A One Health genomic approach to *Escherichia coli* clonality and antimicrobial resistance from Bangladesh

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“Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world.”

- Louis Pasteur -

Table of contents

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Abstract

Antimicrobial resistance (AMR) in *Escherichia coli* represents a critical global health challenge, particularly in low- and middle-income countries where extensive antibiotic use and abuse in human medicine, livestock farming, and environmental contamination accelerates the spread of AMR. In Bangladesh, where population density, poor sanitation and waste management practices provide favourable conditions for the dissemination of AMR bacteria, systematic genomic studies on *E. coli* across interconnected sources remain scarce. This thesis characterises the genomic diversity, resistance mechanisms, and virulence potential of *E. coli* at the human–animal–environment interface within a One Health framework and examines the role of horizontal gene transfer in the dissemination of clinically significant resistance genes.

A One Health sampling strategy (Chapter 3) captured *E. coli* from clinical patients, healthy volunteers, poultry, domestic animals, and environmental sources, establishing a multi-sectoral dataset to evaluate transmission dynamics. Phenotypic susceptibility testing against 17 clinically important antibiotics revealed high-levels of resistance to β-lactams, aminoglycosides, and fluoroquinolones (Chapter 4).

Genomic analyses demonstrated extensive clonal diversity, with several globally recognised high-risk sequence types (ST131, ST410, ST648, and ST1193) detected across reservoirs (Chapter 5). Phylogroup distributions overlapped between human and non-human sources, underscoring potential cross-reservoir transmission. AMR was predominantly mediated by $bla_{\text{CTX-M-15}}$, $bla_{\text{NDM-5}}$, *qnrS1*, and genes conferring resistance to aminoglycosides. Virulence profiling revealed widespread presence of extra-intestinal pathogenic *E. coli*-associated determinants, often co-localising with AMR genes.

A key finding was the detection of the plasmid-mediated *tet*(X4) gene, conferring tigecycline resistance, and carbapenem resistance mediated by $bla_{\text{NDM-5}}$. Plasmid characterisation and genomic context analyses (Chapter 6) confirmed that both genes were disseminated via horizontal gene transfer across diverse clonal backgrounds, highlighting the risk of mobile resistance elements in amplifying AMR.

These findings demonstrate the extensive genomic diversity and AMR burden of *E. coli* circulating in Bangladesh and emphasise the critical role of horizontal gene transfer in the emergence of resistance to last-resort antibiotics. This thesis provides the first comprehensive One Health genomic snapshot of *E. coli* in Bangladesh and delivers essential baseline evidence to support integrated AMR interventions and stewardship strategies at national and global levels.

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Declaration

I declare that this thesis is my own work, undertaken with appropriate support and assistance from my supervisors and colleagues. Specific assistance I have received from others in relation to this thesis is outlined below.

Sampling for this study was collected by the author with the assistance of a team from Bangladesh Agricultural University and Mymensingh Medical College Hospital, who provided support during the sample collection process. I acknowledge the assistance from the team members, especially Saifur Rahman. 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.

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Presentation at international conferences

1. **Pondit, A.,** *et al*. Linking carbapenem-resistant *E. coli* in hospital-admitted patients and the environment in Bangladesh: An emerging public health threat. Poster presentation at the 35th ESCMID Global, April 2025, Vienna, Austria.

2. **Pondit, A.,** *et al*. Genomic epidemiology of third-generation cephalosporin resistance *E. coli* and *K. pneumoniae* from urinary tract infections in Bangladesh. ePoster presentation at the 34th ECCMID, April 2024, Barcelona, Spain.

List of abbreviations

3GC	Third-generation cephalosporin
AMK	Amikacin
AMP	Ampicillin
AMR	Antimicrobial resistance
AMU	Antimicrobial usage
AMX-CL	Amoxicillin-clavulanic acid
APEC	Avian pathogenic *E. coli*
ARB	Antibiotic-resistant bacteria
ARGs	Antimicrobial resistance genes
AST	Antibiotic susceptibility testing
AWEEC	Animal Welfare and Experimentation Ethics Committee
AZT	Aztreonam
BARA	Bangladesh AMR Response Alliance
BAU	Bangladesh Agricultural University
BBS	Bangladesh Bureau of Statistics
BDT	Bangladeshi taka
BF	Bird faeces
bla	β-lactamase gene
BLAST	Basic Local Alignment Search Tool
BLASTn	Basic Local Alignment Search Tool for nucleotides
ble	Bleomycin resistance gene
BRIG	BLAST ring image generator

CCS	Chicken cloacal swab
CDC	Communicable Disease Control
CEF	Cefepime
CF	Conversion factor
CFU	Colony forming unit
CGE	Centre for Genomic Epidemiology
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standard Institute
CMS	Chicken meat swab
COL	Colistin
CREC	Carbapenem-resistant *E. coli*
CRF	Case record form
CRGs	Carbapenem-resistance genes
CTX	Cefotaxime
CTZ	Ceftazidime
CTZ-AVI	Ceftazidime-avibactam
DAEC	Diffusely adhering *E. coli*
DARS	Domestic animal rectal swab
DGDA	Directorate General of Drug Administration
DGHS	Directorate General of Health Services
DMSO	Dimethyl sulfoxide
EAEC	Enteroaggregative *E. coli*
ECDC	European Centre for Disease Prevention and Control

ECOFF	Epidemiological cut-off
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ESBLs	Extended-Spectrum β-Lactamases
ETEC	Enterotoxigenic *E. coli*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ExPEC	Extraintestinal pathogenic *E. coli*
FAO	Food and Agriculture Organization
FOS	Fosfomycin-glucose-6-phosphate
GEN	Gentamicin
GIT	Gastrointestinal tract
GLASS	Global Antimicrobial Resistance and Use Surveillance System
HAIs	Hospital-acquired infections
HGT	Horizontal gene transfer
HRS	Human rectal swab
HUS	Haemolytic uremic syndrome
IBM SPSS	International Business Machines Statistical Package for the Social Sciences
IEDCR	Institute of Epidemiology, Disease Control and Research
IOI	Ineos Oxford Institute for Antimicrobial Research
IPC	Infection prevention and control
IPEC	Intestinal pathogenic *E. coli*

IPM	Imipenem
IRB	Institutional Review Board
IS	Insertion sequence
iTOL	Interactive Tree of Life
KPC	*Klebsiella pneumoniae* carbapenemase
LEE	Locus of enterocyte effacement
LEV	Levofloxacin
LMICs	Low- and middle-income countries
LT	Heat-labile
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionization–Time of Flight Mass Spectrometry
mcr	Mobile colistin resistance genes
MDR	Multidrug-resistant
MER	Meropenem
MGEs	Mobile genetic elements
MICs	Minimum inhibitory concentrations
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

NDM-5NEC	*bla*$_{\text{NDM-5}}$ negative *E. coli*
NDM-5PEC	*bla*$_{\text{NDM-5}}$ positive *E. coli*
NMEC	Neonatal meningitis-associated *E. coli*
OD	Optical density
ONT	Oxford nanopore technologies
OXA	Oxacillinase
OxTREC	Oxford Tropical Research Ethics Committee
PAI	Pathogenicity island
PATRIC	Pathosystems Resource Integration Centre
PBP	Penicillin binding protein
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PMQR	Plasmid-mediated quinolone resistanc
qnr	Quinolone resistance gene
Qs	Quarters
QRDR	Quinolone resistance-determining regions
RND	Resistance-nodulation-division
r	Pearson correlation coefficient
SDG	Sustainable Development Goal
SNP	Single-nucleotide polymorphism
SOPs	Standard operating procedures
SSI	Surgical site infection

ST	Sequence type
STEC	Shiga toxin-producing *E. coli*
tet	Tetracycline resistance gene
TIG	Tigecycline
TZP	Piperacillin-tazobactam
UK	United Kingdom
UN	United Nations
UNEP	United Nations Environment Programme
UNICEF	United Nations Children's Fund
UPEC	Uropathogenic *E. coli*
UTI	Urinary tract infection
VAGs	Virulence-associated genes
VFs	Virulence factors
WASH	Water, sanitation, and hygiene
WGS	Whole-genome sequencing
WHO	World Health Organisation
WOAH	World Organisation for Animal Health
WP	Work packages
XDR	Extensively drug-resistant
χ^2	Chi-square

Chapter 1

General introduction

1.1 The discovery and development of antibiotics

The term antibiotic originated from antibiosis, meaning "against life," and was later coined as "antibiotics" by Selman Waksman in 1947 (Clardy *et al*., 2009; Waksman, 1947). Initially, the term referred specifically to compounds produced by microorganisms that inhibit the growth or kill bacteria. Over time, its meaning has broadened to encompass any substance capable of suppressing bacterial growth or survival, regardless of its origin (Hutchings *et al*., 2019; Davies and Davies, 2010; Scholar and Pratt, 2000). Antibiotics revolutionised medicine in the 20th century, beginning with the discovery of penicillin by Alexander Fleming in 1928, and their transformative role was firmly established during the Second World War (Ligon, 2004). These agents not only reduced mortality from bacterial infections but also enabled the success of modern medical interventions such as surgery, chemotherapy, and organ transplantation.

1.2 Classification of antibiotics

Antibiotics can be classified by their mechanism of action, chemical structure, or spectrum of activity.

Based on their effect, they are either bactericidal (killing bacteria) or bacteriostatic (inhibiting bacterial growth) (Ishak *et al*., 2025). Furthermore, antibiotics are grouped as narrow-spectrum, acting on a limited set of bacteria (e.g., only Gram-positive or Gram-negative), or broad-spectrum, active against a wide range of pathogens (Baran *et al*., 2023). Antibiotics act through five primary modes: inhibition of cell wall synthesis (e.g., β-lactams, glycopeptides), alteration of the cell membrane (e.g., polymyxins), inhibition of protein synthesis (e.g., tetracyclines, aminoglycosides, macrolides), inhibition of nucleic acid synthesis (e.g., fluoroquinolones), or disruption of folate

synthesis (e.g., sulfonamides, trimethoprim) (Baran *et al*., 2023; Schwalbe, 2007; Finberg *et al*., 2004) (Figure 1.1).

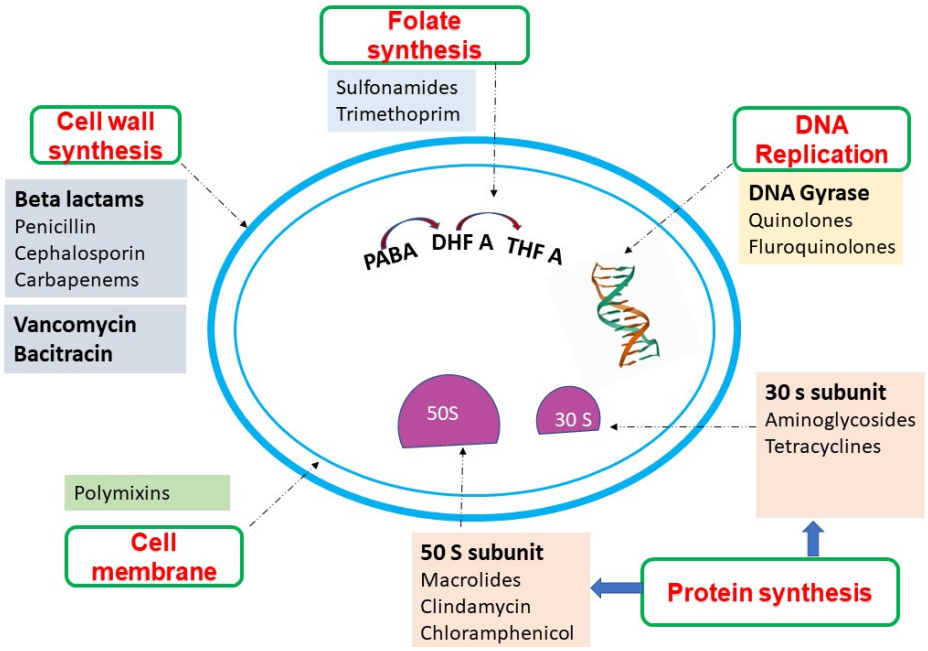


Figure 1.1 Mechanisms of action of different antibiotic classes targeting bacterial cells. (The figure was created with BioRender.com).

The timeline of antibiotic discovery and resistance emergence further illustrates how resistance typically follows shortly after the introduction of each new antibiotic class. For example, penicillin was discovered in 1928, but resistance was reported by 1940; similarly, resistance to tetracyclines appeared within a decade of their introduction (Laxminarayan *et al*., 2013). This pattern highlights the ongoing evolutionary arms race between bacterial adaptation and the development of antibiotics (Figure 1.2).

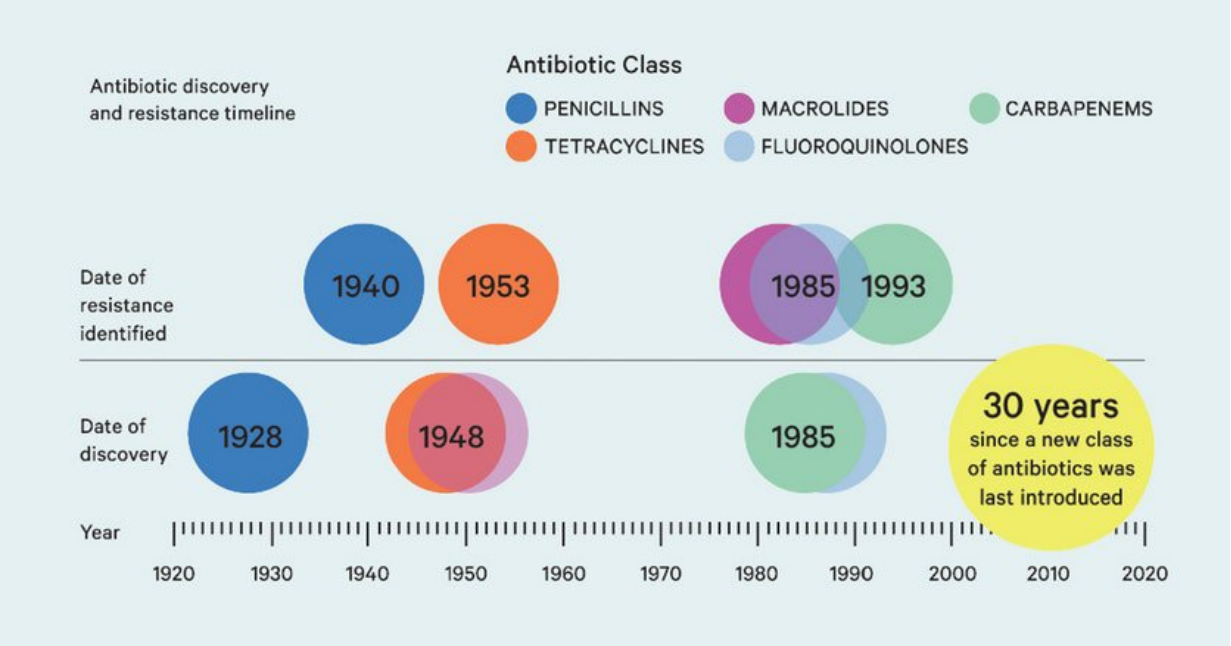


Figure 1.2 Timeline of antibiotic discovery and emergence of resistance across major classes of antibiotics [Adapted from Yu *et al*., 2021, licensed under Creative Commons Attribution (CC BY 4.0)].

1.3 Antimicrobial resistance (AMR) and its underlying mechanisms

AMR is defined as the ability of microorganisms, such as bacteria, viruses, fungi, and parasites, to withstand the effects of antimicrobial agents that would normally kill or inhibit their growth. In bacteria, resistance arises through genetic mutations or horizontal gene transfer and is rapidly accelerated by the misuse and overuse of antibiotics in human medicine, agriculture, and animal husbandry (Davies & Davies, 2010). Overuse refers to the excessive or unnecessary prescription and consumption of antibiotics, such as prescribing them for viral infections (e.g. influenza or the common cold), where they have no therapeutic benefit. Misuse encompasses inappropriate practices, including incorrect dosing, incomplete treatment courses, and the use of broad-spectrum antibiotics when narrow-spectrum agents would be sufficient. In agricultural and animal

husbandry settings, antibiotics are frequently administered prophylactically or as growth promoters in livestock, often at sub-therapeutic doses, creating ideal conditions for the selection and spread of resistant bacterial strains. These practices increase selective pressure on microbial populations, facilitating the emergence and dissemination of antibiotic resistance. In addition to the use in humans and animals, environmental contamination with antibiotics presents a significant driver of AMR within a One Health framework. Effluents from pharmaceutical manufacturing facilities, as well as hospital and municipal wastewater, can contain high concentrations of active antibiotic compounds, creating environmental hotspots that exert strong selective pressure on environmental and commensal bacteria (Larsson, 2014).

Bacteria employ multiple strategies to evade the action of antibiotics. These include: enzymatic inactivation, modification or destruction of the antibiotic molecule (e.g., β-lactamases), target site modification (e.g., methylation of 23S rRNA by *erm* genes conferring resistance to macrolides), structural changes in the bacterial target that prevent antibiotic binding (e.g., mutations in DNA gyrase leading to fluoroquinolone resistance), target protection or bypass, including the production of alternative penicillin-binding proteins such as PBP2a encoded by *mecA* in methicillin-resistant *Staphylococcus aureus*, production of alternative proteins that substitute for antibiotic targets such as altered dihydrofolate reductase conferring resistance to trimethoprim and reduced drug accumulation, decreased permeability through porins or active efflux via efflux pumps (Elshobary *et al*., 2025; Belay *et al*., 2024).

These mechanisms, often working in combination, severely compromise the effectiveness of antibiotic therapy. A summary of the major resistance mechanisms is shown in Figure 1.3.

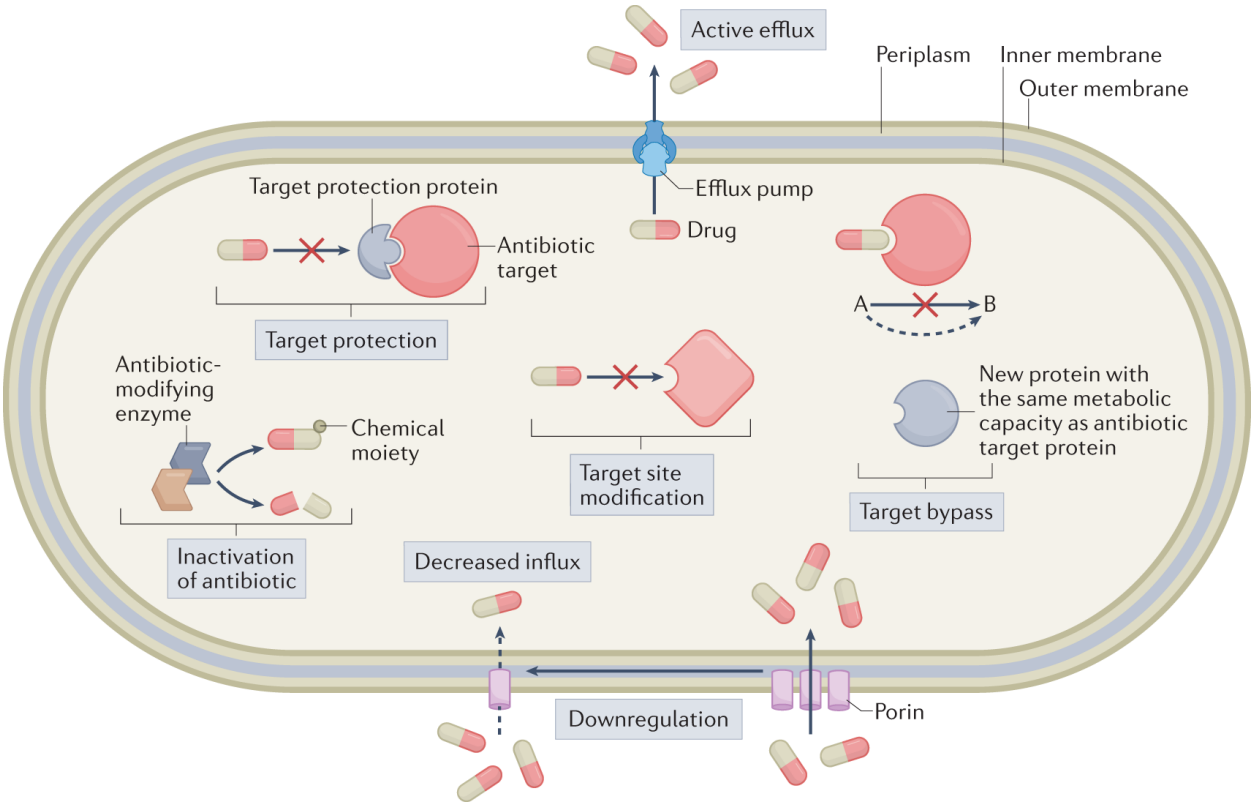


Figure 1.3 Major mechanisms of bacterial antimicrobial resistance, including antibiotic inactivation, target modification, target protection, target bypass, decreased influx, and active efflux (Adapted from Darby *et al*., 2023, with permission from Springer Nature).

AMR has emerged as one of the most pressing global public health threats. In 2019, an estimated 4.95 million deaths were associated with bacterial AMR, including 1.27 million directly attributable to AMR (Murray *et al*., 2022). More recent analyses show that in 2021 alone, 4.71 million deaths were associated with AMR, with 1.14 million deaths directly attributable. While AMR-related mortality has declined in children under five, it has risen dramatically in adults over 70, reflecting both an ageing population and a rising burden of resistant infections in older age groups (Antimicrobial Resistance Collaborators, 2022). Figure 1.4 illustrates the global distribution of AMR mortality, with South Asia and Latin America projected to bear the highest

burdens by 2050. The geographical differences in projected AMR-attributable mortality may reflect disparities in healthcare access, antimicrobial stewardship, and sanitation infrastructure across regions. Areas with higher infectious disease burden and limited WASH services might therefore experience greater selection and transmission of resistant pathogens. These patterns highlight the importance of context-specific One Health interventions.

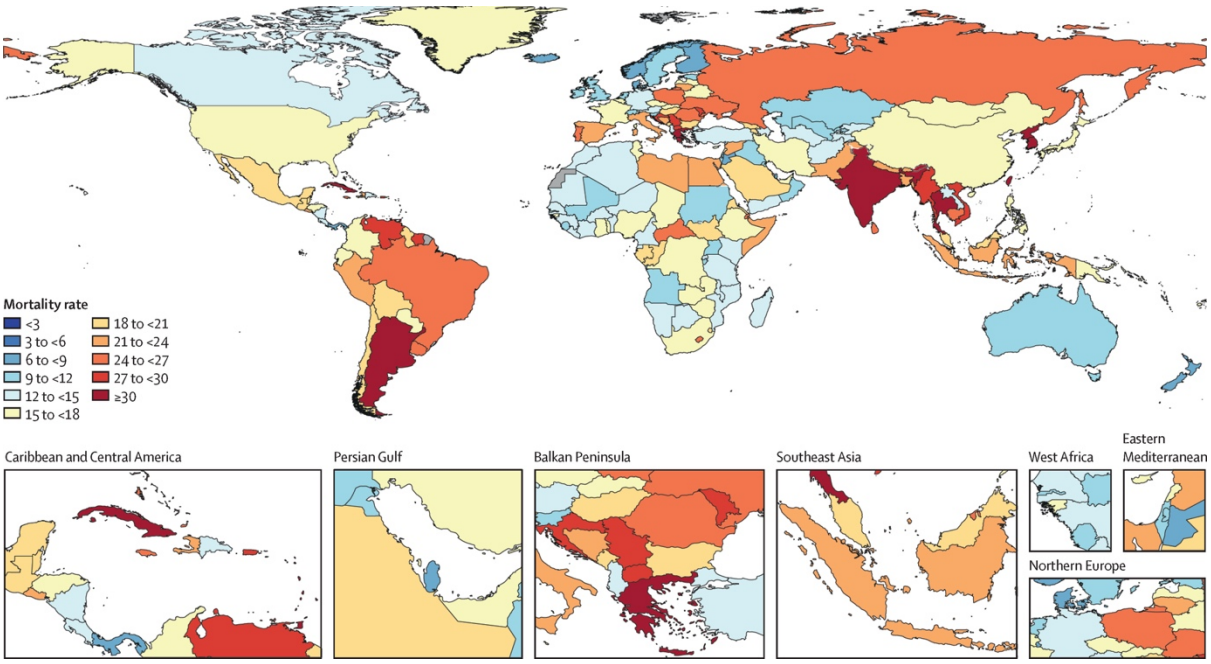


Figure 1.4 Global distribution of AMR-attributable death rates per 100,000 population projected for 2050, highlighting disproportionate burdens in South Asia, sub-Saharan Africa, and Latin America. [Adapted from Antimicrobial Resistance Collaborators, 2024, licensed under Creative Commons Attribution (CC BY 4.0)].

Notably, resistance to carbapenems among Gram-negative bacteria and to methicillin in *Staphylococcus aureus* has expanded most rapidly in recent decades (Murray *et al*., 2022). Forecasts predict up to 10 million annual deaths by 2050 if urgent action is not taken, underscoring

the importance of infection prevention, stewardship of existing antibiotics, and development of new drugs.

1.4 AMR in the context of the Sustainable Development Goals (SDGs)

AMR is not only a public health threat but also a cross-cutting issue that impedes progress towards the United Nations Sustainable Development Goals (SDGs). It is linked to nearly all of the goals in some way. It is explicitly incorporated in SDG 3 (Good health and Well-being) under indicator 3.d.2, which tracks the percentage of bloodstream infections caused by selected antimicrobial-resistant organisms (World Health Organisation, 2025). However, its impact extends to several other goals. For example, AMR threatens poverty reduction (SDG 1) and food security (SDG 2) through its negative effects on livelihoods, healthcare costs, and agriculture (Van Boeckel *et al*., 2019). Education and gender equality (SDGs 4 and 5) are also disproportionately affected, as women and children in low and middle-income countries (LMICs) bear a higher burden of resistant infections (Jasovský *et al*., 2016). Moreover, clean water and sanitation (SDG 6) are directly linked to AMR, since poor hygiene, untreated wastewater, and inadequate access to safe water facilitate the spread of resistant pathogens (Fuhrmeister *et al*., 2023; United Nations Environment Programme [UNEP], 2023). At the same time, AMR exacerbates global inequalities (SDG 10) and undermines responsible production and consumption in agriculture and health systems (SDG 12), while also threatening the sustainability of terrestrial ecosystems (SDG 15) through environmental contamination and antimicrobial overuse (WHO, 2024; Jasovský *et al*., 2016). Finally, the global response to AMR requires strong multisectoral collaboration, directly aligns directly with SDG 17 (Partnerships for the Goals) (Jasovský *et al*., 2016).

