজ্ঞানিক গবেষণাপত্র প্রকাশের মাধ্যমে, এবং অংশগ্রহণকারীদের সাথে গবেষণার ফলাফল সরবরাহ করা হবে।

অংশগ্রহণ এবং প্রত্যাহারঃ এই গবেষণায় আপনার অংশগ্রহণ সম্পূর্ণ স্বেচ্ছামূলক এবং আপনি স্বাধীনভাবে গবেষণায় অংশগ্রহণে অস্বীকৃতি জানাতে পারেন। এমনকি এই গবেষণায় অংশগ্রহনের সিদ্ধান্ত নেয়ার পরও কোন প্রকার ব্যাখ্যা ছাড়া যে কোন সময় আপনি গবেষণা থেকে নিজেকে প্রত্যাহার করে নিতে পারেন।

গবেষণা সম্পর্কিত প্রশ্নঃ যদি আপনার এই গবেষণা নিয়ে কোন প্রশ্ন থাকে, নির্দ্বিধায় আমাকে অথবা আমাদের দলের অন্য কোন গবেষককে জিজ্ঞাসা করতে পারেন।

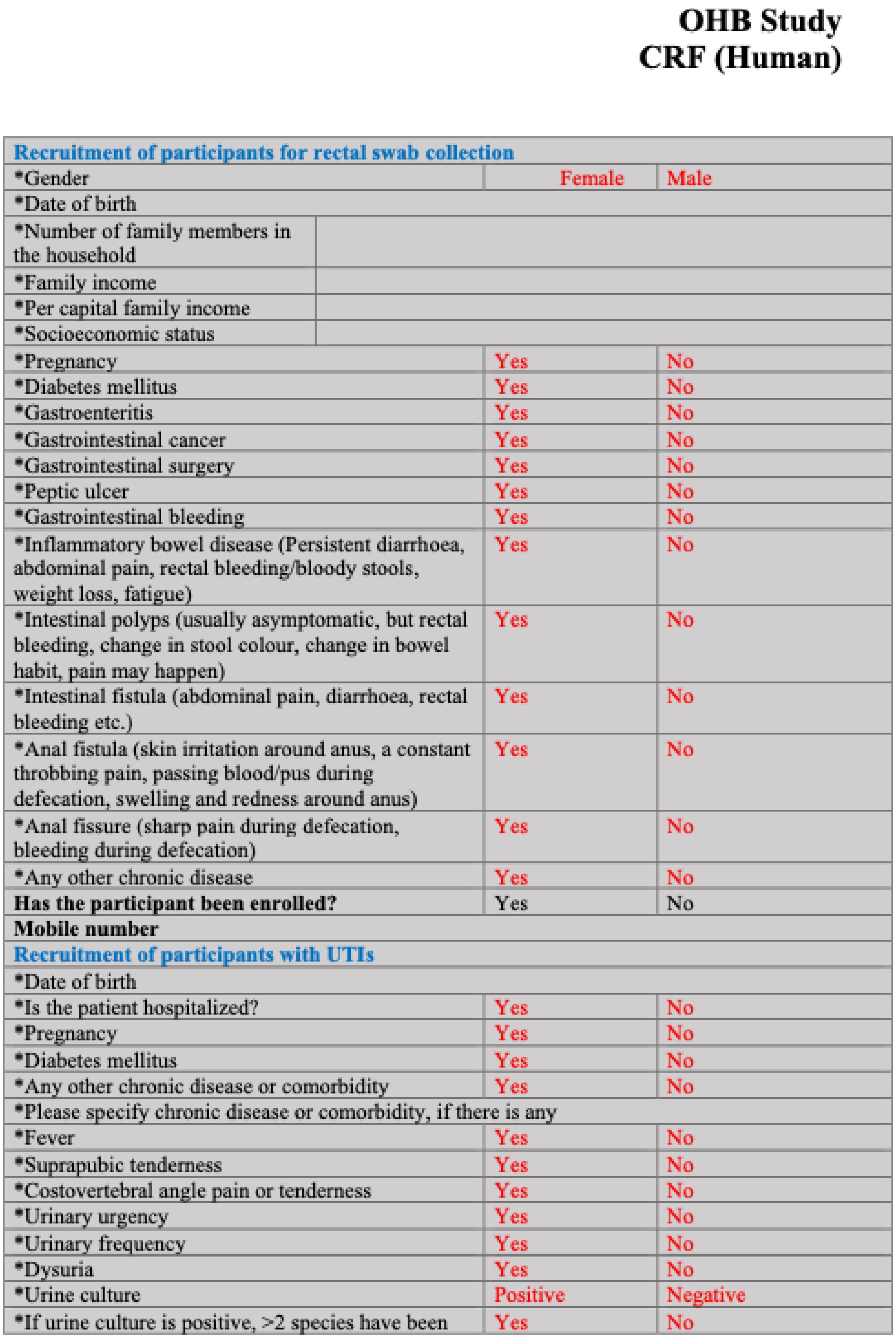
অংশগ্রহণকারী হিসেবে অধিকারঃ এই গবেষণা প্রকল্পটি ময়মনসিংহ মেডিকেল কলেজ হাসপাতাল এর গবেষণা রিভিউ কমিটি কর্তৃক অনুমোদনপ্রাপ্ত। অংশগ্রহণকারী হিসেবে আপনার অধিকার অথবা আপনার প্রতি গবেষক দলের মনোভাব নিয়ে যে কোন প্রশ্ন থাকলে আমাদের সাথে যোগাযোগ করতে পারেন।