Figure 1.5 Sustainable Development Goals (SDGs) with direct relevance to AMR. (Adapted from United Nations, 2015, Sustainable Development Goals).

1.5 The One Health approach: background and relevance to AMR

The One Health approach acknowledges the intricate interdependence between human, animal, and environmental health systems and emphasises a transdisciplinary framework for tackling global health challenges. Initially conceptualised to address zoonotic diseases, One Health has

broadened to encompass a wide range of interconnected issues such as AMR, emerging infectious diseases, food safety, and ecosystem health (Destoumieux-Garzón *et al*., 2018).

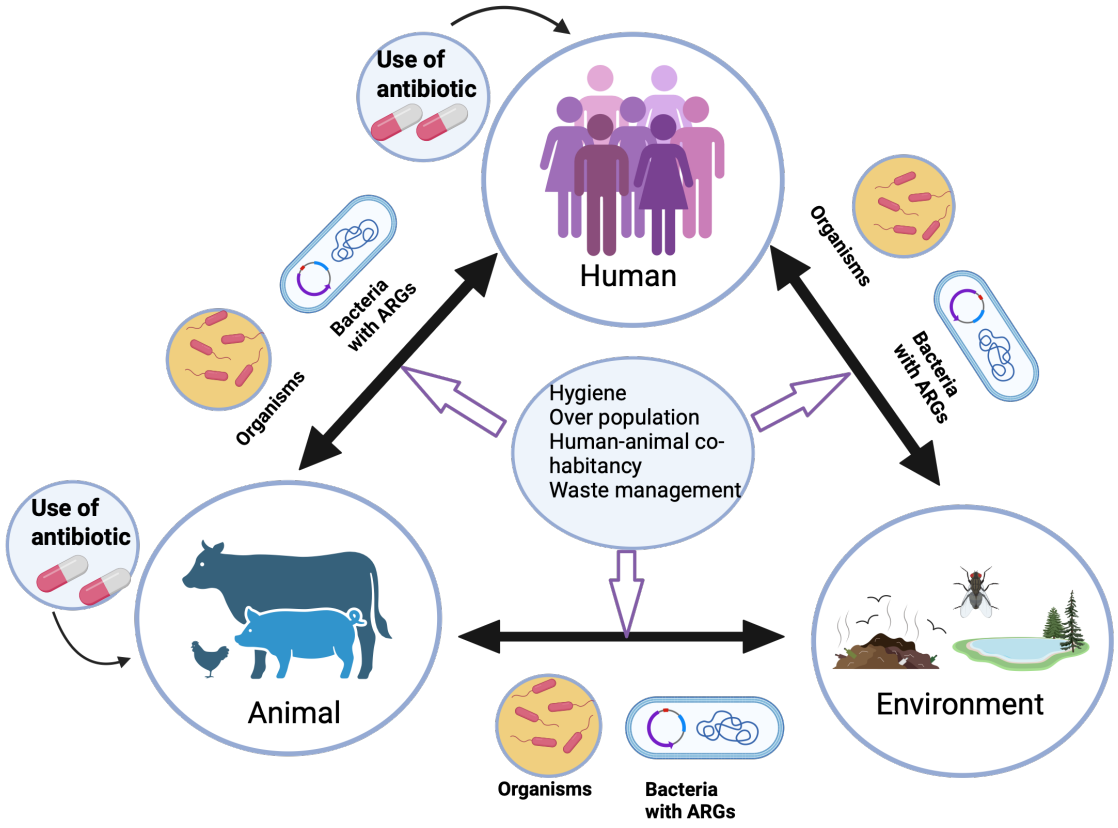


Figure 1.6 The One Health framework of AMR transmission. Human, animal, and environmental sectors interact through antibiotic use and poor hygiene, facilitating the spread of resistant organisms and antimicrobial resistance genes (ARGs). (The figure was created with BioRender.com).

Rather than being confined to a single sector, AMR exemplifies the complexity of health challenges that transcend traditional boundaries. Resistant pathogens and their genes can move

between humans, animals, and the environment through diverse pathways such as food chains, direct contact, and contaminated water sources (Nadimpalli *et al*., 2021). *E. coli*, in particular, is widely recognized as a sentinel organism for AMR surveillance because it readily acquires and disseminates resistance determinants through mobile genetic elements like plasmids, integrons, and transposons (Nasrollahian *et al*., 2024; Mencía-Ares *et al*., 2022). The shift in the global AMR landscape has also been marked by the rise of multidrug- and extensively drug-resistant Gram-negative bacteria, especially Enterobacteriaceae. Resistance mechanisms such as extended-spectrum β-lactamases, carbapenemases (e.g., NDM-1), and mobile colistin resistance genes (*mcr)* have rendered many infections increasingly untreatable (Walsh, 2018).

In LMICs like Bangladesh, the One Health interfaces are often more porous due to socio-economic, infrastructural, and policy challenges. Factors such as dense human and animal populations, indiscriminate antimicrobial use in human and veterinary practices, limited biosecurity in farming systems, and weak wastewater and waste management contribute to the widespread dissemination of resistant bacteria and genes across environments (Tasneem, 2024; Williams *et al*., 2023).

The World Health Organization (WHO), Food and Agriculture Organization (FAO), and World Organisation for Animal Health (WOAH) have all advocated for integrating One Health principles into national and global AMR action plans. This collaboration is formalised in the One Health Joint Plan of Action (2022–2026), which guides coordinated multisectoral efforts to tackle AMR across human, animal, and environmental health sectors (WHO, FAO, UNEP, & WOAH, 2022).

1.6 The burden of AMR in Bangladesh

Bangladesh faces a mounting AMR challenge, with multidrug-resistant (MDR) infections increasingly reported across human, animal, and environmental sectors. The country's high population density, widespread over-the-counter antibiotic use, and weak diagnostic and stewardship infrastructures accelerate the emergence and spread of resistant organisms (Azim *et al*., 2023; Hoque *et al*., 2020; Khan *et al*., 2020).

In the clinical sector, AMR poses a major public health threat. Surveillance reports indicate that common bacterial pathogens, particularly *Enterobacterales*, show high levels of resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides (DGHS, IEDCR, 2024; Chowdhury *et al*., 2023). Alarmingly, carbapenem-resistant strains carrying bla_{NDM} and other carbapenemase genes are emerging, severely limiting treatment options (Farzana *et al*., 2023).

In the animal health sector, routine use of antimicrobials as growth promoters and for prophylaxis in poultry, livestock, and aquaculture has fueled resistance development. Resistant bacteria isolated from animals frequently harbour extended-spectrum β-lactamases (ESBLs), colistin resistance genes (*mcr-1*, *mcr-3*), and plasmid-mediated quinolone resistance markers (Chowdhury *et al*., 2021; Sobur *et al*., 2019). Weak veterinary drug regulation and poor biosecurity practices in farming systems are major drivers of AMR spread, facilitating rapid emergence and cross-species transmission. Indeed, FAO's AMR Action Plan links inadequate regulatory frameworks to elevated AMR risk, and reviews of LMICs agricultural sectors reveal persistent non-therapeutic antimicrobial use in industrial farms. In Bangladesh, research indicates that commercial poultry farms compensate for biosecurity gaps by using prophylactic antibiotics, thereby increasing the potential for resistant bacteria to emerge and spread (FAO, 2016). At the environmental interface,

resistant bacteria have been documented in surface water, sewage, soil, and street food samples, often carrying mobile resistance determinants with zoonotic or clinical relevance (Hossain *et al*., 2025; Nadimpalli *et al*., 2021). The discharge of untreated hospital effluents, agricultural waste, and domestic sewage into rivers and open drains creates reservoirs for resistance gene exchange and persistence (Hossain *et al*., 2021).

While Bangladesh has launched a National Action Plan on AMR (2017–2022) and is participating in the Global AMR Surveillance System, existing surveillance remains predominantly focused on human health. Veterinary and environmental AMR surveillance efforts are emerging but remain fragmented and underrepresented (DGHS, 2022). Moreover, genomic epidemiology, a critical tool for understanding transmission dynamics, clonal spread, and resistance gene evolution, has only recently been applied in Bangladesh, leaving significant gaps in One Health–integrated AMR monitoring.

1.7 Genomic epidemiology and its role in AMR surveillance

The rapid emergence and spread of AMR have complicated the management of infectious diseases globally, especially in LMICs where surveillance systems remain under-resourced (Delpy *et al*., 2024; Iskandar *et al*., 2021). Traditional AMR monitoring has largely depended on phenotypic antibiotic susceptibility testing, which, though useful for clinical decision-making, falls short in capturing the evolutionary trajectories, horizontal gene transfer events, and global dissemination patterns of resistant organisms (MacLean & San Millan, 2019). Genomic epidemiology, powered by advances in whole-genome sequencing (WGS), has transformed the surveillance of AMR by enabling high-resolution detection of resistance determinants, virulence factors, and mobile genetic elements across pathogens and reservoirs (Hendriksen *et al*., 2019a; Didelot *et al*., 2012).

Genomic tools such as multilocus sequence typing (MLST), core-genome phylogenetics, single-nucleotide polymorphism (SNP) analysis, and plasmid replicon typing provide critical insights into bacterial population structure and allow the tracking of high-risk clones across clinical, veterinary, and environmental sectors (Gardy & Loman, 2018). Importantly, genomics allows the simultaneous identification of co-occurring resistance and virulence genes, thereby providing a more comprehensive risk assessment of strains with epidemic or zoonotic potential (Wyres & Holt, 2018).

Several global initiatives have integrated genomic epidemiology into AMR monitoring frameworks. The WHO's Global Antimicrobial Resistance Surveillance System (GLASS) has expanded beyond phenotypic testing to include genomic approaches such as whole-genome sequencing, supported by a global network of collaborating centres. These developments aim to harmonize surveillance and enhance data comparability across countries (Hope, 2024; WHO, 2020). Similarly, open-access genomic platforms such as Pathogenwatch, NCBI Pathogen Detection, and PATRIC facilitate the rapid identification of resistance mechanisms and track the international dissemination of AMR clones (Hendriksen *et al.,* 2019b; Wattam *et al*., 2017).

In South Asia and Bangladesh, the adoption of genomic epidemiology remains limited but is gaining momentum. Whole-genome sequencing studies have revealed the circulation of globally disseminated *E. coli* lineages such as ST131, ST410, and ST648, alongside the presence of plasmid-borne carbapenemases (e.g., bla_{NDM}), colistin resistance genes (e.g., *mcr-1*), and tigecycline resistance genes [e.g., *tet*(X4)] in both clinical and animal isolates (Nisa *et al*., 2024; Ahmed *et al*., 2020).

Despite its transformative potential, genomic epidemiology faces challenges in LMIC contexts, including high sequencing costs, limited local sequencing and bioinformatics capacity, and restricted intersectoral coordination. However, strengthening genomic infrastructure, investing in workforce training, and promoting global data-sharing initiatives could help overcome these barriers (WHO, 2022a). By integrating genomic epidemiology into routine AMR surveillance, LMICs like Bangladesh can better map resistance dynamics, detect emerging threats in real time, and design evidence-based interventions that align with both national action plans and global AMR strategies (Struelens *et al*., 2024; Ahmed *et al*., 2022).

1.8 *Escherichia coli*: taxonomy, biology, and clinical relevance

Within the context above, *E. coli* emerges as an ideal sentinel organism for One Health–based genomic surveillance. Its ubiquity across human, animal, and environmental niches, coupled with its remarkable genomic plasticity and capacity to acquire and disseminate resistance determinants, makes it a valuable proxy for studying AMR transmission and evolutionary dynamics. Before exploring its role as an indicator species, it is necessary to understand *E. coli's* biology, pathogenic potential, and resistance mechanisms.

1.8.1 Taxonomy, discovery, and genetic characteristics of *E. coli*

E. coli is a Gram-negative, rod-shaped, facultatively anaerobic bacterium belonging to the family *Enterobacteriaceae* (Tenaillon *et al*., 2010). It was first described in 1885 by the German Austrian paediatrician Theodor Escherich, who isolated the bacterium from infant faeces and initially named it *Bacterium coli commune* (Escherich, 1889). Later, in recognition of his work, the organism was renamed *Escherichia coli*.

Taxonomically, *E. coli* belongs to the domain Bacteria, phylum *Proteobacteria*, class *Gammaproteobacteria*, order *Enterobacterales*, and genus *Escherichia* (Adeolu *et al*., 2016). Within the genus *Escherichia*, *E. coli* is the type species, closely related to *E. albertii*,

E. fergusonii, and *E. marmotae*. Genomic studies suggest high diversity, with average genome sizes ranging between 4.5–5.5 Mb and harbouring ~4,000–5,500 coding sequences (Touchon *et al*., 2009; Blattner *et al*., 1997). The species displays remarkable genetic plasticity, which underlies its ability to adapt to diverse ecological niches and acquire mobile genetic elements, including plasmids, transposons, and bacteriophages.

The first complete genome sequence of *E. coli* K-12 strain MG1655 was published in 1997, representing a landmark in bacterial genomics (Blattner *et al*., 1997). Since then, thousands of *E. coli* genomes have been sequenced, revealing its extraordinary genomic diversity and evolutionary dynamics.

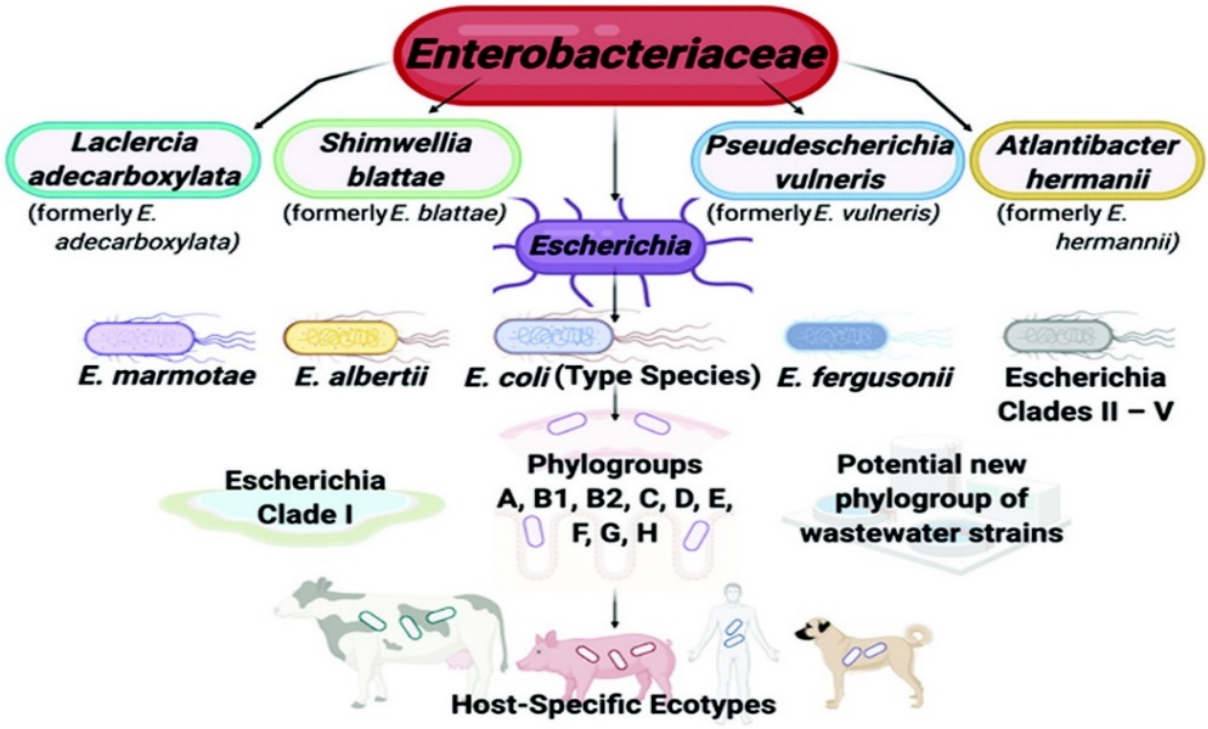


Figure 1.7 Taxonomic diversity and phylogroups within the genus Escherichia [Adapted from Yu *et al*., 2021, licensed under Creative Commons Attribution (CC BY 4.0)].

1.8.2 *E. coli* as a commensal and indicator organism

E. coli is one of the earliest colonisers of the gastrointestinal tract (GIT) of humans and animals, often acquired within hours of birth through contact with the mother and environment (Nowrouzian *et al*., 2001). In healthy hosts, *E. coli* exists predominantly as a commensal, residing in the colon and cecum, where it can reach concentrations of 10^7–10^9 CFU per gram of faeces in humans and slightly lower densities (10^4–10^6 CFU/g) in animals (Leimbach *et al*., 2013; Penders *et al*., 2006).

The relationship between host and bacterium is usually defined as commensalism. The bacterium benefits from nutrients, warmth, and shelter within the gut, while the host is largely unaffected.

However, commensal *E. coli* also provides benefits by competing with pathogenic bacteria for adhesion sites and producing antimicrobial peptides such as colicins and microcins, which contribute to colonization resistance (Sassone-Corsi & Raffatellu, 2015).

Importantly, *E. coli* plays a central role as an indicator organism of faecal contamination. Because it is abundant in the intestines of warm-blooded animals and readily detected in water, food, and environmental samples, *E. coli* has long been used to monitor sanitary quality and the risk of enteric pathogen transmission (WHO, 2022b). Detection of *E. coli* in drinking water is widely interpreted as evidence of recent faecal contamination (WHO, 2022b); recent field work also documents frequent detection of *E. coli* across household media, including drinking water (Deblais *et al*., 2025). Thus, *E. coli* simultaneously represents a harmless commensal, a sentinel of environmental hygiene, and, in some cases, a reservoir for AMR genes, which makes it uniquely relevant in both microbiology and public health contexts.

1.8.3 Pathogenesis of *E. coli*

Although most *E. coli* strains exist as harmless commensals, certain lineages have acquired distinct virulence factors that allow them to colonize new niches, evade host defenses, and cause a wide spectrum of diseases in humans and animals. These virulence attributes are often encoded on mobile genetic elements such as plasmids, bacteriophages, and pathogenicity islands, which can be transferred between strains, creating unique combinations of pathogenic traits (Desvaux *et al*., 2020; Croxen *et al*., 2013; Kaper *et al*., 2004).

Pathogenic *E. coli* strains are broadly categorized into intestinal pathogenic *E. coli* (IPEC), which primarily cause diarrheal diseases, and extraintestinal pathogenic *E. coli* (ExPEC) (Figure 1.8), which are associated with infections outside the gastrointestinal tract (Pokharel *et al*., 2023).

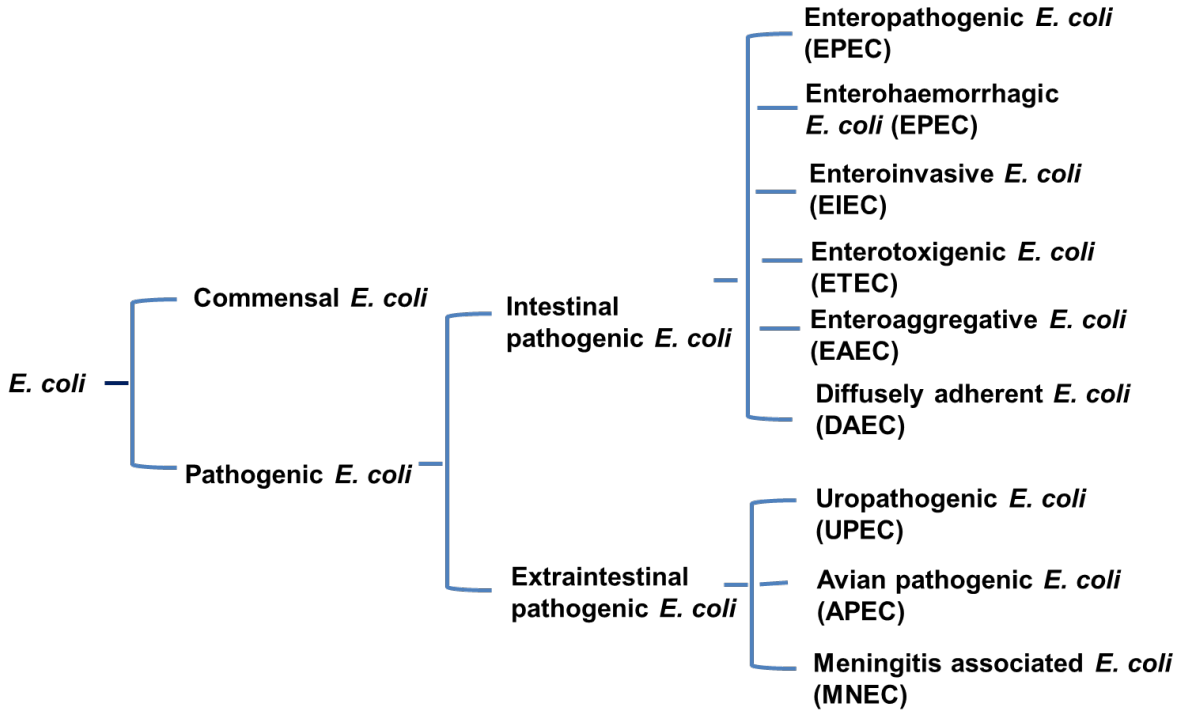


Figure 1.8 Classification of pathogenic *E. coli.* The figure was created with Microsoft PowerPoint.

Intestinal pathogenic *E. coli* (IPEC)

Six major pathotypes of diarrheagenic *E. coli* are recognised, each defined by characteristic virulence factors and mechanisms of pathogenesis:

- Enterotoxigenic *E. coli* (ETEC): Produces heat-labile (LT) and/or heat-stable (ST) enterotoxins, often associated with travelers' diarrhea in humans and diarrhea in calves and piglets (Alhadlaq *et al*., 2024).

- Enteropathogenic *E. coli* (EPEC): Characterized by the formation of attaching and effacing (A/E) lesions on intestinal epithelial cells mediated by the locus of enterocyte effacement (LEE) pathogenicity island. EPEC is a significant cause of infantile diarrhoea in LMICs (Alhadlaq *et al*., 2024).
- Enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC): Produces Shiga toxins (Stx1 and Stx2) that can cause hemorrhagic colitis and hemolytic uremic syndrome (HUS). The serotype O157:H7 is the most notorious, often linked to contaminated beef and dairy products (Alhadlaq *et al*., 2024; Lee *et al*., 2024).
- Enteroaggregative *E. coli* (EAEC): Forms aggregative "stacked brick" adherence on epithelial cells, associated with persistent diarrhoea and malnutrition in children (Alhadlaq *et al*., 2024; Pokharel *et al*., 2023; Hebbelstrup Jensen *et al*., 2014).
- Diffusely adherent *E. coli* (DAEC): Characterised by diffuse adherence patterns mediated by adhesins, linked to diarrhoea in children (Alhadlaq *et al*., 2024).
- Enteroinvasive *E. coli* (EIEC) invades intestinal epithelial cells and causes a dysentery-like illness similar to that of *Shigella* spp., as the two share a close genetic similarity (Alhadlaq *et al*., 2024).

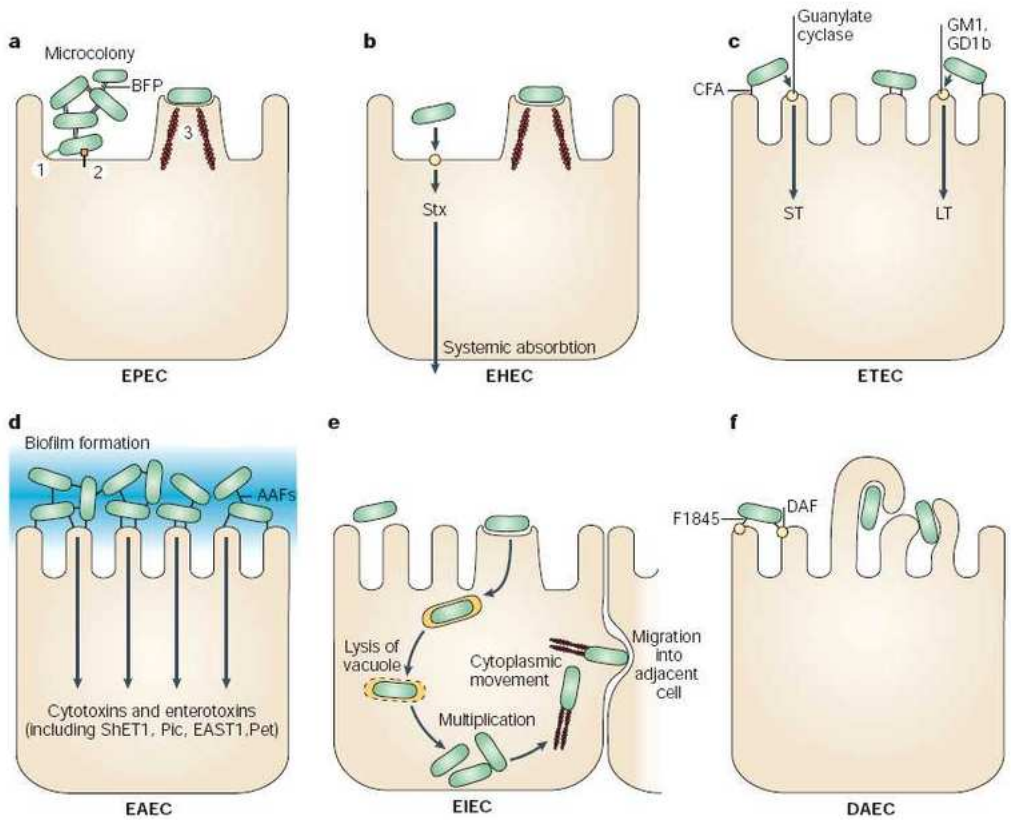


Figure 1.9 Pathogenic schema of intestinal *E. coli*, illustrating their interaction with intestinal epithelial cells (Adapted from Kaper *et al*., 2004, with permission from Springer Nature). AAF, aggregative adherence fimbriae; BFP, bundle-forming pilus; CFA, colonization factor antigen; DAF, decay-accelerating factor; EAST1, enteroaggregative *E. coli* ST1; GM1, GD1b, Ganglioside; LT, heat-labile enterotoxin; ShET1, *Shigella* enterotoxin 1; ST, heat-stable enterotoxin.

Extraintestinal pathogenic *E. coli* (ExPEC)

ExPEC strains are responsible for a range of infections outside the gut and are often equipped with adhesins, siderophores, toxins, and capsules that allow them to survive in extraintestinal environments. Major ExPEC pathotypes include:

- Uropathogenic *E. coli* (UPEC): The leading cause of urinary tract infections (UTIs), responsible for cystitis, pyelonephritis, and occasionally urosepsis. UPEC strains frequently express adhesins (P fimbriae, type 1 fimbriae), toxins (α-hemolysin), and siderophores (García-García *et al*., 2025; Sung *et al*., 2024).
- Neonatal meningitis-associated *E. coli* (NMEC): A primary cause of neonatal meningitis, often associated with the K1 capsular antigen that enables crossing of the blood–brain barrier (Sun *et al*., 2025; Nguyen *et al*., 2024).
- Avian pathogenic *E. coli* (APEC): Causes colibacillosis in poultry, leading to respiratory infections, septicemia, and pericarditis. APEC shares many virulence genes with ExPEC strains from humans, raising zoonotic concerns (Watts & Wigley, 2024; Li *et al*., 2024).

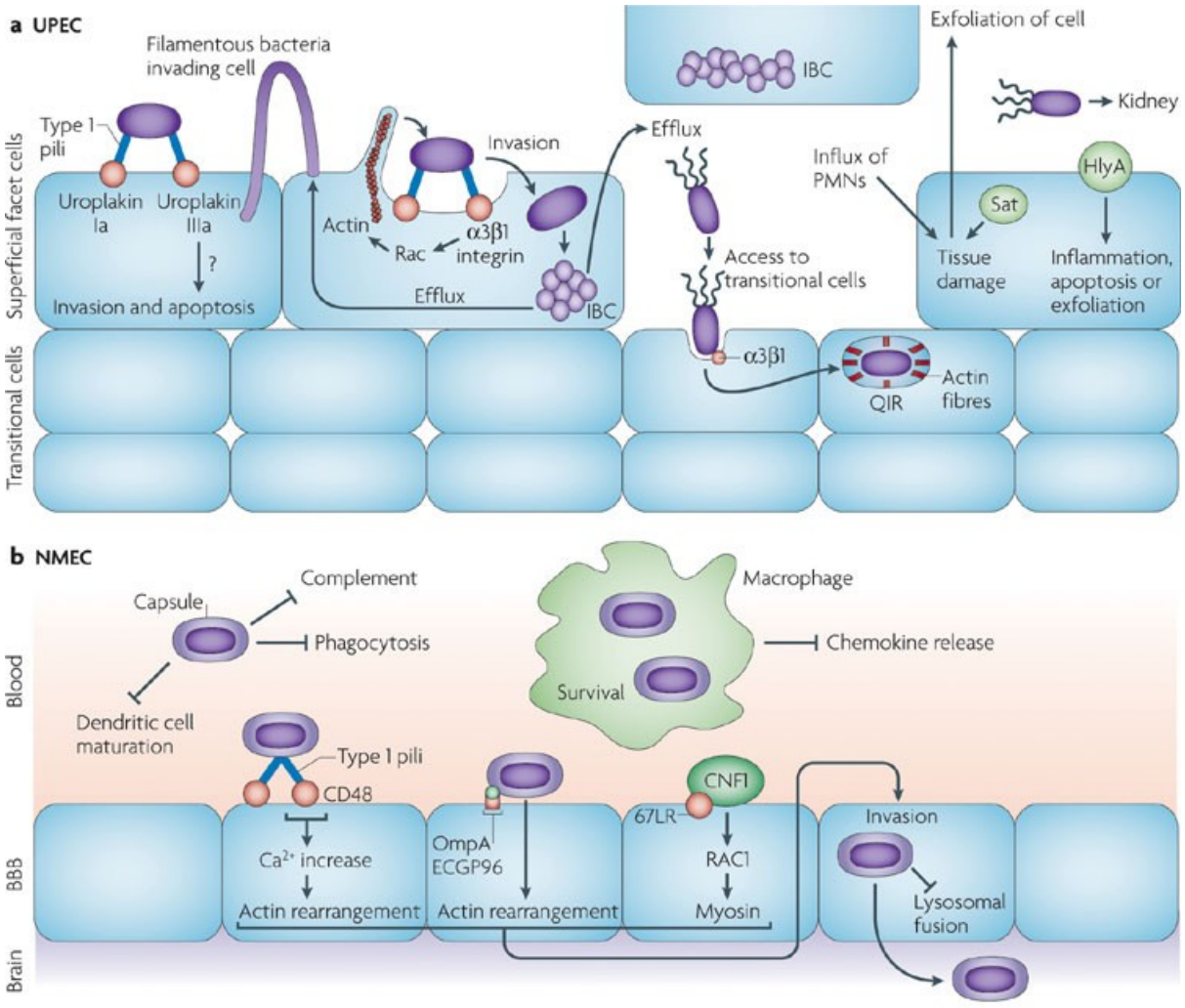


Figure 1.10 Pathogenic mechanisms of extraintestinal *E. coli* (Adapted from Croxen and Finlay, 2010, with permission from Springer Nature). BBB, blood-brain barrier; CNF1, Cytotoxic Necrotising Factor 1; HlyA, Hemolysin A; IBCs, intracellular bacterial communities; PMN, polymorphonuclear leukocyte; OmpA, Outer Membrane Protein A; Sat, secreted autotransporter toxin.

In addition to these, *E. coli* is a frequent cause of bloodstream infections, surgical site infections, and hospital-acquired infections, with increasing multidrug resistance complicating treatment (Bucataru *et al*., 2023).

Together, these diverse pathotypes underscore the dual nature of *E. coli*: while a majority exist as harmless commensals, pathogenic strains exploit virulence factors and horizontal gene transfer to cause severe, sometimes life-threatening, infections in humans and animals.

1.8.4 Virulence factors of *E. coli*

The ability of *E. coli* to cause disease is determined by its diverse arsenal of virulence factors (VFs), which enable colonization, immune evasion, and host damage. Key virulence determinants include adhesins such as fimbriae (e.g., type 1 fimbriae, P fimbriae, curli) that promote attachment to mucosal and urinary tract epithelia, surface antigens (O, H, and K antigens) that aid in immune evasion, and toxins such as Shiga toxins, heat-labile and heat-stable enterotoxins, and α-hemolysin, which disrupt host cell function (Edison *et al*., 2024; Croxen *et al*., 2010). Many ExPEC strains also produce siderophores (e.g., enterobactin, aerobactin, yersiniabactin) to acquire iron in extraintestinal niches, while EAEC and EPEC exploit type III secretion systems for intimate host–cell interactions. The distribution of these virulence factors varies across phylogroups: strains from phylogroups B2 and D are often more virulent, whereas A and B1 groups are commonly commensal (Tenaillon *et al*., 2010; Clermont *et al*., 2000). Importantly, the clustering of multiple virulence factors within single clones, such as ST131, enhances pathogenic potential and explains their global epidemiological success (Pitout & DeVinney, 2017).

1.8.5 Clonality of *E. coli*

The concept of bacterial clonality refers to populations derived from a single ancestral cell through non-sexual reproduction, leading to genetically similar lineages with shared evolutionary traits. In *E. coli*, clonality plays a central role in the global dissemination of AMR. While commensal

strains are genetically diverse, several high-risk clones have emerged over the last two decades, combining multidrug resistance with virulence traits (Arredondo-Alonso *et al*., 2025).

Among these, ST131 is the most prominent global clone, associated with extended-spectrum β-lactamase (ESBL) production, particularly *bla*$_{\text{CTX-M-15}}$, and fluoroquinolone resistance (Mathers *et al*., 2015; Nicolas-Chanoine *et al*., 2014). Genomic analyses suggest that ST131 diversified into distinct subclades (A, B, and C), with one clade emerging in the early 2000s and rapidly disseminating worldwide, driven largely by plasmid acquisition (Stoesser *et al*., 2016). This diversification resulted in the emergence of the C1 and C2 subclades, which have been identified as the most successful and globally disseminated ST131 lineages, strongly associated with fluoroquinolone resistance and carriage of *bla*$_{\text{CTX-M-15}}$. Other widely distributed ESBL-producing clones include ST405, ST648, ST410, and ST38, each linked to resistance plasmids and multidrug phenotypes (Mazumder *et al*., 2021; Pitout & Peirano, 2017).

Carbapenem resistance has also been linked to emerging clones, such as ST167, which commonly carries *bla*$_{\text{NDM-5}}$ *metallo*-β-lactamase genes, making it a notable high-risk lineage across Asia and beyond (Walker *et al*., 2025). Similarly, clones such as ST1011 have been associated with *mcr-1*-mediated colistin resistance, raising alarm due to their potential for pan-resistance (Li *et al*., 2022). Although carbapenemases like KPC-2 are less frequent in *E. coli* compared to *Klebsiella pneumoniae*, their presence within ST131 and related clones demonstrates the fluidity of horizontal gene transfer across Enterobacterales (Huang *et al*., 2024).

1.8.6 AMR in *E. coli*

Although *E. coli* is naturally susceptible to many antibiotics, it has become one of the most prominent bacterial species associated with AMR due to its remarkable genetic plasticity and ability to acquire resistance genes through horizontal gene transfer (HGT). Mobile genetic elements, including plasmids, integrons, transposons, and bacteriophages, are crucial for enabling *E. coli* to acquire and spread resistance genes across human, animal, and environmental niches (Mangroliya *et al*., 2025; Chekole *et al*., 2025; Poirel *et al*., 2018).

At the molecular level, plasmid-borne resistance genes confer antimicrobial resistance through several well-characterised mechanisms. Extended-spectrum β-lactamases (ESBLs), such as $bla_{\text{CTX-M}}$ variants, hydrolyse the β-lactam ring of third-generation cephalosporins, rendering these antibiotics ineffective. Carbapenemases, including bla_{NDM}, $bla_{\text{OXA-48}}$, and bla_{KPC}, further expand this activity to carbapenems, compromising last-resort therapies. Plasmid-mediated colistin resistance genes (*mcr*) encode phosphoethanolamine transferases that modify the lipid A component of lipopolysaccharide, reducing colistin binding to the bacterial outer membrane. Plasmid-mediated quinolone resistance (PMQR) genes, such as *qnr*, protect DNA gyrase and topoisomerase IV from fluoroquinolone inhibition, while *aac*(6′)-Ib-cr enzymatically modifies certain fluoroquinolones, leading to reduced susceptibility (Liu *et al*., 2016; Cantón *et al*., 2012; Nordmann *et al*., 2011; Robicsek *et al*., 2006). Together, these mechanisms enable plasmids to confer resistance across multiple antibiotic classes simultaneously. High-level aminoglycoside resistance is often conferred by 16S rRNA methylases (Jacoby *et al*., 2014; Doi & Arakawa, 2007). Chromosomal mutations in the quinolone resistance-determining regions (QRDRs) of *gyr*A and *par*C represent the most common mechanism of fluoroquinolone resistance in *E. coli*, occurring

more frequently than plasmid-mediated resistance and playing a key role in the emergence and global success of high-risk clones such as ST131 (Nicolas-Chanoine *et al*., 2014).

From a One Health perspective, the AMR problem in *E. coli* is exacerbated by its widespread exposure to antimicrobials across sectors. In human health, *E. coli* is a leading cause of urinary tract infections, bloodstream infections, and surgical site infections. Surveillance data show alarming increases in resistance to third-generation cephalosporins, aminoglycosides, and fluoroquinolones (Murray *et al*., 2022). In animal health, antimicrobials are extensively used for therapy, prophylaxis, and as growth promoters, especially in poultry and aquaculture. Extensive and often sub-therapeutic use of antimicrobials in poultry production creates sustained selective pressure that favours resistant *E. coli* over susceptible populations. Continuous antibiotic exposure eliminates sensitive bacteria while enabling resistant strains and resistance-carrying plasmids to persist, amplify, and spread. High-density poultry farming further facilitates horizontal gene transfer through frequent bacterial contact, allowing resistance genes to disseminate rapidly within flocks and into surrounding environments. These resistant bacteria and plasmids can then be transmitted to humans via direct animal contact, contaminated food products, and environmental pathways, reinforcing the One Health cycle of antimicrobial resistance (Van Boeckel *et al*., 2015; Woolhouse *et al*., 2015; Marshall & Levy, 2011). This has resulted in widespread carriage of ESBL- and *mcr*-positive *E. coli* in livestock (Malik *et al*., 2025; Odey *et al*., 2024). At the environmental interface, resistant *E. coli* is frequently recovered from rivers, sewage, wastewater, street food, and soil, often carrying high-risk clones and mobile resistance genes (Nadimpalli *et al*., 2021). These overlapping reservoirs highlight the continuous gene flow across humans, animals, and the environment.

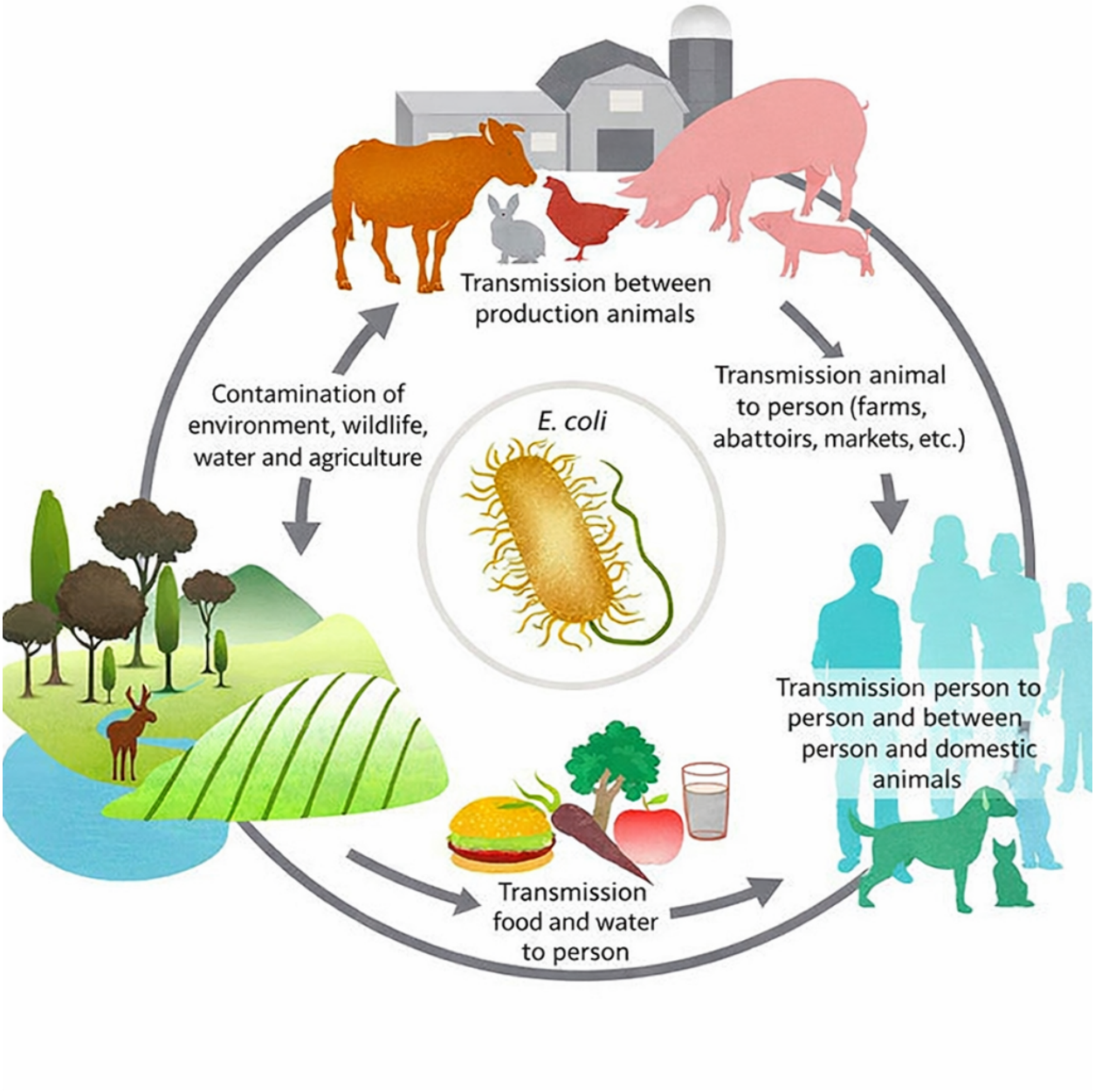


Figure 1.11 Transmission pathways of *E. coli* across the One Health spectrum. Schematic representation of how resistant *E. coli* circulates between humans, animals, food, and the environment through interconnected routes [Adapted from *E. coli* Lab, (n.d), reproduced with permission].

The situation is particularly concerning in Bangladesh, where dense human and animal populations, unregulated antibiotic use, and poor wastewater management accelerate resistance

dissemination. Poultry production, which represents a major contributor to national meat supply, frequently relies on broad-spectrum antimicrobials (e.g., β-lactams, fluoroquinolones, aminoglycosides, sulfonamides), often at sub-therapeutic levels without veterinary oversight (Flatgard *et al*., 2024; Samad *et al*., 2023). This has resulted in high prevalence of MDR *E. coli* isolates from poultry, livestock, aquaculture, and companion animals. In clinical settings, *E. coli* with bla_{NDM} and ESBL genes are increasingly reported, while wastewater and surface waters act as reservoirs for resistant clones and plasmids (Asaduzzaman *et al*., 2022).

A key feature that makes *E. coli* such an effective reservoir and disseminator of resistance is its ability to acquire and exchange genetic material. This capacity is largely driven by mobile genetic elements, particularly plasmids, which serve as the primary vehicles of horizontal gene transfer.

Plasmids are extrachromosomal DNA elements that play a key role in the dissemination of AMR genes. However, not all plasmids are mobile. Only conjugative plasmids that carry transfer (tra) genes and an origin of transfer (oriT) can self-transfer through conjugation. In contrast, non-conjugative plasmids can be mobilized in trans by co-existing conjugative plasmids. In *E. coli*, both conjugative and mobilisable plasmids contribute to AMR spread and persistence under antibiotic selection pressure. Plasmids often carry multiple resistance genes, facilitating the emergence of multidrug-resistant strains in both clinical and non-clinical environments (Partridge *et al*., 2018; Carattoli, 2013; Smillie *et al*., 2010).

Several incompatibility (Inc) plasmid types have been strongly associated with high-risk AMR genes. For instance, IncF-type plasmids are frequently linked to $bla_{\text{CTX-M}}$, *aac*(6′)-Ib-cr, and virulence genes in extraintestinal pathogenic *E. coli* (ExPEC) lineages such as ST131 (Phan *et al*., 2015; Woodford *et al*., 2011). IncX3 and IncX4 are notable for mobilizing carbapenemase

(*bla*$_{NDM}$) and colistin resistance (*mcr*) genes, respectively (Wu *et al*., 2019). Meanwhile, broad-host-range plasmids like IncA/C2, IncHI2, and IncI1 have been found in isolates from animals, humans, and the environment, highlighting their importance in cross-sectoral AMR spread under the One Health umbrella (Rozwandowicz *et al*., 2018; Carattoli, 2009).

Horizontal gene transfer facilitated by plasmids occurs not only within species but also across genera, enhancing the adaptability of bacterial communities. Mobile genetic elements, such as insertion sequences (IS), transposons, and integrons, often accompany plasmids, further increasing the potential for gene rearrangements and resistance amplification. In Bangladesh, studies have shown that food animals, flies, surface water, and even vegetables harbour *E. coli* with plasmids carrying *bla*$_{NDM}$, *bla*$_{OXA-48}$, *mcr-1*, and *tet*(X4) (Johura *et al*.,2022).

Genomic epidemiology has enabled detailed plasmid profiling through replicon typing, sequencing, and plasmid reconstruction (Orlek *et al*., 2017). This has revealed complex plasmidomes with diverse replicon types, structural variants, and co-localisation of resistance and virulence determinants. Understanding the role of these plasmids in AMR ecology is critical for risk assessment, outbreak tracing, and developing effective interventions, especially in LMICs where surveillance systems are still developing (Walas *et al*., 2023).

The presence of identical or similar plasmids in *E. coli* from multiple host types (human, animal, and environmental) reinforces the idea that plasmid-mediated resistance is not confined to a single domain. Rather, it circulates across ecosystems, mirroring the interconnectedness central to the One Health paradigm (Rozwandowicz et al., 2018; Carattoli, 2013). Among the diverse resistance determinants disseminated via plasmids, carbapenemases, mobile colistin resistance genes, and the

emerging tigecycline resistance determinant *tet*(X) represent particularly urgent threats, which will be discussed in the following section (He *et al*., 2019).

1.9 Critical antimicrobial resistance determinants in *E. coli*

Beyond conferring resistance to commonly used antimicrobials, plasmids have enabled the global spread of resistance genes against "last-resort" antibiotics, including carbapenems, colistin, and tigecycline. The emergence of plasmid-borne determinants such as $bla_{\text{NDM-5}}$, *mcr-1*, and *tet*(X4) has redefined the threat landscape of AMR by raising the possibility of pan-resistant bacterial strains with no effective therapeutic options. Understanding the origins, mechanisms, and dissemination of these resistance determinants is therefore critical for contextualising the AMR burden in Bangladesh within global trends. To place these critical resistance determinants within a clinical and stewardship framework, Table 1.1 summarises commonly used antibiotics according to the WHO AWaRe (Access, Watch, and Reserve) classification, together with their mechanisms of action and major resistance mechanisms relevant to *E. coli.*

Table 1.1 WHO AWaRe classification of antibiotics showing Access, Watch, and Reserve group of antibiotics

WHO AWaRe group	Antibiotic class	Common examples	Mechanism of action	Common resistance mechanisms
Access (first-line)	Penicillins	Amoxicillin, Ampicillin	Inhibit cell wall synthesis by binding PBPs	β-lactamases (TEM, SHV), altered PBPs
	Aminopenicillin + β-lactamase inhibitor	Amoxicillin–clavulanate	Cell wall synthesis inhibition	ESBLs, inhibitor-resistant β-lactamases
	Folate pathway inhibitors	Trimethoprim–sulfamethoxazole	Inhibit folate synthesis	Target modification
	Tetracyclines	Doxycycline	Inhibit protein synthesis (30S ribosome)	Efflux pumps (*tetA*), ribosomal protection
Watch (higher resistance potential)	Fluoroquinolones	Ciprofloxacin, Levofloxacin	Inhibit DNA gyrase and topoisomerase IV	QRDR mutations (*gyrA*, *parC*), PMQR genes
	3rd-generation cephalosporins	Cefotaxime, Ceftazidime	Inhibit cell wall synthesis	ESBLs (e.g. $bla_{\text{CTX-M}}$)
	Aminoglycosides	Gentamicin, Amikacin	Inhibit protein synthesis (30S ribosome)	Aminoglycoside-modifying enzymes, 16S rRNA methylases
	Macrolides	Azithromycin	Inhibit protein synthesis (50S ribosome)	Target modification, efflux pumps
Reserve (last-resort)	Carbapenems	Imipenem, Meropenem	Broad-spectrum inhibition of cell wall synthesis	Carbapenemases (bla_{NDM}, bla_{KPC}, $bla_{\text{OXA-48}}$)
	Polymyxins	Colistin	Disrupt outer membrane (lipid A binding)	Lipid A modification (*mcr* genes)
	Glycylcyclines	Tigecycline	Inhibit protein synthesis (30S ribosome)	Efflux pumps, enzymatic inactivation [*tet*(X)]
	Oxazolidinones	Linezolid	Inhibit protein synthesis (50S ribosome)	Target mutation, methylation (*cfr*)

1.9.1 Carbapenem resistance and the *bla*$_{\text{NDM-5}}$ gene

Carbapenems are β-lactam antibiotics introduced in the 1980s and considered one of the most reliable treatments against multidrug-resistant Gram-negative infections (Sreejith *et al*., 2023; Papp-Wallace *et al*., 2011). They exert their bactericidal activity by binding to penicillin-binding proteins, thereby inhibiting bacterial cell wall synthesis and displaying high stability against most β-lactamases. Resistance to carbapenems arises predominantly through carbapenemase enzymes, among which the New Delhi metallo-β-lactamase (NDM) family has had the greatest global impact. The first *bla*$_{\text{NDM}}$ gene was reported in *Klebsiella pneumoniae* and *E. coli* isolated from a Swedish patient with prior hospitalisation in India (Kumarasamy *et al*., 2010; Yong *et al*., 2009). Since its initial discovery, the NDM family has rapidly diversified through amino acid substitutions, resulting in numerous variants with differing enzymatic properties. According to the National Center for Biotechnology Information (NCBI) β-lactamase database, 67 distinct NDM variants have been recognised [(NCBI, (accessed on 26 October 2024); Al-Marzooq *et al*., 2024] with *bla*NDM-5 being particularly significant.