সাক্ষাৎকার গ্রহণকারীর নাম:
সাক্ষাৎকার গ্রহণকারীর স্বাক্ষর:
তারিখঃ

অংশগ্রহণকারীর নাম:
অংশগ্রহণকারীর স্বাক্ষর:
তারিখঃ

Appendix E

Case Record Form (CRF) for collecting data from human participants.



OHB Study
CRF (Human)

Recruitment of participants for rectal swab collection		
*Gender	Female	Male
*Date of birth		
*Number of family members in the household		
*Family income		
*Per capital family income		
*Socioeconomic status		
*Pregnancy	Yes	No
*Diabetes mellitus	Yes	No
*Gastroenteritis	Yes	No
*Gastrointestinal cancer	Yes	No
*Gastrointestinal surgery	Yes	No
*Peptic ulcer	Yes	No
*Gastrointestinal bleeding	Yes	No
*Inflammatory bowel disease (Persistent diarrhoea, abdominal pain, rectal bleeding/bloody stools, weight loss, fatigue)	Yes	No
*Intestinal polyps (usually asymptomatic, but rectal bleeding, change in stool colour, change in bowel habit, pain may happen)	Yes	No
*Intestinal fistula (abdominal pain, diarrhoea, rectal bleeding etc.)	Yes	No
*Anal fistula (skin irritation around anus, a constant throbbing pain, passing blood/pus during defecation, swelling and redness around anus)	Yes	No
*Anal fissure (sharp pain during defecation, bleeding during defecation)	Yes	No
*Any other chronic disease	Yes	No
Has the participant been enrolled?	Yes	No
Mobile number		
Recruitment of participants with UTIs		
*Date of birth		
*Is the patient hospitalized?	Yes	No
*Pregnancy	Yes	No
*Diabetes mellitus	Yes	No
*Any other chronic disease or comorbidity	Yes	No
*Please specify chronic disease or comorbidity, if there is any		
*Fever	Yes	No
*Suprapubic tenderness	Yes	No
*Costovertebral angle pain or tenderness	Yes	No
*Urinary urgency	Yes	No
*Urinary frequency	Yes	No
*Dysuria	Yes	No
*Urine culture	Positive	Negative
*If urine culture is positive, >2 species have been	Yes	No