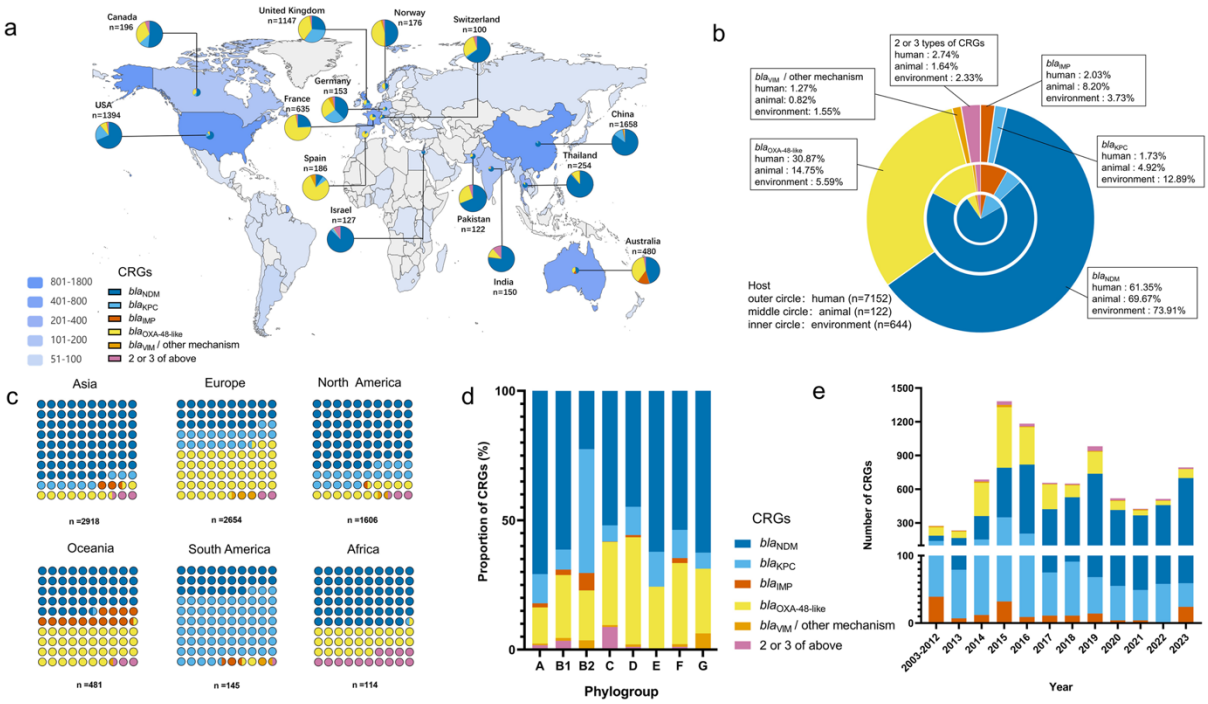


Figure 1.12 a: The global distribution of carbapenem-resistant *E. coli* (CREC) and the proportion of different carbapenem-resistant genes (CRGs) in countries where strains exceeding 100 have been reported. **b:** A circular plot displaying the distribution of CRGs across various sources is represented as percentages. **c:** the distribution of CRGs across the six continents. **d:** the diversity of CRGs across different phylogroups of *E. coli*. **e:** A histogram is presented above, illustrating the distribution of various CRGs expressed as percentages from 2003 to 2023, CRGs, carbapenem resistance genes [Adapted from Huang *et al*., 2024, licensed under Creative Commons Attribution (CC BY 4.0)].

bla$_{\text{NDM-5}}$ was first identified in *E. coli* in the United Kingdom in 2011 from a patient with a history of travel to India (Hornsey *et al*., 2011). Compared to *bla*$_{\text{NDM-1}}$, it carries two amino acid substitutions (Val88Leu and Met154Leu) that enhance hydrolytic efficiency and confer higher levels of resistance to carbapenems and extended-spectrum cephalosporins. These genes are often

carried on IncX3 and IncF plasmids, which are highly transmissible across bacterial hosts (Qamar *et al*., 2025; Ma *et al*., 2024).

Globally, *bla*$_{\text{NDM-5}}$-positive *E. coli* has been reported in diverse reservoirs, including clinical isolates, livestock, companion animals, wastewater, and food. High-risk clones such as ST167, ST410, and ST131 are strongly associated with *bla*$_{\text{NDM-5}}$ (Chanchaithong *et al*., 2025).

In Bangladesh, NDM-producing Enterobacteriaceae are increasingly reported, with *E. coli* among the most common carriers (Mazumder *et al*., 2021). However, most studies rely on phenotypic detection or PCR-based screening, and detailed genomic epidemiology, including plasmid characterisation, clonal analysis, and transmission mapping, remains limited. This creates a critical gap in understanding the dynamics of *bla*$_{\text{NDM-5}}$ in Bangladesh, particularly in hospital settings where carbapenems are widely used and in the community, where weak antibiotic stewardship exacerbates the emergence of resistance.

1.9.2 Colistin resistance and the *mcr-1* gene

Colistin (polymyxin E) was introduced in the 1950s but was largely abandoned due to nephrotoxicity and neurotoxicity, only to re-emerge in the 2000s as a last-line antibiotic against carbapenem-resistant bacteria (Binsker *et al*., 2022; El-Sayed Ahmed *et al*., 2020; Falagas & Kasiakou, 2005). Colistin exerts its bactericidal activity by binding to the lipid A component of lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria, disrupting membrane integrity and leading to cell death.

The discovery of *mcr-1* in *E. coli* from pigs, pork, and human samples in China in 2015 revolutionised the field (Liu *et al*., 2016). *mcr-1* encodes a phosphoethanolamine transferase that

modifies lipid A of lipopolysaccharide, decreasing colistin binding affinity and thus conferring resistance. Unlike chromosomal mutations, plasmid-borne *mcr-1* can spread horizontally between bacteria, rapidly disseminating resistance across species and ecosystems.

To date, at least ten *mcr* variants (*mcr-1* to *mcr-10*) have been reported, with *mcr-1* remaining the most widespread (Ye *et al*., 2016). Globally, *mcr-1* has been detected in clinical, veterinary, food, and environmental *E. coli* isolates from over 50 countries (Matamoros *et al*., 2017). High-risk plasmid types, including IncI2, IncHI2, and IncX4, have been major vehicles for *mcr-1* dissemination (Mei *et al*., 2024).

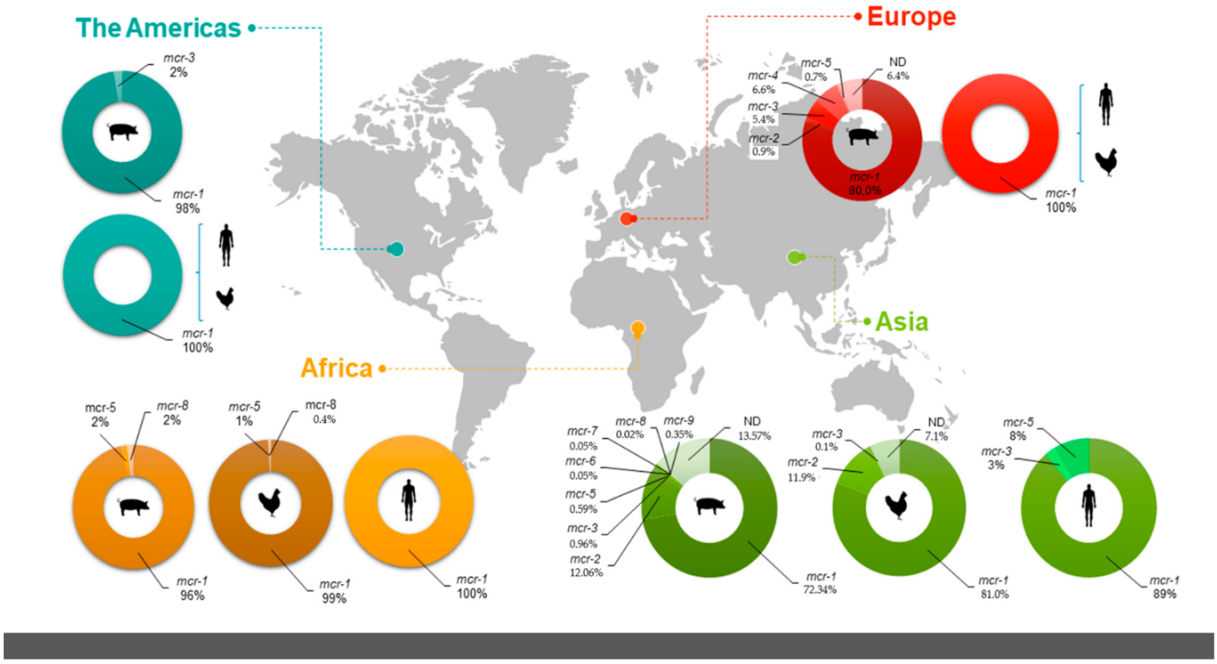


Figure 1.13 Global distribution of prevalence and diversity of *mcr* variants of *E. coli* isolates described in community studies of healthy humans, pigs, and chickens. The colored pie charts represent the percent distribution of *mcr* variants in each continent, light green, Asia; red, Europe; yellow, Africa; dark green, the Americas. The small figure silhouettes indicate the hosts (healthy

humans, pigs, or chickens). [Adapted from Bastidas-Caldes *et al*., 2022, licensed under Creative Commons Attribution (CC BY 4.0)].

In Bangladesh, colistin resistance has been documented across multiple reservoirs, including *E. coli* from poultry, aquaculture, hospital wastewater, and clinical isolates, underscoring the widespread circulation of *mcr*-positive strains (Sharif *et al*., 2025; Tanzin *et al*., 2024; Ali *et al*., 2024).

Although the Directorate General of Drug Administration (DGDA) officially banned the use of colistin in livestock and poultry production in 2019, reports of resistant isolates continue to emerge, reflecting the persistence of resistance genes in animal and environmental reservoirs and the possibility of unauthorised use (Al Asad *et al*., 2024; DGDA, 2019).

1.9.3 Tigecycline resistance and the *tet*(X4) gene

Tigecycline, a glycylcycline antibiotic derived from minocycline, was approved in 2005 and specifically developed to overcome traditional tetracycline resistance mechanisms, including efflux pumps and ribosomal protection proteins (Su *et al*., 2024). Tigecycline binds to the 30S ribosomal subunit with high affinity, thereby inhibiting protein synthesis and retaining activity against MDR organisms such as extended-spectrum β-lactamase (ESBL)-, carbapenem-, and colistin-resistant *E. coli* (Stein & Babinchak, 2013). For over a decade, tigecycline resistance was thought to arise exclusively from chromosomal mechanisms, primarily overexpression of multidrug efflux systems and other resistance-nodulation-division (RND) family pumps (Linkevicius *et al*., 2016). The World Health Organisation (WHO), through its AWaRe classification framework, designates tigecycline as a "Reserve" antibiotic, meaning it should be preserved for the treatment of confirmed or suspected infections due to multidrug-resistant

pathogens when no other alternatives are available (Wang & Li, 2024; WHO, 2019). Globally, its clinical use is increasing, particularly in tertiary care hospitals managing severe MDR infections.

A turning point occurred in 2019 when He *et al*. identified two plasmid-mediated tigecycline resistance genes, *tet*(X3) and *tet*(X4), in *E. coli* and *Acinetobacter* spp. from livestock and the environment in China (He *et al*., 2019). These genes encode flavin-dependent monooxygenases that enzymatically inactivate tigecycline and other next-generation tetracyclines, including eravacycline and omadacycline, by oxidizing the antibiotic's active structure (Sun *et al*., 2020; He *et al*., 2019). The presence of *tet*(X4) on conjugative plasmids such as IncQ1, IncX1, and IncFII has accelerated its dissemination potential, as these plasmids exhibit high transferability between bacterial species and across ecosystems (Sun *et al*., 2020; Fang *et al*., 2020).

Since its discovery, *tet*(X4) has spread rapidly across Asia, being reported in *E. coli* from swine, poultry, retail meat, wastewater, and even human clinical isolates in China, Vietnam, Laos, and Singapore (Liu *et al*., 2024; Zhang *et al*., 2022). Meta-analyses suggest that livestock-associated *E. coli* is acting as a major reservoir for *tet*(X4), with evidence of zoonotic spillover through the food chain (Fan *et al*., 2024).

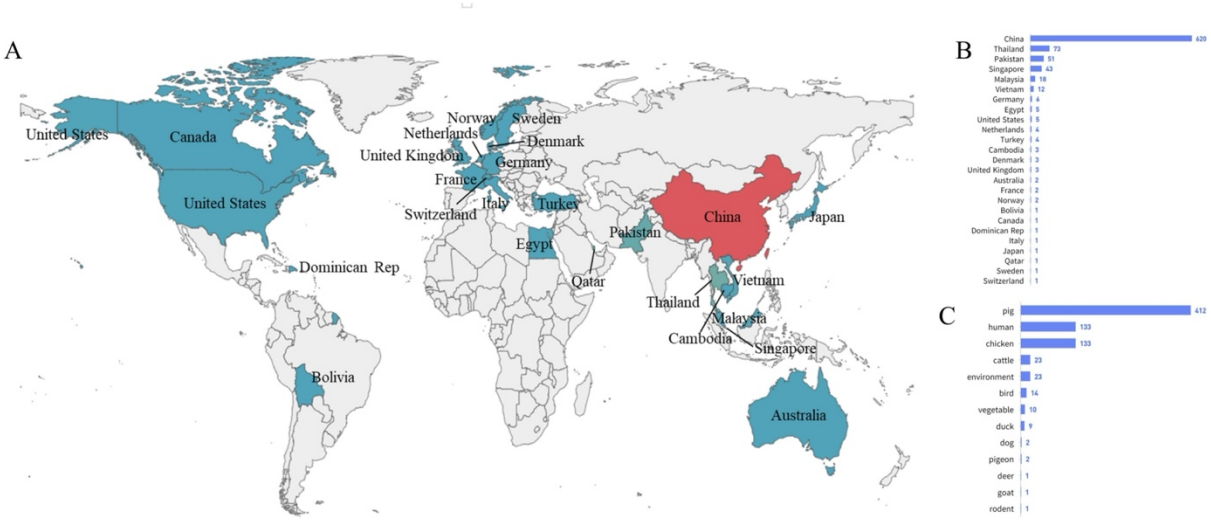


Figure 1.14 Global distribution of 864 *tet*(X)-positive *E. coli* isolates. (A) Global distribution of *tet*(X)-positive *E. coli*. The redder the color, the greater the number of *tet*(X)-positive *E. coli* isolated in that country. (B) Bar graph of the number of *tet*(X)-positive isolates by different countries. (C) Bar graph of the number of *tet*(X)-positive isolates by different hosts (Adapted from Li *et al*., 2023, with permission from Elsevier).

Globally, *tet*(X4) remains concentrated in Asia, but sporadic reports from Europe and North America indicate its emerging spread (Zhang *et al*., 2022; Fang *et al*., 2020). The risk of global dissemination is heightened by international trade in food animals, meat products, and the potential for environmental contamination through agricultural waste.

1.10 Objectives and scope of the thesis

This thesis aims to explore the genomic epidemiology of *E. coli* AMR within a One Health framework in Bangladesh. The overarching objective is to investigate the clonal diversity, resistance profiles and plasmid characteristics of *E. coli* across human, animal, and environmental

reservoirs using both phenotypic and genotypic approaches, with a focus on understanding the underlying drivers of resistance transmission and evolution.

Specific objectives:

1. To establish and describe a One Health-based sampling framework in Bangladesh, including site selection and sample size determination across human, animal, and environmental sources, to generate representative data for AMR analysis.
2. To determine the AMR profiles and epidemiology of *E. coli* isolates from diverse One Health sources, including human clinical infections, livestock, food animals, water, flies, and environmental samples from Bangladesh.
3. To characterise the genomic diversity of *E. coli* isolates using molecular typing methods (such as MLST and phylogroup analysis), and to identify the dominant clonal lineages across different reservoirs. At the same time, to investigate how key virulence genes are distributed among these isolates, and whether their presence is associated with particular sequence types (STs), phylogroups, or sources of isolation.
4. To identify the plasmid replicon types and associated mobile genetic elements (MGEs), including the carriage of important AMR genes. To assess the contribution of plasmids and horizontal gene transfer in the spread of AMR traits within and between One Health sectors.

Research questions:

Addressing the objectives of this study, the following research questions were formulated:

Research question 1: How can a One Health–based sampling framework be established across human, animal, and environmental sources to generate representative data for antimicrobial resistance analysis?

Research question 2: What are the antimicrobial resistance profiles of *E. coli* isolates obtained from diverse One Health sources, including human clinical infections, livestock, food animals, insects, water, and environmental samples from Bangladesh?

Research question 3: What is the genomic diversity of *E. coli* isolates across One Health reservoirs as determined by molecular typing methods, and which clonal lineages predominate in different sources? How are the key virulence genes distributed among these isolates, and are they associated with specific sequence types, phylogroups, or sources of isolation?

Research question 4: Which plasmid replicon types and mobile genetic elements are present in *E. coli* isolates, and how do plasmids and horizontal gene transfer contribute to the dissemination of antimicrobial resistance within and between sampling sectors?

Scope of the study:

This research examines 1634 *E. coli* isolates collected from a wide range of One Health sources. Both phenotypic antimicrobial susceptibility testing and WGS have been used to provide a comprehensive view of resistance. The study not only investigates known resistance genes and plasmid types but also investigates new genomic features that may be emerging in local bacterial

populations. Emphasis is placed on sequence types such as ST131, ST648, and ST410, which are recognised globally for their pathogenicity and resistance. By mapping resistance across different reservoirs, this thesis contributes to national AMR surveillance and informs One Health policymaking. Ultimately, the findings aim to improve our understanding of the complex ecology of AMR in *E. coli*, providing insights necessary for targeted interventions in human health, animal health and environmental management.

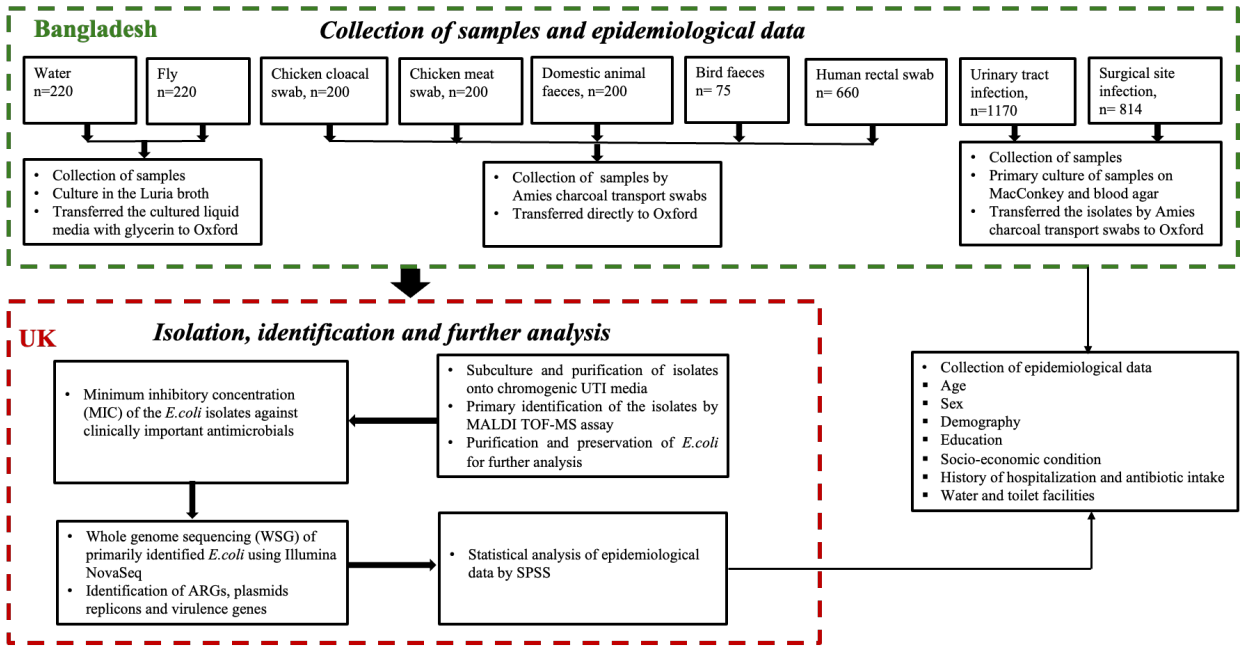
Chapter 2

Materials and Methods

2.1 Study design and sampling overview

This study was designed as a prospective cross-sectional investigation under a One Health framework, integrating the human, livestock, and environmental sectors. A detailed description of the study design, sampling site selection, and collection period is provided in Chapter 3 (One Health sampling strategies for investigating AMR transmission dynamics in Bangladesh). In brief, samples were collected in the Mymensingh district of Bangladesh over four seasonal terms between December 2021 and March 2023.

The sampling framework included human, animal, and environmental reservoirs. Human samples comprised urine, surgical site infection swabs, and rectal swabs from healthy volunteers. Farm samples included chicken cloacal swabs, chicken meat swabs, rectal swabs from domestic animals, and faeces from free-flying birds around farms. Environmental samples were obtained from water sources and flies collected in and around farms and the Mymensingh Medical College Hospital.



❖ MALDI TOF-MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry
Chi-square tests were performed to analyze the relationship between categorical variables and one-way ANOVA to analyze trends of different time points

Figure 2.1 Integration of work packages (WP)

2.2 Collection of samples

Clinical samples (urine and surgical site infection swabs) were collected from Mymensingh Medical College Hospital (MMCH). The farm samples included chicken cloacal swabs (CCS), chicken meat swabs (CMS), domestic animal rectal swabs (DARS) from animals reared around the farms, and free-flying bird faeces (BF) found near the farm. The environmental samples water and flies were collected from and around the farms and MMCH.

Ethical approval for both human and farm sample collection was obtained from the Mymensingh Medical College, Animal Welfare and Experimentation Ethics Committee (AWEEC) of Bangladesh Agricultural University and from the Oxford Tropical Research Ethics Committee (OxTREC) at the University of Oxford (Details Provided in 3.2.5). The number of total collected samples is shown in Table 2.1.

Table 2.1 Total number of collected samples in this study

Sample type	Number of collected samples				
	Quarter1	**Quarter2**	**Quarter3**	**Quarter4**	**Total**
Water	55	55	55	55	**220**
Fly	55	55	55	55	**220**
Chicken cloacal swab	50	50	50	50	**200**
Chicken meat swab	50	50	50	50	**200**
Domestic animal rectal swab	50	50	50	50	**200**
Bird faeces	6	22	18	29	**75**
Urinary tract infection	216	391	301	262	**1170**
Surgical site infection	94	196	336	188	**814**
Human rectal swab	165	165	165	165	**660**
Total	**741**	**1034**	**1080**	**904**	**3759**

2.2.1 Collection of farm samples

All these samples were collected using Amies transport swabs with charcoal. All necessary hygienic and safety measures were taken during the collection of each type of sample, and proper precautions were taken to ensure that no animal or bird was harmed. Broiler and layer farms from each subdistrict were selected based on the inclusion criteria (detailed in Section 3.2.6)

For collecting CCS, the sterile swab stick was used. Each bird was held securely, and the entire tip of the swab was gently inserted into the cloaca through the vent. The cloacal mucosa was sampled by rotating the swab two to four times while applying gentle pressure against the mucosal surface. Following collection, the swab was immediately placed into the transport reagent tube, the lid was tightly secured, and the tube was labelled with the appropriate identification code.

To collect CMS, the sterile swab stick was moistened with sterile phosphate-buffered saline (PBS). The swab was subsequently wiped over the carcass, both vertically and horizontally, while applying gentle pressure against the surface. The swab was placed in the sample tube and closed firmly and labelled with the appropriate identification code.

Rectal swabs from domestic animals were collected using Amies transport swabs. After carefully opening the swab package, the swab was inserted 4-5 cm into the anus and gently rotated for 10-15 seconds pressing on the mucus membrane. After taking out the stick, it was placed in the sample tube, and the lid was closed tightly and labelled with the appropriate identification code.

To collect the bird faeces, a clean sheet was placed near the farms, under the trees where birds defecated. Then, approximately 1 gram of freshly voided faeces was collected using a sterile swab. The sample was placed into an Amies transport swab tube containing charcoal, and the lid was closed tightly and labelled with the appropriate identification code.

2.2.2 Collection and processing of environmental samples

Water samples were collected from the designated sites using sterile 50 ml Falcon tubes. For each sample, 30 ml of water was obtained by inserting the tubes horizontally to a depth of approximately 30 cm below the surface. The tubes were immediately sealed, labelled, and transported to the microbiology laboratory at Bangladesh Agricultural University (BAU) for immediate processing. In the laboratory, samples were centrifuged at 3600 rpm for 10 minutes. After centrifugation, the supernatant was removed, and the pellet was washed twice with 10 mL of Luria broth (Sigma-Aldrich, Missouri, USA). For storage preparation, 1 mL of the pellet was combined with 1.4 mL of Luria broth and 600 µL of glycerol in a screw-cap tube, which was securely closed and wrapped with paraffin tape. The tubes were labelled and stored at −80°C until transport to the United Kingdom.

Fly traps were deployed near the waste pit inside and outside the farms and the hospital and left in place for 12 hours. After exposure, traps were collected and placed into separate sterile zipper bags before being transferred to the BAU microbiology laboratory. Each fly was carefully removed from the trap, using sterile forceps to recover as much of the specimen as possible, and placed into a 25 mL screw-cap sterile test tube containing 3 mL of Luria broth. Tubes were labelled and incubated overnight at 37°C in a shaking incubator set to 200 rpm. Following incubation, 2.4 mL of the cultured broth and 600 µL of glycerol were transferred to a sterile 5 mL screw-cap tube for preservation. This preparation was stored at −80°C until it was transported to the United Kingdom.

2.2.3 Collection and processing of clinical samples

Two types of clinical samples, i.e., urine and post-surgical infection wound swabs, were collected from outdoor patients and hospital-admitted patients, respectively. The urine specimens referred to the microbiology laboratory of Mymensingh Medical College (MMC) from outdoor patients

suspected of infection by physicians at MMCH were included in this study. Similarly, in the case of surgical site infection, the specimens (wound swabs) sent to the microbiology laboratory for culture, suspected of infection in the surgical site, by the physician (meeting the inclusion criteria of this study) were considered as samples. In both cases, the sample size was determined by the number of specimens referred to the laboratory per term. The specimens were plated primarily at the microbiology laboratory of MMC and were broadly divided into two groups: 1) positive culture, and 2) no growth. Positive cultures obtained at the laboratory of MMC were transferred to Oxford University for further analysis.

The SSI sampling procedure involved collecting swabs from infection sites when the wound was opened for dressing. The inclusion and exclusion criteria were detailed in 3.2.13. Swabs were taken before dressing, and the surrounding skin and mucosal surfaces were cleansed to prevent contamination during the swabbing process. Swabs were sent to the MMC microbiology lab for culture and sensitivity testing. Primary cultures were performed on MacConkey agar and blood agar at 37°C for overnight. If the culture was positive and relatively pure, colonies from any bacteria were then transferred to Amies charcoal transport swabs and stored until they could be sent to the UK.

Urine samples were collected in sterile, screw-top containers that were appropriately labelled according to standard procedures. The inclusion and exclusion criteria were detailed in 3.2.14. Patients were instructed on the proper method for urine collection. The containers were then stored in a refrigerator or cool box at temperatures of 2 to 8°C until they were transferred to the laboratory. From each sample tube, 30 ml of urine 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 on MacConkey and blood agar media using a micropipette. The media

containing the samples were incubated at 37°C overnight. After incubation, the media plates were inspected for growth. If the culture was positive and relatively pure, colonies of any bacteria were then transferred to Amies charcoal transport swabs and stored until they could be sent to the UK.

2.2.4 Collection of human rectal swabs

HRS were collected from healthy human volunteers residing within a 10 km radius of the selected farms and MMCH with their consent. Samples were collected according to the inclusion and exclusion criteria of this study (detailed in Section 3.2.12). Amies charcoal transport swabs were used for collecting HRS. The rectal swab collecting procedure involves inserting the swab stick 3-4 cm into the anus, gently rotating it for 10 seconds, and then placing the stick into the Amies charcoal swab tube. Each participant was given a unique identifier number, which was anonymised. Amies charcoal transport swabs were stored until they could be sent to the UK.

2.2.5 Collection of demographic and clinical data from healthy volunteers and patients

During the collection of HRS, SSI, and UTI samples, demographic and clinical histories were obtained from the patients using the case record form (CRF) (Attached in Appendix B). The data collected from the patients included the patients' name, age, sex, locality, family member, family income, clinical symptoms or reason for hospitalisation, admitting wards, outcome (discharge or death), date of admission, date of sample collection, date of outcome, and ongoing antibiotics used during hospitalisation. For rectal swabs, the data collected from healthy volunteers included their name, age, sex, locality, family member, family income, socio-economic status, rearing of animals, sanitary status, previous hospitalisation history, and previous or current antibiotic usage. The metadata collected in this study are described in Table 2.2.

Table 2.2 Metadata definitions

Metadata	**Description**
Gender	Male or female
Per capita family income	Total income of the family was divided by the number of the family members
Village	Based on locality
Occupation	Whether the participants were either crop farmer or livestock farmer or unemployed
Education	Whether the participants were educated or uneducated
Methods of waste disposal	Either the household waste was disposed near the house or away from the house in the waste pit
Raising domestic animal	Whether the participants raise any domestic animal or not
Own farm	Whether the participants were either farm owner or not
Drinking water availability	Source of daily-drinking water (filtered water/ tube well water/ well water or tap water
Consumption of protein	How many days of a week the participants could have main protein source fish or chicken
Toilet facilities	Either communal or 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 toilet

Previous antibiotic usage history within three months	Whether the participants either had history of taking antibiotics within 3 months of sampling or not
Antibiotic taken with or without prescription of 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 last six months of sampling
Outcome	Whether the patients were discharged or dead

2.3 Transfer of biological specimens

Clinical isolates, rectal swabs, and all farm samples were collected in Amies transport swabs with charcoal (COPAN Diagnostics, VWR, UK) and transported to the UK in a UN3373 container (UN3373, Lelystad, the Netherlands) with appropriate labelling and proper documentation. For the fly and water samples, dry ice was used to maintain a cool temperature.

2.4 Isolation and identification of bacteria

After collection in Bangladesh, all samples and isolates were transferred to the laboratory at Oxford University for further processing. Clinical isolates from UTIs and SSIs were sub-cultured on chromogenic UTI agar (Thermo Fisher Scientific, UK) supplemented with vancomycin (10 mg/L) (Liofilchem, Roseto, Italy). Rectal swabs and farm-derived samples transported in Amies charcoal medium were similarly plated on chromogenic UTI agar with vancomycin. 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 sample 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. Pink colonies were selected based on colony colour and morphology on chromogenic UTI agar plates and sub cultured to obtain purified isolates of *E. coli*. Species-level identification was performed using Matrix-Assisted Laser Desorption/Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany). Only a single colony from each sample was taken and used for downstream analysis. 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) for downstream analyses. *E. coli* isolates identified by MALDI-TOF MS were subsequently confirmed by whole-genome sequencing at the Illumina platform (NovaSeq 6000, Illumina Inc., San Diego, USA), and only WGS-confirmed *E. coli* strains were included in further analyses.

2.5 Determination of phenotypic resistance pattern

Minimum inhibitory concentration (MIC) was used to assess phenotypic resistance in *E. coli*. Antimicrobial susceptibility of *E. coli* isolates was determined according to the guidelines outlined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (v.12.0) (EUCAST, 2022). The agar dilution method was used to determine the MIC of clinically relevant antimicrobials (Andrews, 2001). Seventeen antimicrobials (Amikacin, Amoxicillin-Clavulanic acid, Ampicillin, Aztreonam, Cefepime, Cefotaxime, Ceftazidime, Ceftazidime-Avibactam, Ciprofloxacin, Colistin, Fosfomycin, Gentamycin, Imipenem, Levofloxacin, Meropenem, Piperacillin-Tazobactam and Tigecycline) were tested for MIC in this study (Table 2.3).

Table 2.3 Name of the antibiotics with their solvents and diluents

Name	Abbreviation	Manufacturer	Solvent	Diluent
1. Amikacin	AMK	Sigma-Aldrich, Missouri, USA	Water	Water
2. Amoxicillin-clavulanic acid	AMX-CL	Sigma-Aldrich, Missouri, USA	DMSO+ NaHCO3/ Water	Water
3. Ampicillin	AMP	Sigma-Aldrich, Missouri, USA	NaHCO3	Water
4. Aztreonam	AZT	Sigma-Aldrich, Missouri, USA	NaHCO3	Water
5. Cefepime	CEF	Thermo Fisher Scientific Inc. MA, USA	Water	Water
6. Cefotaxime	CTX	MedChemExpress, NJ, USA	Water	Water
7. Ceftazidime	CTZ	MedChemExpress, NJ, USA	NaHCO3	Water
8. Ceftazidime-avibactam	CTZ-AV	MedChemExpress, NJ, USA	NaHCO3(Ceftazidime) Water (Avibactam)	Water
9. Ciprofloxacin	CIP	Sigma-Aldrich, Missouri, USA	1 ml HCL+ Water	Water
10. Colistin	COL	Sigma-Aldrich, Missouri, USA	Water	Water

11. Fosfomycin-Glucose-6-Phosphate	FOS	Sigma-Aldrich, Missouri, USA	Water	Water
12. Gentamicin	GEN	Sigma-Aldrich, Missouri, USA	Water	Water
13. Imipenem	IPM	Sigma-Aldrich, Missouri, USA	NaHCO3	Water
14. Levofloxacin	LEV	Sigma-Aldrich, Missouri, USA	Water	Water
15. Meropenem	MER	Sigma-Aldrich, Missouri, USA	Water	Water
16. Piperacillin-tazobactam	TZP	LKT Laboratories, MN, USA	DMSO (Piperacillin) NaHCO3 (Tazobactam)	Water
17. Tigecycline	TIG	Sigma-Aldrich, Missouri, USA	Water	Water

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 plate showing no growth was considered the MIC of the strain. *Escherichia coli* ATCC 25922 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 2.4). Stock solutions were prepared using the following formula:

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

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 2.4 Serial dilution of antimicrobials to reach 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
	880µL of stock B in 35 ml agar	2
	440 µL of stock B in 35 ml agar	1
	220 µL of stock B in 35 ml agar	0.5
	110 µL of stock B 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 B in 35 ml agar	0.06

2.6 Whole genome sequencing

2.6.1 Short read sequencing

For short-read sequencing Illumina platform (NovaSeq 6000) (Illumina Inc., San Diego, USA) was used. Preparation of isolates for short-read sequencing was performed by reviving the isolates from -80°C and culturing them on UTI chromogenic agar plates at 37°C. After overnight incubation, a single pure colony from the UTI chromogenic agar plate was mixed with 200 µL of 1×PBS. From there, 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 at 200 rpm for 6 hours to achieve an optical density (OD600) of 10. The harvested bacterial cells in broth were then centrifuged at 8000 rcf for 3 mins. After discarding the supernatant, the bacterial pellet was washed twice with 1 mL PBS. The extra PBS was also removed from the pellet by a pipette. The pellet was then resuspended in 0.5 mL of 1x DNA Shield buffer, provided by the sequencing service (https://microbesng.com/). The bacterial cells in the DNA Shield buffer were transferred into 2 mL screw-capped tubes, which were labelled earlier and shipped for sequencing to MicrobesNG, Birmingham, with the proper shipment conditions. The isolates were sequenced on the NovaSeq 6000 platform (Illumina Inc., San Diego, CA). DNA libraries were prepared for paired-end sequencing using 2 x 250 bp kits. Quality control of raw reads included fastqc (0.11.9). Per batch 93 samples were sequenced.

2.6.1.1 Bioinformatic analysis of raw reads (short-read sequencing)

Reads were assembled into contigs (. fasta) using Shovill v1.1.0 which incorporates adapter trimming, assembly with SPAdes, and correction of minor assembly errors (1.1.0-foss-2018b-Python-2.7.15). Assembly metrics were evaluated using Quast (v2.1). The *de novo* assemblies were then annotated using Bakta (version 1.9.3).

2.6.2 Long read sequencing

Long-read sequencing was performed on the PromethION platform (Oxford Nanopore Technologies, Oxford, UK). Ninety-six samples were sequenced per run.

2.6.2.1 DNA extraction

QIAcube (Qiagen, Hilden, Germany) was used for extracting DNAs. For DNA extraction, the isolates were revived from -80°C by culturing them on UTI chromogenic agar at 37°C overnight. Then, a pure isolated bacterial colony was sub cultured 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. Automated DNA extraction was undertaken using QIAcube (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The extracted DNA was stored at -20 °C until use.

2.6.2.2 DNA quantification

The resulting gDNA was quantified using the Qubit 3.0 (Thermo Fisher Scientific, Waltham, USA). For each standard and DNA sample, 200 µL of working solution was prepared by diluting the Qubit reagent 1:200 in Qubit buffer (Thermo Fisher Scientific, USA). Assay tubes for standards were prepared by mixing 10 µL of the standard with 190 µL of the working solution. From each DNA, 2 µL of DNA was added to 198 µL of the 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. The Minimum acceptance criteria for DNA concentration were 10 ng/μL before proceeding to sequencing.

2.6.2.3 Preparation of genomic libraries

For long-read sequencing on the PromethION platform (Oxford Nanopore Technologies, Oxford, UK) genomic libraries were prepared using Rapid Barcoding Kit 96 V14Q20+ Kit14 (SQK-RBK114.96) and barcoded DNA loaded onto R10.4.1 flow cell (FLO-PRO114M). PromethION 2 Solo was connected to the MinKNOW Software (24.02.8) to obtain the raw reads.

2.6.2.4 Bioinformatic analysis of raw reads (long-read sequencing)

Raw data (.pod5) were processed with the Dorado basecaller (version 0.7.2) to generate raw reads in FASTQ format. I performed duplex base-calling using dna_r10.4.1_e8.2_400bps_sup@v4.2.0 model. The reads were assembled into contigs using Flye (2.9.4) followed by assembly polishing using Medaka (1.12.0). Unicycler (0.5.0) was used to yield hybrid assemblies using both raw short reads (.fastq) and long reads (.fastq).

2.6.3 Genomic characterisation of isolates

Whole-genome assemblies were analysed using a combination of pipelines and databases from the Centre for Genomic Epidemiology (CGE) and other curated resources. Genome assemblies were generated using the Shovill pipeline (v1.1.0), which performs de novo assembly using the SPAdes assembler. MLST was performed on the assembled contigs using the mlst command-line tool, which assigns sequence types based on the PubMLST *E. coli* (Achtman) scheme. ResFinder (v-4.6.0) was used to identify acquired AMR genes, while CARD (Comprehensive Antibiotic Resistance Database, v-4.0.1) was additionally used to cross-validate resistance gene predictions. Virulence-associated genes were detected using VirulenceFinder (v-3.2.0), and PlasmidFinder (v-2.1.1) was used to screen for plasmid

replicon types. Species identification was confirmed using KmerFinder (v-3.0.2). Phylogroup assignment was performed *in silico* using the Clermont *E. coli* phylogrouping scheme (ClermonTyping) based on whole-genome sequence data.

2.6.4 Phylogenetic analysis

To investigate the evolutionary relationships among the *E. coli* isolates, a phylogenetic tree was constructed using a combination of genomic mapping, tree-building, and visualization tools. Initially, core genome alignments were generated using the Bactmap (v.1.0.0) pipeline, which facilitated the extraction and alignment of conserved genomic regions across isolates. The aligned sequences were then processed with the VeryFastTree (v.4.0), an optimized variant of the FastTree algorithm, to construct an approximately maximum-likelihood (ML) phylogenetic tree.

VeryFastTree was chosen due to its scalability and computational efficiency, enabling the analysis of large bacterial genomic datasets with reduced runtime (Piñeiro and 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. SNP calling was performed using Snippy (v4.4.5), followed by recombination removal using Gubbins (v2.3.4) (Croucher *et al*., 2015), and pairwise SNP calculation using pairsnp (v.0.0.7) (GitHub, 2018).

2.7 Plasmid analysis and visualization

Plasmid assemblies and annotations were conducted in Geneious Prime (version 2025.2.2, Biomatters Ltd.), allowing detection of plasmid backbones, AMR genes, and associated mobile elements. To compare plasmid sequences, the BLAST Ring Image Generator (BRIG, version

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 PyGenomeViz (v1.6.1), a Python-based genome visualisation platform, to highlight regional similarities across plasmids. Together, these tools supported comparative plasmid analysis and produced detailed graphical representations of genomic features.

2.8 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 or Fisher's exact test as appropriate. Statistical significance was defined as $p < 0.05$. Correlations between variables were examined using the Pearson correlation coefficient (r).

Chapter 3

One Health sampling strategies for investigating antimicrobial resistance transmission dynamics in Bangladesh

3.1 Introduction

AMR is a complex global health threat that necessitates a holistic, multi-sectoral approach, particularly in densely populated LMICs like Bangladesh (Hoque *et al.*, 2020). A "One Health" approach, which acknowledges the interconnectedness of human, animal, and environmental health, is crucial for effectively tackling this complex issue (World Health Organisation [WHO] - One Health, 2023). To effectively capture the AMR epidemiology across various sectors, it is necessary to implement optimised sampling strategies that will inform targeted interventions and policies (Norström *et al.*, 2023). Such optimisation is crucial not only for selecting an appropriate sampling method and determining a suitable sample size based on study duration and available resources but also for ensuring the precision of study findings. A sample that is too small may yield imprecise and potentially misleading results, while a huge sample may prove resource-intensive and could highlight statistically significant but irrelevant differences. Therefore, the initiative to optimise sampling strategies is instrumental in developing an operational sampling protocol that is both effective and resource-efficient (European Centre for Disease Prevention and Control [ECDC], 2023).

Bangladesh is a developing country and is the 8th most populous country globally, accounting for 2.13% of the global population. The average population density is approximately 1,350 people per square kilometre (3,496 people per square mile) (Worldometer, Bangladesh population, 2024). In Bangladesh, poultry and other domestic animals frequently coexist in proximity to human populations, a characteristic common to both rural and urban settings. Except for a few large poultry farms, most are situated near residential areas, which heightens the risk of cross-species transmission of antibiotic-resistant bacteria, thus contributing to the spread of AMR (Figure 3.2; Flatgard *et al.*, 2024).

Additionally, the waste management systems in many urban areas are often inadequately organised, resulting in unmanaged waste being dispersed near roads, hospitals, residences, and water bodies (Figure 3.3). Improper waste disposal can attract animals and facilitate the transmission of diseases, ultimately contributing to the dissemination of AMR (Vikesland *et al*., 2019). Moreover, the misuse of antimicrobials in human medicine, poultry, agriculture, and aquaculture, exacerbated by unregulated access to antibiotics, adds to the AMR issue in the country (WHO, 2023; Hoque *et al*., 2020). Given this scenario, Bangladesh recognises the critical importance of a One Health approach in tackling AMR. The country has established a National One Health Strategy that highlights AMR as a multisectoral issue and has implemented various integrated activities, including the development of a national antibiotic surveillance system and a laboratory network. The One Health Plus Integrated Antimicrobial Stewardship Approach in Bangladesh emphasises the creation of a One Health AMR Data Dashboard at the One Health Secretariat, which is designed to forecast data on antimicrobial susceptibility (AST) patterns of key pathogens recovered from human infections, livestock, and the environment (CDC Bangladesh, 2022; One Health Trust, 2018). Despite progress in adopting a One Health approach to combat AMR, several challenges persist, including inconsistent data collection, a lack of standardised protocols, and resource constraints such as inadequate infrastructure and funding. Surveillance efforts in human and animal health in Bangladesh are currently operating independently, lacking integration of sampling across livestock, humans, and the environment, which limits the ability to obtain valuable insights into cross-transmission dynamics (Ahmed *et al*., 2022).

This study adopts the One Health sampling framework outlined in publications by Walsh (2018) and Zhou (2022), which has been optimised to align with the local context in Bangladesh (Zhou *et al*., 2022b; Walsh *et al*., 2018). Sampling sites, sectors, and types were strategically selected, and a sampling protocol was designed that ensures objectivity while

providing representative findings for both urban and rural areas. This protocol incorporates AMR surveillance across the human-animal-environment interface at both community and hospital levels. Engaging rural communities in AMR surveillance presents significant challenges, particularly in regions where awareness and understanding of AMR issues are limited. Moreover, identifying appropriate environmental indicators and creating effective methods for environmental sampling are complex tasks that necessitate careful consideration of local behaviours and environmental factors (Franklin *et al*., 2024; Berendonk *et al*., 2015). I aimed to collect valuable data to contribute to the AMR landscape in Bangladesh. This chapter will detail the approach to designing the study and developing the sampling protocol.

3.2 Results

3.2.1 Study design

A prospective cross-sectional study was designed within a One Health perspective, which encompassed sampling from human populations, the livestock chain, and the surrounding environment. Human samples represented multi-drug resistant (MDR) *E. coli* from healthy carriage, community-acquired infections and healthcare-associated infections. To elucidate the dynamics of AMR spread, healthy volunteers residing within a 10 km radius of the selected poultry farms were enrolled. Concurrently, environmental sampling was conducted within the same radius, encompassing the poultry farms and hospitals involved in this study. Sample collection and patient enrolment were conducted by the author, supported by the local team.

A schematic diagram of the sampling strategy is shown in Figure 3.1, which represents the conceptual, preparatory, and operational stages of an AMR surveillance study grounded in the One Health approach, emphasising the interconnectedness of human, animal, and environmental health.

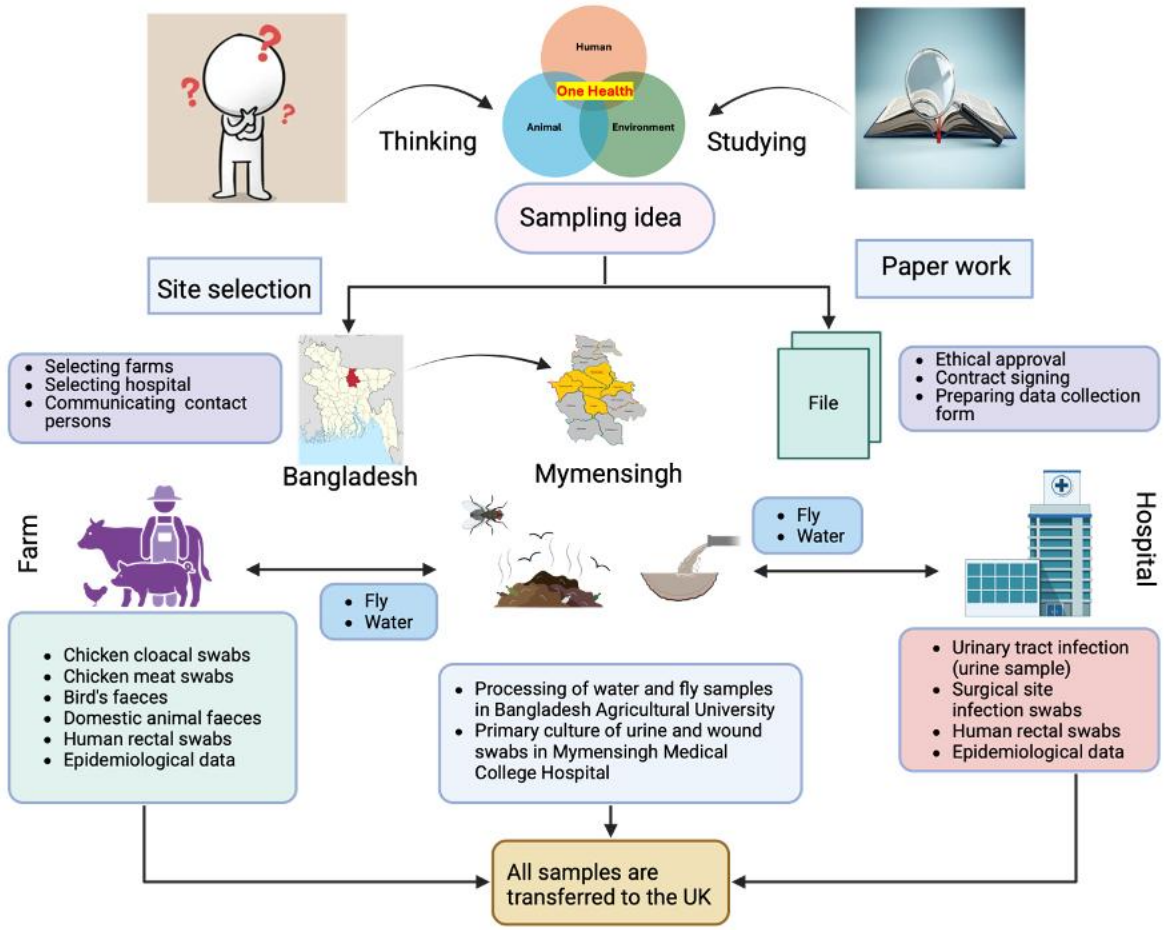


Figure 3.1. Overview of study design and sampling strategy for One Health-based AMR surveillance in Mymensingh, Bangladesh. (The Figure was created with BioRender.com).

3.2.2 Selection of study location

Bangladesh has a land area of 130,170 square kilometres (50,259 square miles). As of 2025, its total population is 174,731,016. The average population density is approximately 1,350 people per square kilometre (3,496 people per square mile) (source: https://www.worldometers.info/world-population/bangladesh-population/). High population density, inadequate waste management, frequent natural calamities, and the close cohabitation of humans and animals have made Bangladesh an ideal setting for a One Health study on the AMR dissemination. Figures 3.2 and 3.3 show the cohabitation of human-animal and poor waste management respectively. Bangladesh has 64 districts and eight administrative divisions. Mymensingh is one of the oldest cities in Bangladesh and is under the Mymensingh division,

covering 4,363.48 km² in northern Bangladesh and typified by small valleys amidst high forests and shares borders with India's Meghalaya state to the north and several Bangladeshi districts to the south, east, and west. In addition to its diverse, multicultural population, Mymensingh contains a major tertiary hospital and an agricultural university. According to the Mymensingh District Animal Resources Office, there are currently 438 registered commercial broiler farms, while another 5,227 are unregistered. In terms of layer chicken farms, 315 out of 4,783 have been registered with the authorities (bdnews24.com, 2022). Mymensingh City, a densely populated area with mixed residential and commercial infrastructure, reflects typical Bangladeshi urban settings (Figure 3.4b). These conditions provided a relevant context for assessing the potential transmission dynamics of antimicrobial-resistant bacteria at the human–environment interface. Mymensingh has 13 subdistricts, and a total of five subdistricts were selected for the sampling, which included Mymensingh Sadar, where the tertiary hospital is located, and four adjacent subdistricts: Gauripur, Muktagachha, Trishal, and Tarakanda (Figure 3.5). Two farms were selected from the subdistricts to collect farm, healthy human rectal swab samples and environmental samples, which complemented clinical samples from MMCH.

Figure 3.2a Human-animal sharing the same environment. Figure 3.2a illustrates the cohabitation of domestic animals and farmers; here, the house of a farmer is shown (on the left, red circle), and the animal is reared very close to the farmer's house. Photograph by the author.

Figure 3.2b One of the poultry farms sampled in this study. The farm shed is shown, and the farmer's house is visible behind the shed (left, red circle). Photograph by the author.

Figure 3.3a Poor waste management system (improper waste disposal near hospital walls, [MMCH] attracting animals searching for food). Photograph by the author.

Figure 3.3b Poor waste management system. (Inappropriate disposal of wastes near waterbodies, such as drains and rivers in Mymensingh city, which contaminates these water bodies). Photograph by the author.

Figure 3.3c Poor waste management system. (A waste pit near a poultry farm shed, from where flies and other vectors can easily spread organisms to the poultry). Photograph by the author.