identified						
*If urine culture is positive, ≤2 species have been identified	Yes	No				
*If urine culture is positive, at least one of bacterium count is $\geq 10^5$ CFU/ml	Yes	No				
Has the participant been enrolled?	Yes	No				
Mobile number						
Recruitment of participants with post-surgical wound infections						
*Date of birth						
*Is the patient hospitalized?	Yes	No				
*History of surgery	Yes	No				
*Date of most recent surgery						
*Reason for the recent surgery (clinical diagnosis)						
*Pregnancy	Yes	No				
*Diabetes mellitus	Yes	No				
*Any other chronic disease or comorbidity	Yes	No				
*Please specify chronic disease or comorbidity, if there is any						
*Redness and pain around the surgical site	Yes	No				
*Discharge of cloudy fluid from the surgical wound	Yes	No				
*Fever	Yes	No				
Has the participant been enrolled?	Yes	No				
Mobile number						
Serial number						
Study ID						
Date of sample collection						
Date of interview						
Type of sample						
Area of residence						
Village						
District						
Latitude						
Longitude						
Education	Primary	Secondary	Tertiary	Others		
Please specify (Education), if 'others'						
Occupation	Farmer (plants)	Farmer (livestock)	housewife	Unemployed	Others	
Please specify (Occupation), if 'others'						
Access to electricity	Yes	No				
Methods of disposal of household waste						
Do you raise domestic animals?	Yes	No				
If 'yes' (raise domestic animal), please specify						
Cat	Dog	Goat	Cow	Chicken	Duck	Others
Please specify (domestic animal), if 'others'						

Do you own farm?	Yes	No		
If 'yes' (own farm), please answer the following				
Type of animal (cow)	Yes	No		
How many numbers of cows?				
Type of animal (goat)	Yes	No		
How many numbers of goats?				
Type of animal (chicken)	Yes	No		
How many numbers of chickens?				
Type of animal (duck)	Yes	No		
How many numbers of ducks?				
Please mention 'type of animal', if not in the list above:	Yes	No		
Please specify 'type of animal', if 'others'				
How many numbers of 'others?				
If 'no' (own farm), please mention the distant between participant's home and nearest farm (in meter)				
Do you have any underlying disease?	Yes	No		
If 'yes' (underlying disease), please specify				
Drinking water	Filter water	Boiled water	Bottle water	Tap water
	Well water	Tube well water	Others	
Please specify, if 'others' (drinking water)				
Consumption Chicken or duck	Yes	No		
Approximate total number of times per week eat chicken or duck				
Consumption Fish	Yes	No		
Approximate total number of times per week eat fish				
Toilet facilities available at home_1	Private (own household only)	Communal (Share between households)		
Toilet facilities available at home_2	Toilets connected to sewers or septic systems	Water-based toilets that flush into pits		
	Simple pit latrines with slabs	Ventilated improved pit latrines		
	Open defecation	Others		
Please specify, if 'others' (Toilet facilities available at home_2)				
Access to water & soap in toilet at home	Yes	No		
Previous Antibiotics Use within 3 months?	Yes	No		
If 'yes' (Previous Antibiotics Use within 3 months), please answer the following (Please enter the data for all antibiotics taken by the participants)				
Name of antibiotic_1				

Did you take without a prescription (antibiotic_1)?	Yes	No
Was the antibiotic prescribed by doctor (antibiotic_1)?	Yes	No
Did you discontinue the antibiotic when the symptoms were improved (antibiotic_1)?	Yes	No
Did you take the antibiotic until the course was completed (antibiotic_1)?	Yes	No
Date of start of antibiotic_1		
Please mention date when antibiotic_1 was discontinued		
Dose of antibiotic_1		
Previous Hospital Admission within 6 months?	Yes	No
If 'yes', (Previous Hospital Admission within 6 months), please answer the following		
Name of the hospital		
Date of admission		
Date of discharge		
Reason of hospitalization (clinical diagnosis)		
Please specify the name of antimicrobials during hospitalization		
Withdrawal from the study		
Does the participant choose withdraw from the study?	Yes	No
Please specify the reason of withdrawal		

*Data should be collected first randomization.

Appendix F

K. pneumoniae assembly QC metrics derived from Illumina sequencing data are provided in Appendix F (Supplementary Table S1) as a separate Excel file.