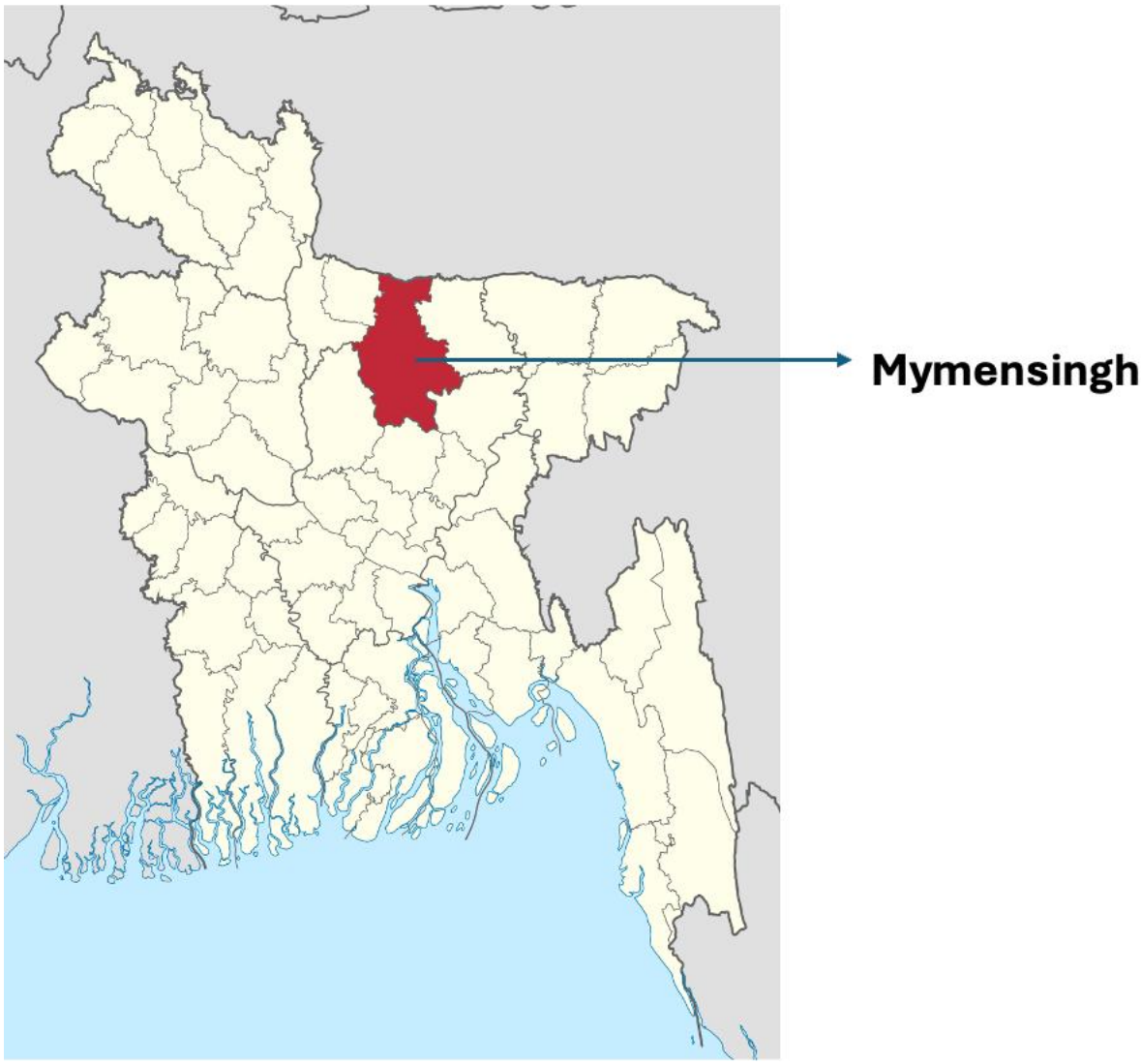


Figure 3.4a Location of the sampling site in Bangladesh.

The map highlights Mymensingh district in red, which served as the sampling location for this study.

Figure 3.4b Urban setting of Mymensingh City, the study location. Photograph by the author.

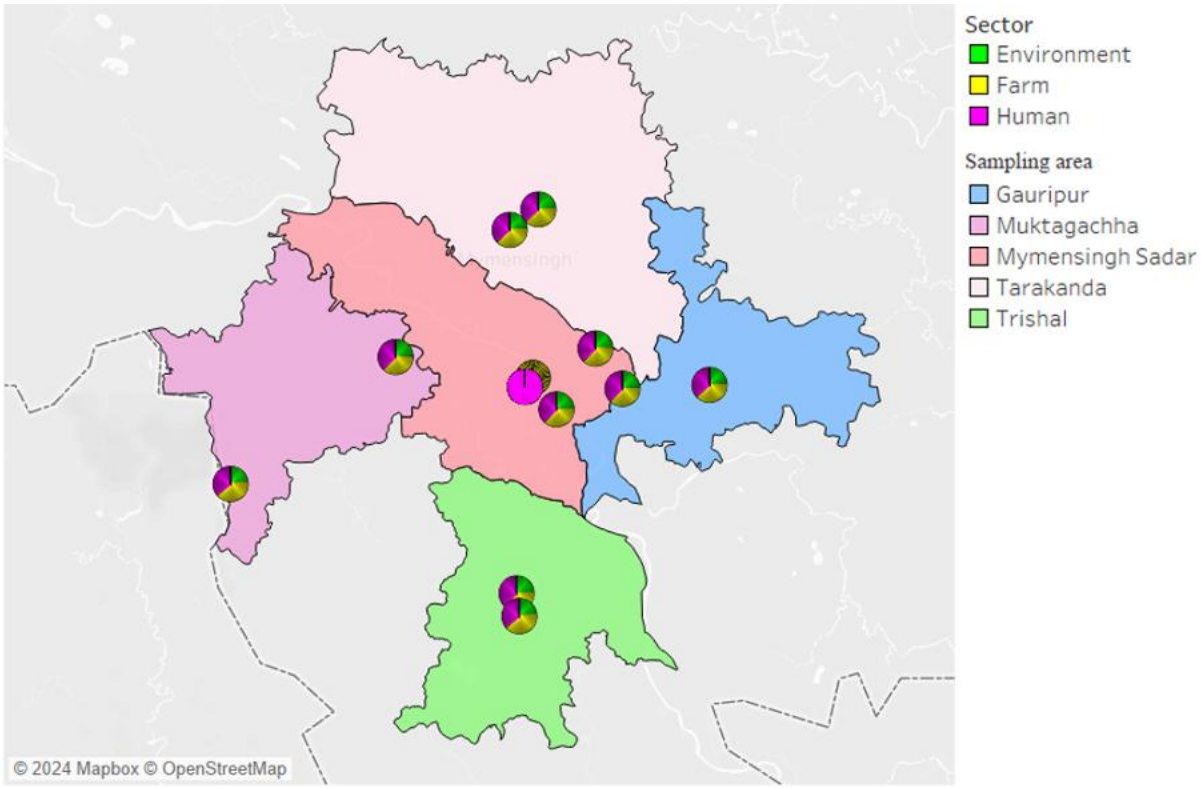


Figure 3.5 Geographical distribution of One Health sampling sites across five sub-districts in Mymensingh, Bangladesh and the types of samples collected from the sites. This map was created with Tableau Desktop.

3.2.3 Sampling period

The total duration of this D.Phil. project is four years (October 2021 to September 2025) with a sampling period spanning from December 2021 to March 2023. The sampling period was divided into four quarters (Qs): Q1 from December 2021 to March 2022; Q2 from April 2022 to July 2022; Q3 from August 2022 to November 2022; Q4 from December 2022 to March 2023. Bangladesh experiences six distinct seasons, with three primary periods: summer (March to June), monsoon (July to November), and winter (December to early March). In this study, Q1 and Q4 corresponded to winter, Q2 to summer, and Q3 to monsoon. The main purpose of including all seasons is to identify any seasonal variation in the isolation and resistance patterns of the organisms. Sampling was conducted simultaneously from poultry, healthy humans and

the environment at a given period during each quarter. Clinical samples from UTIs and SSIs were collected throughout the entire sampling period from the enrolled patients according to the inclusion criteria who visited or were admitted to MMCH. It is worth noting that consistently the same farms were enrolled in each subdistrict for poultry sampling in each quarter; however, the sampling from the poultry and human subjects was non-repetitive.

3.2.4 Sample size calculation

The statistical analyses assessing both the prevalence and trend of resistance mechanisms rely on the assumptions of chi-square testing. To ensure adequate sampling, sample size determination for the chi-square of homogeneity was performed. The following assumptions were made for sample size determination:

- Significance alpha, α was set to 0.05, and beta, β was set to 0.2 making power equal to 0.8.
- A buffer of 15% was included to account for dropouts and unanticipated missingness
- Three scenarios were calculated for different Cohen's d values (low = 0.10, medium = 0.30, large = 0.50).
- As sampling was conducted at four time points for each source group, the required sample sizes per time point, with and without buffer, as well as the overall totals, were calculated.

Sample size estimations were initially performed using Chi-squared tests of homogeneity to determine the minimum number of observations required across farm, environmental, and human sources (Table 3.1).

Table 3.1 Sample size calculation scenarios for Chi-square test of homogeneity**.**

Source group	**Effect size Cohen's d**	**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 swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; BF, bird faeces; UTIs, urinary tract infections; SSI, surgical site infections; HRS, human rectal swab.

The recommended sample size was associated with the smallest effect size (Cohen's d = 0.10), resulting in a total of 1656 observations (184 for each of the 9 sources and 46 for each time of measurement). The total sample size collected in this study is shown in Table 3.2.

Table 3.2 Total number of samples collected in this study.

Sector	**Recommended samples size**	**Number of samples collected**
SSI	184	814
UTI	184	1170
HRS	184	660
Fly	184	220
Water	184	220
CCS	184	200
CMS	184	200
DARS	184	200
BF	184	75
Total	**1696**	**3759**

CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swabs, BF; bird faeces; HRS, human rectal swab; UTI, urinary tract infection; SSI, surgical site infection.

3.2.5 Ethical approval, consent, and data protection

Ethical approval was taken for both human and farm samples. Ethical approval from the Institutional Review Board (IRB) of Mymensingh Medical College [Memo no. MMC/IRB/2021/420] was granted for human samples. Ethical approval for animal samples was secured from the Animal Welfare and Experimentation Ethics Committee (AWEEC) of Bangladesh Agricultural University [Approval no. AWEEC/BAU/2021(23)]. Additionally,

ethical approval was obtained from the Oxford Tropical Research Ethics Committee (OxTREC) at the University of Oxford [OxTREC Ref no. 30-21]. Written consent was obtained from all individuals who participated in this study, including consent for the use of photographs containing identifiable individuals for academic and publication purposes. Participants' and patients' data were anonymised using a spreadsheet that was protected by encryption and password protection. Every farm owner enrolled in the study was contacted and discussed the perspective of this study with them, obtaining their consent for collecting samples from their farms. Contact persons from MMCH were also approached for their consent and collaboration with the sampling and processing of clinical samples in the microbiology lab at MMC. A collaboration agreement has been established between the University of Oxford and Bangladesh Agricultural University (BAU) to facilitate sample processing in the university's microbiology laboratory. This agreement outlines the management of funding provided by the Ineos Oxford Institute for Antimicrobial Research (IOI), Oxford, to support sampling-related expenses and issues related to data security and protection, copyright, and authorship for any publications resulting from this research. All ethical approval certificates are attached in Appendix A.

3.2.6 Collection of chicken cloacal swabs (CCS)

Standard operating procedures (SOPs) were created for collecting samples from each category. Two poultry farms were selected from each sub-district, with one broiler and one layer farm chosen to collect chicken cloacal swabs. The inclusion criteria for selecting the farms were:

- Farms having >3000 birds (small-scale farms have been excluded).
- Farms raise birds of single age groups at one time, and all birds are consigned to a single batch and provided feed/drink from a common source.

Hygienic measures were strictly followed during sample collection and processing. The cloacal samples were collected using Amies charcoal transport swabs (Barber *et al*, 1998). Figure 3.6 shows the collection of chicken cloacal swabs.

Figure 3.6a Photograph of a shed at one of the poultry farms sampled in this study. Photograph by the author.

Figure 3.6b Photograph showing the interior of a poultry farm included in the sampling. Photograph by the author.

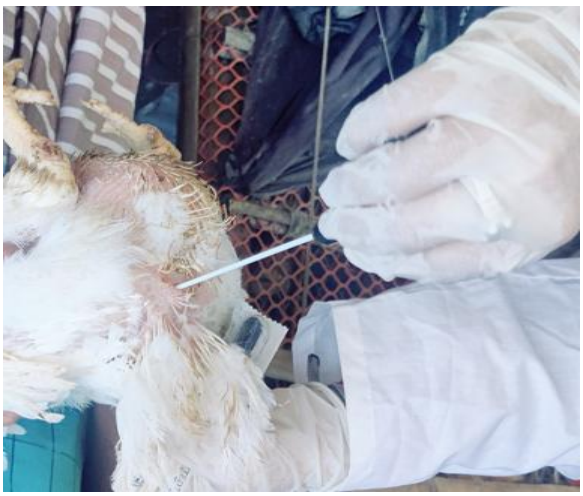


Figure 3.6c Collection of chicken cloacal swabs. Photograph taken by a member of the sampling team.

3.2.7 Collection of chicken meat swabs (CMS)

Chicken meat swabs were collected from live bird markets located within a 10 km radius of the selected farms. In Bangladesh, live bird markets often involve the slaughter and processing of birds on-site. A picture of a live bird market and the meat swab sampling process is illustrated in Figure 3.7. For the collection of meat swabs, Amies charcoal transport swabs were used.

Figure 3.7 Live bird slaughtering and sampling process. The left image shows the typical live bird market setting in Bangladesh, where chickens are often slaughtered without proper hygienic conditions that facilitate cross-contamination and the spread of pathogens. The right image shows the collection of chicken meat swabs immediately after slaughter, following aseptic technique with sterile swabs. Photograph taken by a member of the sampling team.

3.2.8 Collection of domestic animal rectal swab (DARS)

Domestic animal rectal swabs were collected from grazing animals near poultry farms, primarily focusing on goats and cattle (Figure 3.8). The samples were collected using Amies charcoal transport swabs.

Figure 3.8a Domestic animals grazing in close proximity to farms and farmers' houses: goats (left) and cattle (right). Photograph by the author.

Figure 3.8b Collection of domestic animal rectal swabs from a goat. Photograph taken by a member of the sampling team.

3.2.9 Collection of bird faeces (BF)

Free-flying birds near the selected poultry farm and the hospital were the source of the bird faeces samples. The bird droppings collected in this study were mostly from pigeons, sparrows, and common mynas. Sterile paper sheets were placed in specific areas, such as around sheds on the farms, under trees within the farm premises, and near the dustbins at MMCH. The collection procedure BF is explained in the methodology chapter, section 2.2.1. The number of bird faeces samples collected was inconsistent due to the birds' unavailability, which could not be controlled. Consequently, it was not possible to collect bird faecal samples from all locations or in a uniform quantity. A total of 75 samples were collected across the four sampling periods.

3.2.10 Collection of water samples

Water samples were collected from various sources around the farms and the hospital, including drains, ponds, rivers, and canals (Figure 3.9). Five samples were collected from each farm and the hospital at each time point. As many water sources as possible were included around the farms and hospital to explore the prevalence in various types of sources.

Hospital drain

Household drain

Farmside drain

Farmside pond

Canal water

River water

Figure 3.9 Sources of water samples collected from areas in and around farms, households, and hospitals. Photograph by the author and a member of the sampling team.

3.2.11 Collection of fly samples

Fly samples were collected from the waste pit near the farms and hospital, as well as from inside and outside. Fly traps were used to collect the flies (Figure 3.10). The number of flies trapped in the fly trap varied depending on the location. Five flies were randomly selected for culture from each location at each time point to maintain a consistent sample size.

Figure 3.10a Waste pit located near a poultry farm that served as a fly sampling site. Photograph by the author.

Figure 3.10b Flies captured using a fly trap placed near sampling sites. Photograph by the author.

3.2.12 Collection of human rectal swab (HRS)

Residents living within a 10 km radius of selected poultry farms and the hospital were chosen for rectal swab collection based on this study's inclusion and exclusion criteria. A prior schedule was established for sampling and interviewing healthy volunteers. They were informed about the purpose and details of the research, and their written consent was obtained prior to providing rectal swabs. The volunteers received thorough instructions on how to collect the samples using Amies charcoal swabs, ensuring that they understood the procedure clearly to perform the sampling correctly. After providing samples, an interview was conducted according to the CRF (Appendix B).

The inclusion and exclusion criteria for selecting potential healthy human participants are as below:

Inclusion criteria:

- Healthy volunteers inhabiting a 10 km radius of the selected poultry farms, including farmers.
- Participants who are willing and able to give informed consent for participation in the study.
- Male or female, aged 18 years or above, who are free of chronic disease.

Exclusion criteria

- Pregnant women
- Diabetic patient
- Children under 18 years
- Participants with gastroenteritis, gastrointestinal cancer, gastrointestinal surgery, peptic ulcer, gastrointestinal bleeding, inflammatory bowel disease (Crohn's disease and ulcerative colitis), intestinal polyps, intestinal fistula, anal fistula or anal fissure.
- Participants with any chronic disease.
- Any individual who is not able to give consent themselves.

3.2.13 Collection of surgical site infection samples (SSI)

Based on the inclusion and exclusion criteria, the target participants were patients with SSIs admitted to MMCH from Dec/2021 to Mar/2023. The inclusion and exclusion criteria are as follows:

Inclusion criteria

- Patients whose wound swabs were sent to Microbiology Lab of MMC for diagnostic purpose
- Patients admitted at MMCH
- If the patient had the following symptoms
    - Redness and pain around the area of surgery
    - Drainage of exudate from surgical wound
    - Fever
- Participants who were willing and able to give informed consent for participation in the study
- Male or female, aged 18 years or above who were free of any chronic disease

Exclusion Criteria

- Pregnant women
- Individuals with diabetes mellitus
- Individuals with malignancy or any other chronic diseases

Once potential participants who met the inclusion criteria were identified, they were contacted by phone to explain the study protocol. Participants were given as much time as they needed to consider the information and were encouraged to ask questions to the investigator, their healthcare provider, or other independent parties to help them decide whether to participate in the study. Following this period of reflection, a visit was scheduled to conduct interviews with the participants.

Figure 3.11 Surgical ward at Mymensingh Medical College Hospital showing overcrowded conditions where SSI samples were collected. Photograph by the author.

3.2.14 Collection of urine samples (UTI)

For UTI samples, patients visiting the outpatient department of MMCH with symptoms of UTI were enrolled in this study, considering the inclusion and exclusion criteria explained below.

Inclusion criteria

- Patients whose urine specimens were sent to the microbiology lab of MMC for diagnostic purposes
- Patients from the outpatient department of MMC were only included (with no history of hospitalisation during the last month)
- Patients meet the following two criteria
    - If the patients had at least one of the following:
        - Fever (>38.0°C)

        - Suprapubic tenderness
        - Costovertebral angle pain or tenderness
        - Urinary urgency
        - Urinary frequency
        - Dysuria
    - If the patient had a urine culture with no more than two species of organisms identified, at least one of which was a bacterium of $\geq 10^5$ CFU/mL

Exclusion criteria

- Pregnant women
- Individuals with diabetes mellitus
- Individuals with malignancy or any other chronic diseases

After identifying individuals who met the inclusion criteria, participants were contacted by phone to inform them of the study procedures and obtain their consent to participate. If they agreed, an interview was scheduled with the participants.

Figure 3.12 Entrance of Mymensingh Medical College Hospital, showing the outpatient department area where patients and visitors gather daily. Photograph by the author.

After collecting and processing the samples, the swabs were transferred to the UK with appropriate documentation, in the UN3373 container.

3.2.15 Collection of demographic and clinical data from patients and healthy volunteers

Epidemiological data were collected from the healthy human volunteer providing rectal swabs and from the patients of UTI and SSI to perform risk assessment. The patient information recorded included demographic and clinical variables such as name, age, sex, residence, household composition, family income, presenting symptoms or reason for admission, hospital ward, treatment outcome (discharge or death), admission date, sample collection date, outcome date, and details of antibiotic use during hospitalization. For healthy volunteers providing rectal swabs, data were collected on demographics (name, age, sex, residence, household and

income), socio-economic status, animal rearing practices, sanitation conditions, history of hospitalisation, and past or current antibiotic use (Figure 3.14). All information captured through the CRF (Attached in Appendix B) was subsequently integrated with microbiological and genomic findings to assess potential risk factors associated with the acquisition of resistant bacteria.

Figure 3.13 Collection of epidemiological data from healthy volunteer participants following human rectal swab sampling. Photograph taken by a member of the field team.

3.3 Discussion

The methodology employed in this study is adapted from a scientific communication by Walsh (2018), which elucidates the components of the One Health strategy in relation to the dynamics of AMR spread. This study has contextualised the sampling sectors accordingly. The approach involves sampling from various sources, including healthy human carriers, healthcare-associated infections, community-acquired infections, animal reservoirs such as live farm poultry, deceased poultry at slaughterhouses, environmental reservoirs such as bird and domestic animal faeces, wastewater, and vectors, including flies in close proximity. This comprehensive approach serves as a representative sampling method. Notably, similar sampling frameworks have been noted in prior studies conducted in Laos, Pakistan, and China (Umair *et al*., 2023; Zhou *et al*., 2022b; Liu *et al*., 2016). However, these earlier studies did not necessarily encompass all sampling sectors, particularly failing to distinguish between healthcare-associated and community-acquired infections in humans, which is crucial for understanding potential transmission modes and drivers of AMR for human infections (Rajagopal *et al*., 2021). In the context of this study, poultry was prioritised for sampling over other food chain components due to its accessibility and affordability, which is recognised as a vital protein source in Bangladesh, especially for economically disadvantaged communities. Beyond its nutritional significance, poultry farming is integral to Bangladesh's agricultural sector, playing a key role in enhancing food security and economic stability (Begum *et al*., 2023). To adequately represent domestic animals within the local context, faeces from cattle or goats have been selected. Cattle and goats are widely reared in rural areas of Bangladesh. Their droppings are readily available, making them convenient samples for research and analysis (Hicks *et al*., 2021). Dog faeces have been excluded from the sampling plan due to the prevalence of street dogs, which complicates the efforts to track potential contamination and ascertain the direction of AMR dissemination (Hasan *et al*., 2024). Moreover, farmers and

residents living in proximity to the participating farms were included as healthy human subjects for rectal swab sampling. The data collected on sanitation practices, animal handling, waste management, and socio-economic factors from these human subjects significantly enhanced the ability to assess the potential transmission of ARGs. The close interactions between humans and animals on these farms contribute substantially to the transmission of antimicrobial resistant bacteria and ARGs. This situation is further aggravated by inadequate sanitation and waste management practices, which lead to environmental contamination (Williams *et al*., 2023).

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. To calculate the sample size, prior studies from Bangladesh focused on the commonly identified resistance mechanisms were used, particularly ESBL and the emerging colistin resistance, *mcr*. In calculating the sample size, three different scenarios were considered based on varying Cohen's d values that reflect different levels of effect size: low (0.10), medium (0.30), and large (0.50). To include a sufficient number of samples from each category, a minimum effect size was calculated. Importantly, this study fulfilled the criteria for estimating the true prevalence of a range of resistance mechanisms in Bangladesh, which sets it apart from other published research in South Asia (Mitra *et al*., 2024; Young *et al*., 2022 Mitchell *et al*., 2021; Hoque *et al*., 2020; Purohit *et al*., 2017).

A limited number of studies have explored an integrated approach that encompasses human, animal, and environmental perspectives to understand AMR epidemiology in Bangladesh. The majority of existing reports have primarily relied on data from individual sampling sources and prediction (Flatgard *et al*., 2024; Khanam *et al*., 2021; Nobel *et al*., 2021; Rousham *et al*., 2018;

Rahman *et al*., 2018). Notably, the recent implementation of the One Health Plus Integrated Antimicrobial Stewardship Approach in Bangladesh seeks to foster collaboration among the clinical, livestock, and environmental sectors; however, the initiation of coordinated periodic sampling remains pending (Institute of Epidemiology, Disease Control and Research [IEDCR], n.d.). While the study conducted by Flatgard *et al*. (2024) incorporated human, poultry, and environmental samples from Bangladesh, it lacked enrolling human subjects to represent clinical infections directly rather compared data with global clinical databases. Compared to other small-scale studies from Bangladesh, this study adhered multiple locations in Mymensingh district, providing a broader and more representative analysis and contributing to a more holistic understanding of AMR in the region (Nobel *et al*., 2021, and Khanam *et al*., 2021; Rahman *et al*., 2018). Given that this study was undertaken as a pilot to inform the design of a controlled trial aimed at representing the entirety of Bangladesh in future research, selecting Mymensingh was particularly strategic, as it serves as a vital agricultural zone, houses a major tertiary care hospital, and represents both urban and rural communities. Studies within neighbouring countries, including India, Nepal, and Sri Lanka, has similarly exhibited limited approaches in delineating the transmission of AMR among human, animal, and environmental sectors, whether concerning sample size, sampling location, sampling sectors or overall study design (Mitra *et al*., 2024; Gunasekara *et al*., 2024; Young *et al*., 2022; Mitchell *et al*., 2021; Purohit *et al*., 2017). This observation highlights the robustness of the current study in providing valuable regional epidemiological insights into AMR, where comprehensive demographic, epidemiological, microbiological, and genomic data will support a robust One Health study design.

Despite the strengths of this integrated approach, several methodological considerations related to sampling and sample size need careful discussion. While formal sample size calculations were performed to guide study design, several limitations should be acknowledged. The chi-

square–based sample size estimation assumes balanced group sizes, independence of observations, and fixed effect sizes, which may not fully capture the heterogeneity of antimicrobial resistance patterns across diverse ecological sources. In practice, unequal numbers of isolates were obtained from different sources, such as clinical and bird faeces. Clinical isolates from UTI and SSI were collected continuously over the study period based on routine diagnostic submissions to the laboratory, and all isolates meeting inclusion criteria were included to maximise statistical power and reflect real-world clinical burden. Consequently, the resulting sample distribution is weighted towards clinical sources and may introduce sampling bias related to healthcare access and diagnostic practices rather than true population prevalence. Environmental and animal sampling, by contrast, was conducted in a structured manner with predefined targets except bird faeces. There is a limitation in this sampling method for collecting bird faeces, as the number of birds defecating was not constant during the period when the sample was collected, so the sample size did not meet the recommended sample size. These differences should be considered when interpreting comparative analyses across sources, particularly with respect to prevalence estimates. Importantly, these sampling considerations must be interpreted within the wider socio-environmental context that shapes antimicrobial use and resistance in Bangladesh.

Studies have indicated that in several LMICs, including Bangladesh, antimicrobials are often available over the counter and without a prescription. This accessibility has contributed to widespread misuse and overuse (Hicks *et al*., 2021). Additionally, research has underscored the impact of agricultural practices, particularly the routine administration of antimicrobials in livestock for growth promotion, as a significant factor in the development and dissemination of AMR (Etienne *et al*., 2025; Samreen *et al*., 2021). Inadequate waste management systems can facilitate the release of both antimicrobials and resistant bacteria into the environment, further exacerbating this public health issue (Musoke *et al*., 2021). By identifying and

characterising these contributing factors, this study aims to inform the formulation of context-specific and evidence-based policies and interventions that address the root causes of AMR in Bangladesh. The findings will highlight the importance of understanding the interconnected dynamics among humans, animals, and the environment in developing targeted treatments for antimicrobial-resistant infections and effective IPC strategies.

Chapter 4

Epidemiology of antimicrobial resistance in *Escherichia coli* within a One Health framework in Bangladesh

4.1 Introduction

AMR is an escalating global health crisis and addressing it through a One Health approach is crucial for understanding its complexities and interconnections (WHO, 2024). The One Health framework highlights the interconnectedness between human health, animal health, and the surrounding environment, recognising that AMR can emerge and spread across these domains (Flatgard *et al.,* 2024). *E. coli* is a versatile organism and constitutes an integral part of the gut flora in both humans and animals, and whilst most strains are harmless, certain pathogenic *E. coli* strains can lead to serious infections (Mohamed *et al.,* 2022; Rubab *et al.,* 2020). *E. coli* can spread to humans through multiple pathways, including contaminated food (such as undercooked beef and raw vegetables), water contaminated with animal or human waste, or direct contact with infected animals or their environments (Pompeyo Ferro *et al.,* 2024; Mandal *et al.,* 2022). The emergence of AMR *E. coli* strains poses a significant challenge as resistance can develop in both human and veterinary medicine, making it essential to monitor resistance patterns in a spatiotemporal manner (Geurtsen *et al.,* 2022).

E. coli is the most common Gram-negative bacterium found in the human gut, leading to intestinal diseases and infections outside the intestine. Diseases linked to *E. coli* in the intestine primarily include diarrhoea, which varies in nature depending on the causative subtypes. Shigatoxin-producing *E. coli* causes severe food-borne diarrhoea in humans. The common infections caused by *E. coli* include UTIs, neonatal sepsis, abdominal and pelvic infections, pneumonia, bacteraemia, and meningitis (Mueller *et al.,* 2023; Sands *et al.,* 2021). Even without showing any clinical signs and symptoms, healthy carriers can infect others (WHO, 2018). UTIs mediated by *E. coli* are the second most diagnosed infection in the community and represent a significant global health issue, impacting nearly 150 million individuals annually (Swathi *et al.,* 2021; Kabugo *et al.,* 2016). Hospital-acquired infections (HAIs) pose a significant global challenge in healthcare systems, contributing to increased mortality, adverse

health conditions, longer hospital stays, higher treatment costs, antibiotic misuse, and the rise of antibiotic resistance. A meta-analysis of research published in Iran in 2018 shows that SSI is one of the most common HAIs, where *E. coli* was one of the main responsible organisms (Ashoobi *et al.,* 2023).

E. coli is considered one of the major causative organisms for a wide range of animal diseases, exhibiting a high level of phenotypic resistance to commonly used antibiotics (Nielsen *et al.,* 2022). *E. coli* is responsible for septicaemia and diarrhoea in young calves, clinical mastitis in dairy cattle, UTI in cats, dogs and horses, colibacillosis in pigs and respiratory infection in horses (Goulart and Mellata, 2022; Nielsen *et al.,* 2022; Bashahun, 2017). Avian pathogenic *E. coli* causes colibacillosis in poultry, leading to significant economic losses in the poultry industry. As a crucial component of the endogenous microflora, *E. coli* can quickly develop resistance to antimicrobials used in poultry. As the poultry industry is continuously expanding, farmers are frequently using antibiotics at sub-therapeutic doses as growth promoters and/or infection prevention in addition to treating sick birds, which leads to a worse scenario of AMR by increasing the selection pressure of resistant *E. coli* (Mandal *et al.,* 2022; Imam *et al.,* 2020).

Animals are potential reservoirs of human infections caused by *E. coli* and a source of disseminating AMR to humans and environmental sectors (Mandal *et al.,* 2022; Henry *et al.,* 2019). Sharing the same household with poultry and domestic animals is a widespread practice in limited-resource settings, particularly in villages where livestock constitutes a major source of income for families. In rural areas in Bangladesh, small- and medium-scale poultry farms (300–5,000 birds) are generally located close to households. The waste generated from these farms is often mixed with household waste and regularly deposited in the environment, including surface water bodies, which act as a medium of organism transmission to animals

and humans as well (Alam *et al.,* 2019). Improper handling of poultry and other animal faeces as fertilisers, or fish feed, can lead to the spread of pathogens to the handler (Rimi *et al.,* 2017).

One Health is a transdisciplinary approach aimed at improving the well-being of humans, animals and the environment, emphasising their interdependence and linked to the UN SDGs (One Health Joint Plan of Action, 2022-2026). The environment acts as a reservoir for the accumulation and transition of nutrients and disease vectors, including bacteria and AMR genes. Flies as a part of our environment, are commonly present in large numbers in places where humans gather, such as hospitals, food markets, slaughterhouses, dining establishments, and farms with poultry and livestock (Akter *et al*., 2020). They can be a nuisance to people, as well as to poultry, livestock, and other farm animals, and additionally serve as potential carriers of diseases. A systematic review found that flies carry over 130 pathogens, including *E. coli* (Khamesipour *et al*., 2018). Flies have been suggested as a possible carrier for infectious diseases and MDR *E. coli* in healthcare settings, especially in LMICs where the hygienic status is not well maintained (Thomson *et al*., 2021; Tufa *et al*., 2020). Nevertheless, the extensive presence of MDR bacteria in food, livestock, animal-derived food items, and environmental samples (such as water, air, and soil) as well as among farmers, indicates the possibility of flies acting as universal vectors disseminating these bacteria across various environments, including hospitals (Zuhora *et al.,* 2023).

Surveillance data is essential for clinicians to administer appropriate antimicrobials, adjust local antibiotic stewardship programs and to inform strategies addressing AMR at local, national, regional and global levels (WHO, 2024). Surveying *E. coli* in the context of AMR can be a useful indicator to determine antibiotic resistance levels in specific geographical regions across certain time frames (Geurtsen *et al*., 2022). The World Health Organisation (WHO) has highlighted a significant deficiency in high-quality data regarding the prevalence

of resistant bacteria across humans, animals, and food sources. This shortfall is particularly evident in the context of community-acquired infections in LMICs (WHO, 2024). A systematic data collection approach following a One Health framework is essential for developing robust stewardship programs and targeted interventions, ultimately striving to enhance public health improvements. This study serves as a pilot project to deepen our understanding of AMR prevalence in the Bangladeshi community. This chapter will describe the prevalence of antibiotic-resistant *E. coli* from a variety of sampling sectors, including human gut flora, human infections, farm animals and the environmental sector.

4.2 Results

4.2.1 Prevalence of *E. coli* in different sources and locations

A detailed description of the sampling sources, study locations and sampling process methodology is stated in Chapter 2 and Chapter 3. In total, 3759 samples were collected from which 1634 *E. coli* isolates were isolated and identified while single colony was picked from each sample. Table 4.1 states the prevalence of *E. coli* isolation from all the sampling locations.

Table 4.1 Prevalence of *E. coli* isolated from different sources

Source	Number of samples	Prevalence of *E. coli* n, (%)
Fly	220	183 (83.2)
Water	220	96 (43.6)
Chicken cloacal swab	200	194 (97)
Chicken meat swab	200	141 (70.5)
Domestic animal rectal swab	200	190 (95)
Bird faeces	75	55 (73.3)
Human rectal swab	660	534 (80.9)
Surgical site infection	814	129 (15.8)
Urinary tract infection	1170	112 (9.6)
Total	**3759**	**1634 (43.47)**

n, number *E. coli* isolates.

The highest prevalence of *E. coli* found in this study was CCS (97%), followed by DARS (95%), fly (83.2%), HRS (80.9%), BF (73.3%), CMS (70.5%), water (43.6%), SSI (15.8%) and UTI (9.6%).

Prevalence of *E. coli* in Mymensingh Sadar

In the Mymensingh Sadar broiler farm, the prevalence of *E. coli* observed in various samples was as follows: flies showed a prevalence of 90% (18/20), water at 70% (14/20), CCS at 95% (19/20), CMS at 80% (16/20), DARS at 90% (18/20), BF at 80.0% (38/47), and HRS at 88.3% (53/60). The layer farm had prevalence rates of 85% (17/20) at flies, 30% (6/20) in water, 100% (20/20) in CCS, 80% (16/20) in CMS, 100% (20/20) in DARS, and 80% (48/60) in HRS. HRS taken around the hospital showed a prevalence of 88.3% (53/60). Water and fly samples collected around the MMCH showed a prevalence of 85% (17/20) and 90% (18/20) respectively. All clinical samples were collected from the hospital located in Mymensingh Sadar. The rates of isolation of *E. coli* from SSI were 15.8% (129/814) and from UTI were 9.6% (112/1170).

Prevalence of *E. coli* in Muktagachha

In the Muktagachha broiler farm, the prevalence of *E. coli* observed in various samples was as follows: flies showed a prevalence of 85% (17/20), water at 35% (7/20), CCS at 90% (18/20), CMS at 45% (9/20), DARS at 90% (18/20) and HRS at 80% (48/60). The Muktagachha layer farm had prevalence rates of 65% (3/20) at flies, 60% (12/20) at water, 100% (20/20) at CCS, 65% (13/20) at CMS, 100% (20/20) at DARS, 44.4% (4/9) at BF and 81.6% (49/60) at HRS. No bird samples were found on the Muktagachha broiler farm. Water samples and CMS in the broiler farm showed comparatively lower prevalence than that of the layer farm, whereas, in the case of fly samples, the prevalence in the broiler farm was higher than that of the layer farm.

Prevalence of *E. coli* in Gauripur

The Gauripur broiler farm had prevalence rates of *E. coli* 90% (18/20) in flies, 30% (6/20) in water, 100% (20/20) in CCS, 70% (14/20) in CMS, 85%, (17/20) in DARS, 60% (3/5) in BF and 68.3% (41/60) in HRS. In the Muktagachha layer farm, the prevalence observed in various samples was as follows: flies showed a prevalence of 95% (19/20), water at 45% (9/20), CCS at 100% (20/20), CMS at 65% (13/20), DARS at 100% (20/20), and HRS at 73.3% (44/60). No bird samples were collected from the Gauripur layer farm. The farm was situated in an area devoid of trees near the bird sheds, and I did not observe any free-flying birds during the sample collection period.

Prevalence of *E. coli* in Trishal

In the Trishal broiler farm, the prevalence of *E. coli* observed in various samples was as follows: flies showed a prevalence of 80% (16/20), water at 60% (12/20), CCS at 85% (17/20), CMS at 70% (14/20), DARS at 95% (19/20) and HRS at 83.3% (50/60). The Trishal layer farm had prevalence rates of 80% (16/20) for fly, 20% (4/20) for water, 100% (20/20) for CMS, 65% (13/20) for CMS, 95% (19/20) for DARS, and 85% (51/60) for HRS. The prevalence in the Trishal layer farm was 50% (4/8), while no bird samples were found around the Trishal broiler farm during the sample collection period. Water samples collected from the broiler farm showed a significantly higher prevalence compared to those from the layer farm (Figure 4.1).

Prevalence of *E. coli* in Tarakanda

In the Tarakanda broiler farm, the prevalence of *E. coli* observed in various samples was as follows: flies showed a prevalence of 75% (15/20), water at 10% (2/20), CCS at 100% (202/0), CMS at 85% (17/20), DARS at 95% (19/20), BF at 100% (4/4) and HRS at 78.3% (47/60). The Tarakanda layer farm had the prevalence rates of 80% (16/20) at flies, 35% (7/20) at water,

100% (20/20) at CCS, 80% (16/20) at CMS, 100% (20/20) at DARS, 100% (4/4) at BF and 85% (51/60) at HRS. Water samples taken from the broiler farm showed a comparatively lower prevalence than those from the layer farm (Figure 4.1b).

The prevalence of *E. coli* in CCS, DARS, and HRS showed a similar pattern across the five sub-districts. In the case of fly samples, the prevalence is comparatively higher in Mymensingh Sadar (88.3%) and Gauripur (92.5%). In BF, the prevalence of *E. coli* in Tarakanda was the highest at 100%, followed by Mymensingh Sadar at 80.8%. In water samples, the highest prevalence was observed in Mymensingh Sadar (61.6%), while the lowest prevalence was found in isolates from Tarakanda (22.5%) (Figure 4.1).

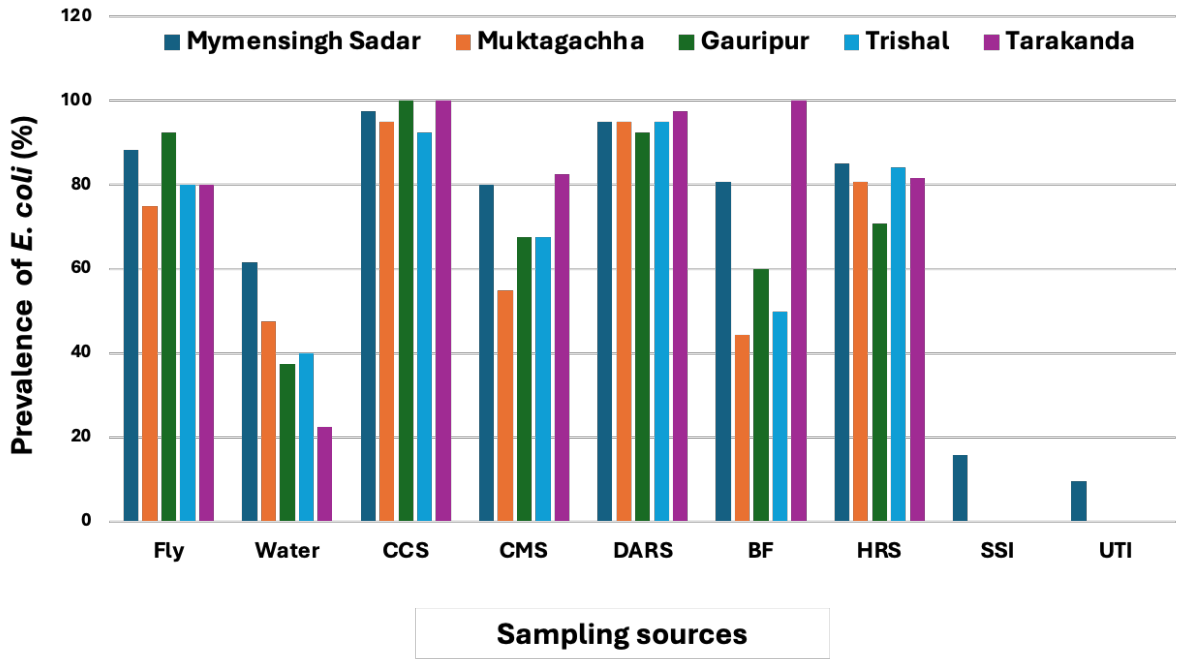


Figure 4.1a Comparison of *E. coli* prevalence across sample types and sub-districts.

BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection.

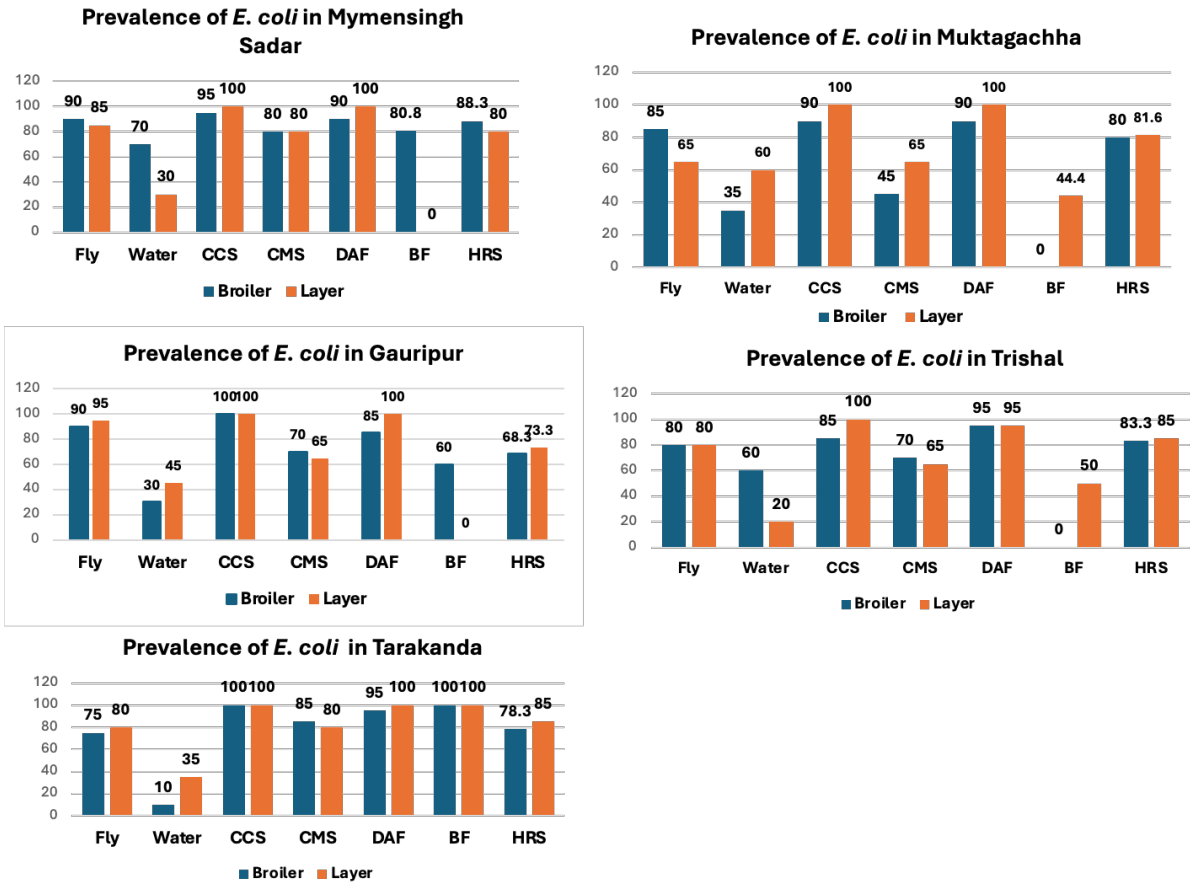


Figure 4.1b Prevalence of *E. coli* in different sample types from broiler and layer farms across selected sub-districts.

BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection.

4.2.2 Prevalence of *E. coli* in different sampling quarters

Samples were collected from Dec/2021 to Mar/2023, and the total sampling period was divided into four quarters: 1st quarter (Dec/2021-Mar/2022), 2nd quarter (Apr/2022-Jul/2022), 3rd quarter (Aug/2022-Nov/2022) and 4th quarter (Dec/2022-Mar/2023). The prevalences of *E. coli* in different quarters were as follows: in the 1st quarter the prevalence was observed at 55.8% (414/741), in the 2nd quarter the prevalence observed at 41.7% (432/1034), in the 3rd quarter the prevalence was observed at 40% (432/1080) and in the 4th quarter the prevalence was

observed at 39.3% (356/904) (Figure 4.2). The highest prevalence of *E. coli* was observed in the 1st quarter (55.8%) of the sampling period, with a downward trend in subsequent quarters.

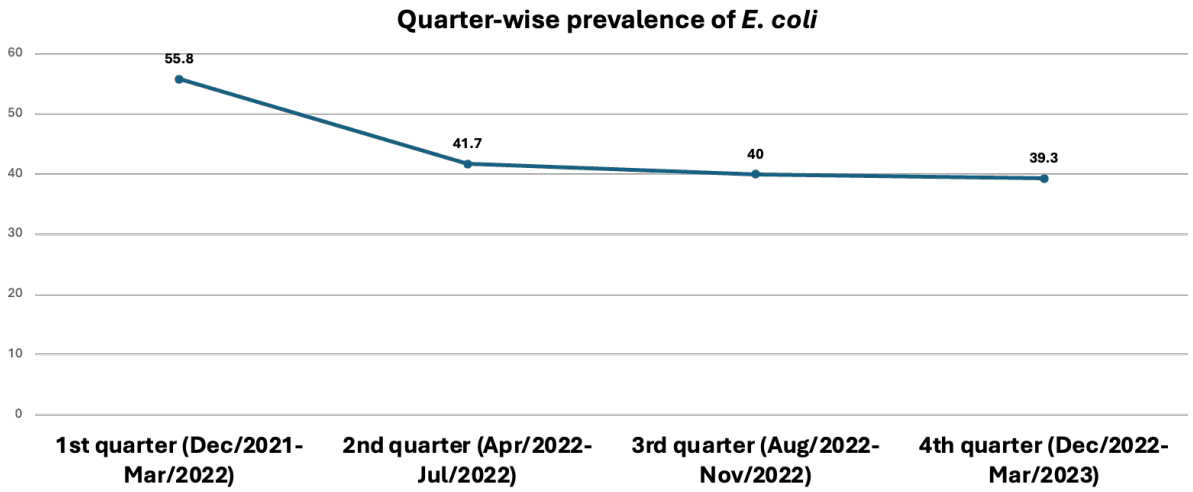


Figure 4.2 Quarter-wise prevalence of *E. coli* across all sampling sources. Line graph showing the prevalence of *E. coli* over four sampling quarters.

Quarter-wise distribution of *E. coli* from different sources

The prevalence of *E. coli* in different sources and quarters is shown in Table 4.2. A chi-square test was performed to determine whether there was any association between a particular season and the prevalence of *E. coli*. Prevalence of *E. coli* from CCS (6.7%), CMS (5.5%), DARS (6.6%) and HRS (21.2%) was significantly higher in the 1st quarter in comparison to other quarters. Prevalence of *E. coli* from SSI was significantly higher (5.2%) in the 3rd quarter compared to other quarters while the prevalence in BF was higher in 4th quarter compared to the others (Table 4.2).

Table 4.2 Comparative analysis of *E. coli* prevalence across different sampling quarters and sources

Attributes	**Sampling period**				
Sample sources	**Quarter 1 (n=741)**	**Others* (n=3018)**	***p*-value**	**OR**	**95% CI**
Fly (n=183)	46 (6.2%)	137 (4.5%)	0.059	1.392	0.986-1.964
Water (n=96)	20 (2.7%)	76 (2.5%)	0.78	1.074	0.652-1.769
CCS (n=194)	50 (6.7%)	144 (4.8%)	0.029	1.444	1.036-2.013
CMS (n=141)	41 (5.5%)	100 (3.3%)	0.004	1.709	1.177-2.481
DARS (n=190)	49 (6.6%)	141 (4.7%)	0.031	1.445	1.033-2.021
BF (n=55)	6 (0.8%)	49 (1.6%)	0.098	0.495	0.211-1.159
HRS (n=534)	157 (21.2%)	377 (12.5%)	0	1.883	1.532-2.316
SSI (n=129)	15 (2%)	114 (3.8%)	0.019	0.526	0.305-0.907
UTI (n=112)	30 (4%)	82 (2.7%)	0.056	1.511	0.987-2.314
Sample sources	**Quarter 2 (n=1034)**	**Others* (n=2725)**	***p*-value**	**OR**	**95% CI**
Fly (n=183)	47 (4.5%)	136 (5%)	0.571	0.907	0.645-1.273
Water (n=96)	19 (1.8%)	77 (2.8%)	0.086	0.644	0.388-1.069
CCS (n=194)	48 (4.6%)	146 (5.4%)	0.376	0.860	0.616-1.201
CMS (n=141)	42 (4.1%)	99 (3.6%)	0.537	1.123	0.777-1.623
DARS (n=190)	50 (4.8%)	140 (5.1%)	0.706	0.938	0.674-1.307
BF (n=55)	20 (1.9%)	35 (1.3%)	0.138	1.516	0.871-2.638
HRS (n=534)	147 (14.2%)	387 (14.2%)	0.991	1.001	0.816-1.229
SSI (n=129)	26 (2.5%)	103 (3.8%)	0.057	0.657	0.424-1.016
UTI (n=112)	33 (3.2%)	79 (2.9%)	0.638	1.104	0.731-1.668
Sample sources	**Quarter 3 (n=1080)**	**Others* (n=2679)**	***p*-value**	**OR**	**95% CI**
Fly (n=183)	48 (4.4%)	135 (5%)	0.443	0.876	0.626-1.228
Water (n=96)	26 (2.4%)	70 (2.6%)	0.718	0.919	0.583-1.450
CCS (n=194)	49 (4.5%)	145 (5.4%)	0.272	0.831	0.596-1.157
CMS (n=141)	38 (3.5%)	103 (3.8%)	0.634	0.912	0.624-1.332
DARS (n=190)	47 (4.4%)	143 (5.3%)	0.212	0.807	0.576-1.131

BF (n=55)	9 (0.8%)	46 (1.7%)	0.041	0.481	0.235-0.986
HRS (n=534)	128 (11.9%)	406 (15.2%)	0.009	0.753	0.609-0.931
SSI (n=129)	56 (5.2%)	73 (2.7%)	0.00	1.952	1.368-2.786
UTI (n=112)	31 (2.9%)	81 (3%)	0.803	0.948	0.623-1.443
Sample sources	**Quarter 4 (n=904)**	**Others* (n=2855)**	***p*-value**	**OR**	**95% CI**
Fly (n=183)	42 (4.6%)	141 (4.9%)	0.722	0.938	0.659-1.335
Water (n=96)	31 (3.4%)	65 (2.3%)	0.056	1.524	0.987-2.354
CCS (n=194)	47 (5.2%)	147 (5.1%)	0.953	1.010	0.721-1.416
CMS (n=141)	20 (2.2%)	121 (4.2%)	0.005	0.511	0.317-0.825
DARS (n=190)	44 (4.9%)	146 (5.1%)	0.768	0.949	0.672-1.341
BF (n=55)	20 (2.2%)	35 (1.2%)	0.031	1.823	1.047-3.174
HRS (n=534)	102 (11.3%)	432 (15.1%)	0.004	0.713	0.567-0.898
SSI (n=129)	32 (3.5%)	97 (3.4%)	0.838	1.043	0.695-1.567
UTI (n=112)	18 (2%)	94 (3.3%)	0.045	0.597	0.358-0.994

Values in parentheses indicate column percentage. Statistical significance was set at $p < 0.05$. Others* denotes the combined prevalence of *E. coli* from the remaining three sampling quarters. Highlighted cells indicate the prevalence of *E. coli,* which was statistically significant ($p < 0.05$).

BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection

4.2.3 Analysing the phenotypic resistance patterns of *E. coli* and comparing the resistance profiles across various antibiotics

To investigate the phenotypic resistance pattern of *E. coli,* MICs were performed against 17 clinically important antibiotics. This selection of antibiotics for susceptibility testing was informed by both research articles on antimicrobial usage in human and veterinary medicine (Rashid *et al.,* 2022; Imam *et al.,* 2020) and data collected from farmers about their antibiotic usage at MMCH and in the farms. The antibiotics commonly used in both animal and human medicine in Bangladesh were prioritised during the selection. The antibiotics used for MIC in this study were ampicillin (AMP), amoxicillin-clavulanic acid (AMC), amikacin (AMK), aztreonam (AZT), cefotaxime (CTX), ceftazidime (CAZ), ceftazidime-avibactam (CAZ-AVI), cefepime (CEF), ciprofloxacin (CIP), colistin (COL), fosfomycin (FOS), gentamicin (GEN), imipenem (IPM), levofloxacin (LEV), meropenem (MER), piperacillin-tazobactam (TZP) and tigecycline (TIG). More than half of the isolates (51.04%, 834/1634), were found to be resistant to AMP, followed by CIP (45.10%, 737/1634) and LEV (43.20%, 706/1634), (Figure 4.3, Table 4.3).

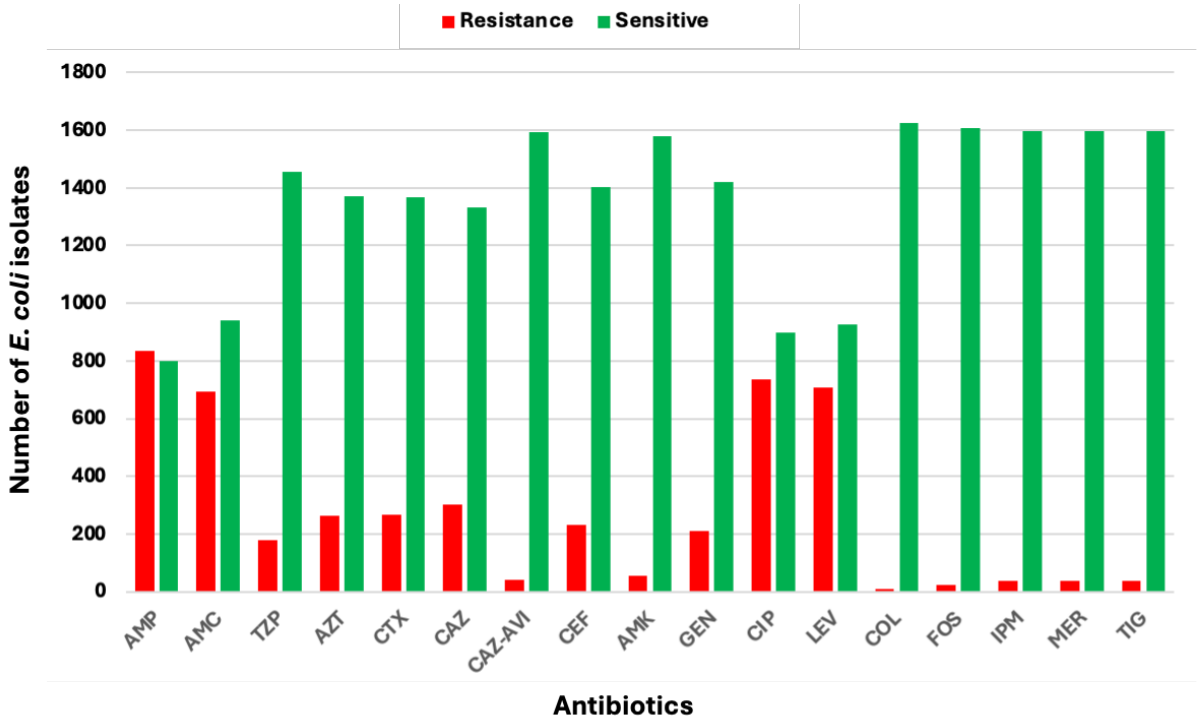


Figure 4.3 Susceptibility pattern of *E. coli* isolates against all antibiotics tested. The bar charts indicate the number of isolates.

AMP, ampicillin; AMC, amoxicillin-clavulanic acid; AMK, amikacin; AZT, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; CEF, cefepime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MER, meropenem; TZP, piperacillin-tazobactam; TIG, tigecycline.

The susceptibility pattern of *E. coli* in various sectors is shown in Table 4.3. The breakpoints for resistance and sensitivity have been determined using the guidelines by EUCAST (v.12.0) (EUCAST, 2022). In the fly sample, the highest level of resistance was observed against AMC (60.1%, 110/183), followed by CIP (59.5%, 109/183), AMP (54.6%, 100/183), and LEV (50.2%, 92/183). Additionally, 28.9% (53/183) of the isolates were found to be resistant to TZP.

The isolates from water samples showed a similar resistance pattern to the isolates from fly samples. The highest resistance was found against AMP (59.3%, 67/96).

In the CCS samples, 88.1% (171/194) of the isolates were found to be resistant to both CIP and LEV. Additionally, 82.9% (161/194) of the isolates showed resistance to AMC, followed by 54.1% (105/194) exhibiting resistance to AMP, and 44.3% (86/194) being resistant to GEN. A similar pattern of resistance was observed in the isolates from CMS samples as in those from CCS isolates, with the highest resistance to CIP (84.4%, 119/141) (Table 4.3).

E. coli isolates from DARS were mostly found to be sensitive to all the antibiotics; 10% (19/190) of isolates were found to be resistant to AMP, which represented the highest level of resistance from this sector. Isolates from BF were also found to be mostly susceptible to the antibiotics. The highest resistance was found to AMP, 20% (11/55), followed by AMC at 16.3% (9/55) (Table 4.3).

E. coli from clinical samples showed a higher resistance pattern compared to the farm and environmental isolates. Over 90% of the isolates from SSI were found to be resistant to AMP, AMC, AZT, CTX, CTZ, and CEF (Table 4.3). The prevalence of resistance to CIP and LEV among SSI isolates was 75.9% (98/129) and 74.4% (96/129), respectively. Additionally, resistance to MER and IPM was observed in 18.6% (24/129) of SSI isolates, a finding that was less common in other sectors. In UTI isolates, the highest prevalence of resistance was against AMP, 68.7% (77/112), and approximately 50% of isolates were resistant to AMC, aztreonam, third and fourth-generation cephalosporins, CIP, and LEV (Table 4.3). In case of HRS isolates, the highest resistance was observed to AMP, 34.4% (184/534), followed by 29.5% (158/534) to AMC and 22.2% (119/534) to CIP.

Overall, the penicillin and fluoroquinolone groups of antibiotics demonstrated greater resistance compared to other groups across all sectors. In contrast, aztreonam and third- and fourth-generation cephalosporins were primarily resistant to clinical isolates, as well as flies and water samples.

Table 4.3 Antimicrobial resistance pattern in *E. coli* in various sources

Source	Resistance to respective antibiotics, n (%)																
	AMP	AMC	TZP	AZT	CTX	CAZ	CAZ-AVI	CEF	AMK	GEN	CIP	LEV	COL	FOS	IPM	MER	TIG
Fly (n=183)	100 (54.6)	110 (60.1)	53 (28.9)	21 (11.4)	22 (12.0)	29 (15.8)	5 (2.7)	14 (7.6)	5 (2.7)	32 (17.4)	109 (59.5)	92 (50.2)	8 (4.3)	7 (3.8)	3 (1.6)	3 (1.6)	11 (6.0)
Water (n=96)	67 (59.3)	50 (52.0)	27 (28.1)	16 (16.6)	17 (17.7)	20 (20.8)	5 (5.2)	15 (15.60	3 (3.1)	14 (14.5)	56 (58.3)	49 (51.0)	0 (0.0)	5 (5.2)	4 (4.1)	5 (5.20	1 (1.0)
CCS (n=194)	161 (82.9)	105 (54.1)	23 (11.8)	7 (3.6)	8 (4.1)	15 (7.7)	0 (0.0)	5 (2.5)	5 (2.5)	86 (44.3)	171 (88.1)	171 (88.1)	2 (1.0)	3 (1.5)	0 (0.0)	0 (0.0)	7 (3.6)
CMS (n=141)	97 (68.7)	76 (53.9)	0 (0.0)	1 (0.7)	2 (1.4)	4 (2.8)	0 (0.0)	1 (0.7)	0 (0.0)	19 (13.4)	116 (82.2)	119 (84.4)	0 (0.0)	4 (2.8)	0 (0.0)	0 (0.0)	13 (9.2)
DARS (n=190)	19 (10.0)	4 (2.1)	0 (0.0)	4 (2.1)	4 (2.1)	4 (2.1)	0 (0.0)	3 (1.5)	0 (0.0)	1 (0.5)	7 (3.6)	7 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
BF (n=55)	11 (20.0)	9 (16.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (5.4)	7 (12.7)	6 (10.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (5.4)
HRS (n=534)	184 (34.4)	158 (29.5)	5 (0.9)	42 (7.8)	42 (7.8)	55 (10.3)	1 (0.1)	34 (6.3)	3 (0.5)	13 (2.4)	119 (22.2)	113 (21.1)	0 (0.0)	3 (0.5)	1 (0.1)	1 (0.1)	1 (0.1)
SSI (n=129)	128 (99.2)	123 (95.3)	59 (45.7)	122 (94.5)	121 (93.8)	122 (94.5)	25 (19.3)	118 (91.4)	28 (21.7)	37 (28.6)	98 (75.9)	96 (74.4)	0 (0.0)	1 (0.7)	24 (18.6)	24 (18.6)	0 (0.0)
UTI (n=112)	77 (68.7)	60 (53.5)	11 (9.8)	49 (43.7)	51 (45.5)	52 (46.4)	4 (3.5)	42 (37.5)	10 (8.9)	7 (6.2)	54 (48.2)	53 (47.3)	0 (0.0)	2 (1.7)	4 (3.50	4 (3.5)	1 (0.8)

Values in parentheses indicate row percentage of resistance. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; AMK, amikacin; AZT, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; CEF, cefepime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MER, meropenem; TZP, piperacillin-tazobactam; TIG, tigecycline

BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection.

MIC_{90} and MIC_{50} values are defined as the lowest antibiotic concentrations at which 90% and 50% of the isolates, respectively, were inhibited. MIC_{50} and MIC_{90} values are often used in antimicrobial susceptibility testing to determine the effectiveness of different antibiotics against specific bacteria (Yamazhan *et al.,* 2005). The MIC_{50} and MIC_{90} values for all antibiotics, along with their respective MIC ranges, are provided in Table 4.4. In the case of ampicillin, 90% of *E. coli* isolates were grown at a concentration of 64 mg/L, while 90% of the isolates were inhibited at 32 mg/L of AMC, GEN, CIP, LEV, and CTX.

Figure 4.4 illustrates the distribution pattern of *E. coli* isolates across different MIC ranges for all tested antibiotics.

Table 4.4 MIC ranges, MIC_{50} and MIC_{90} values of *E. coli* isolates for different antibiotics

Number of *E. coli*, n= 1634	Antibiotics	MIC_{50} (mg/L)	MIC_{90} (mg/l)	Range of MIC (mg/l)	n (%) Resistance
	AMP	16	>64	0.25 to >64	834 (51.04)
	AMC	8	32	0.06 to >64	695 (42.53)
	TZP	1	8	0.06 to >64	178 (10.89)
	AZT	≤0.06	8	0.06 to >64	262 (16.03)
	CTX	≤0.06	32	0.06 to >64	267 (16.34)
	CAZ	0.125	8	0.06 to >64	303 (18.54)
	CAZ-AVI	≤0.06	0.125	0.06 to >64	40 (2.45)
	CEF	≤0.06	4	0.06 to >64	231 (14.14)
	AMK	4	4	1 to >64	54 (3.30)
	GEN	1	32	0.06 to >64	212 (12.97)
	CIP	0.25	32	0.06 to >64	737 (45.10)
	LEV	0.5	32	0.06 to >64	706 (43.20)
	COL	0.125	0.25	0.06 to 16	10 (0.61)
	FOS	1	2	0.06 to >64	25 (1.52)
	IPM	0.125	0.25	0.06 to 64	36 (2.20)
	MER	≤0.06	0.06	0.06 to >64	37 (2.26)
	TIG	0.125	0.25	0.06 to 4	38 (2.32)

n, number of resistant isolates to the respective antibiotics. Values in the parentheses indicate the row percentage. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; AMK, amikacin; AZT, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; CEF, cefepime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MER, meropenem; TZP, piperacillin-tazobactam; TIG, tigecycline.

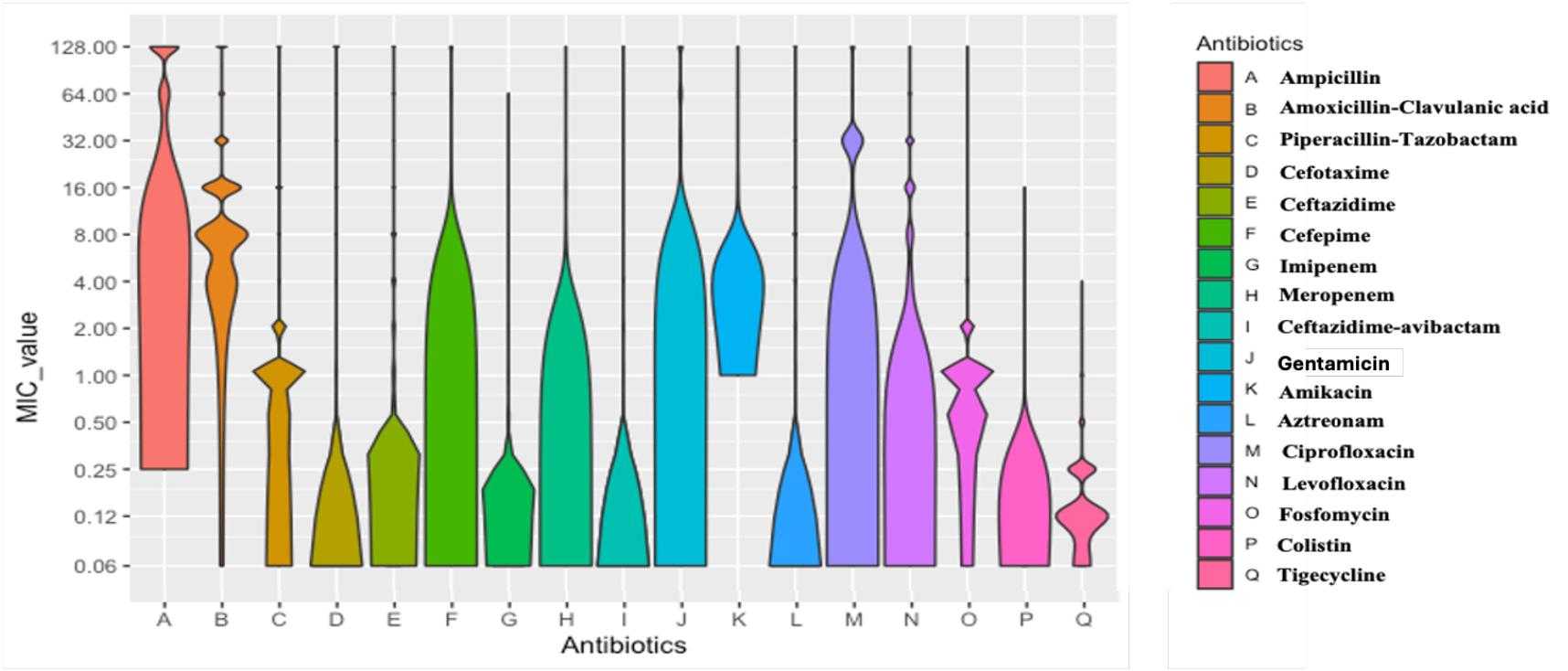


Figure 4.4 Distribution of MIC values of *E. coli* isolates across different antibiotics.

AMP, ampicillin; AMC, amoxicillin-clavulanic acid; AMK, amikacin; AZT, aztreonam; CTX, cefotaxime; CAZ; ceftazidime, CAZ-AVI, ceftazidime-avibactam; CEF, cefepime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MER, meropenem; TZP, piperacillin-tazobactam; TIG, tigecycline.

4.2.4 Investigating the prevalence of MDR *E. coli* isolated from various sampling sources

MDR is defined as nonsusceptibility to at least one agent in three or more antimicrobial categories (Cosentino *et al*., 2023). The MDR *E. coli* isolates found in this study are detailed in Table 4.5. Among the isolates from SSI, 76.7% (99/129) were found to be resistant to multiple classes of antibiotics, while the prevalence of MDR in UTI was 34.8% (39/112). The highest rate of MDR *E. coli* from farm samples was found in CMS, 21.9% (31/141). From fly samples, 29.5% (54/183) of *E. coli* were found to be MDR, and from water samples, 29.1% (28/96) of *E. coli* exhibited MDR phenotypes. In contrast, the percentages of MDR isolates were lower in DARS, HRS, and BF.

Table 4.5: *E. coli* resistant to two or more groups of antibiotics from various sources

Source	**Number of *E. coli* resistant to three or more than three antibiotics n (%)**
Fly (n=183)	54 (29.5)
Water (n= 96)	28 (29.1)
CCS (n= 194)	23 (11.8)
CMS (n= 141)	31 (21.9)
DARS (n= 190)	1 (0.5)
BF (n= 55)	2 (3.6)
HRS (n= 534)	39 (7.3)
SSI (n= 129)	99 (76.7)
UTI (n=112)	39 (34.8)

Values in parentheses indicate row percentage. BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection.

Resistance to various antibiotic combinations was observed across sample types. The combination of antibiotic classes and their prevalence of resistance in different sectors is shown in Table 4.6. Nearly half of the isolates (n= 664) were found to be resistant to penicillin and fluoroquinolones, primarily sourced from water, flies, clinical samples, and specimens from CCS and CMS. Resistance to a combination of penicillin and fluoroquinolones was highest in CCS at 81.4%, followed by SSI at 77.5% and CMS at 68.7%. Other sources exhibited comparatively lower percentages. A significant percentage (75.9%) of isolates from SSI samples exhibited resistance to both cephalosporins and fluoroquinolones, while 32.1% of UTI isolates also showed similar resistance patterns. Notably, five isolates were found to be resistant to both tigecycline and colistin; all the isolates were from fly samples. These five isolates were also found to be resistant to CIP and LEV. All of them were found to be sensitive to AMK, CEF and CAZ-AVI.

Table 4.6 *E. coli* resistant to two or more groups of antibiotics from various sources

Combination of antibiotics	Resistant to respective combination of antibiotics, n (%)								
	Fly n=183	Water n= 96	CCS n=194	CMS n= 141	DARS n= 190	BF n= 55	HRS n= 534	SSI n=129	UTI n=112
Penicillin + Fluoroquinolones (n=664)	100 (54.6)	51 (53.1)	158 (81.4)	97 (68.7)	6 (3.1)	6 (10.9)	97 (18.1)	100 (77.5)	49 (43.7)
Penicillin + Aminoglycosides (n=213)	33 (18)	13 (13.5)	77 (39.6)	17 (12.0)	1 (.5)	2 (3.6)	13 (2.4)	45 (34.8)	12 (10.7)
3GC + Aminoglycosides (n=91)	10 (5.4)	6 (6.2)	10 (5.1)	2 (1.4)	0 (0.0)	2 (3.64)	7 (1.3)	45 (34.8)	9 (8.0)
3GC + Fluoroquinolones (n=229)	21 (11.4)	19 (19.7)	14 (7.2)	4 (2.84)	0 (0.0)	2 (3.64)	35 (6.5)	98 (75.9)	36 (32.1)
3GC + Carbapenem (n=38)	3 (1.6)	5 (5.2)	0 (0.0)	0 (0.0)	0 (0.0)	0(0.0)	1 (.1)	25 (19.3)	4 (3.57)
Aminoglycosides + Fluoroquinolones (n=220)	31 (16.9)	14 (14.5)	86 (44.3)	19 (13.4)	1 (.5)	2 (3.64)	11 (2.0)	44 (34.1)	12 (10.7)
Aminoglycosides + Carbapenem (n=26)	1 (.5)	3 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (.1)	19 (14.7)	2 (1.7)
Fluoroquinolones + Carbapenem (n=37)	3 (1.6)	5 (5.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (.1)	24 (18.6)	4 (3.5)
Tigecycline+ Colistin (n=5)	5 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Fluoroquinolones + Aminoglycosides + Colistin (n=5)	0 (0.0)	3 (3.1)	0 (0.0)	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fluoroquinolones + Aminoglycosides + Carbapenem (n=25)	1 (.5)	3 (3.1)	0 (0.0)	0 (0.0)	0(0.0)	0 (0.0)	1 (0.1)	18 (13.9)	2 (1.7)

Values in parentheses indicate column percentage. BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection.

4.2.5 Antibiotic usage in farms

During the farm sample collection from each sampling point, antibiotic usage data for the previous four months were collected from the farm owners (Table 4.7). Farm data shows that fluoroquinolones, penicillin, and aminoglycosides were the highest administered antibiotics.

Table 4.7 List of antibiotics used in poultry farms included in this study during the sampling period

Farms	**Antibiotics used in farm across the sampling season**			
	Quarter 1	**Quarter 2**	**Quarter 3**	**Quarter 4**
Mymensingh broiler	Usage not identified	Usage not identified	Usage not identified	Usage not identified
Mymensingh layer	Neomycin sulphate (aminoglycosides)	Colistin sulphate (Polymixin)	Usage not identified	Ciprofloxacin (fluoroquinolones)
Muktagachha broiler	Amoxicillin (penicillin)	Amoxicillin (penicillin)	Amoxicillin (penicillin)	Doxycycline (tetracycline)
Muktagacchha layer	Usage not identified	Tilmicosin (macrolids)	Amoxicillin (penicillin)	Doxycycline (tetracycline)
Gauripur broiler	Gentamicin (aminoglycosides)	Gentamicin (aminoglycosides)	Usage not identified	Gentamicin (aminoglycosides)
Gauripur layer	Usage not identified	Doxycycline (tetracycline)	Usage not identified	Doxycycline (tetracycline)

Trishal broiler	Usage not identified	Trimethoprim+ Sulphadiazine (sulphonamides)	Ciprofloxacin (fluoroquinolones)	Amoxicillin (penicillin)
Trishal layer	Norfloxacin (fluoroquinolones)	Levofloxacin (fluoroquinolones)	Levofloxacin (fluoroquinolones)	Doxycycline (tetracycline)
Tarakanda broiler	Usage not identified	Neomycin sulphate (aminoglycosides)	Ciprofloxacin (fluoroquinolones)	Usage not identified
Tarakanda layer	Levofloxacin (fluoroquinolones)	Neomycin sulphate (aminoglycosides)	Amoxycillin (penicillin)	Usage not identified

Values in the parentheses indicate the respective group of antibiotics.

4.2.6 Seasonal effects on resistance pattern

A chi-square test was used to assess the association between antibiotic resistance patterns of the isolates and sampling quarter. No significant association was observed between resistance patterns and sampling time during the 1st quarter (Dec/2021 – Mar/2022) and the 4th quarter (Dec/2022 – Mar/2023). In contrast, colistin resistance showed a significant association with sampling time, with a higher proportion of resistant isolates observed in the 2nd quarter (Apr/2022 – Jul/2022) compared with other quarters ($p < 0.05$; Table 4.8). Similarly, resistance to ceftazidime–avibactam, imipenem, and meropenem was significantly associated with the 3rd quarter (Aug/2022 – Nov/2022), indicating that resistant isolates were more frequently detected during this period than in other sampling quarters ($p < 0.05$; Table 4.8).

Table 4.8 Significant resistance prevalence of antibiotics in various sampling quarters

Quarter 2						
Antibiotic		Others	Quarter-2	*p* value	95% CI Lower	95% CI upper
COL	R, n= 10	4 (40))	6 (60)	0.016	0.066	0.844
	S, n= 1624	1198 (73.8)	426 (26.2)			
Quarter 3						
Antibiotic		Others	Quarter-3	*p* value	95% CI lower	95% CI upper
CAZ-AVI	R, n= 40	23 (57.5)	17 (42.5)	0.02	0.252	0.9
	S, n=1594	1179 (74.0)	415 (26.0)			
IPM	R, n= 36	20 (55.6)	16 (44.4)	0.013	0.226	0.857
	S, n= 1598	1182 (74.0)	416 (26.0)			
MER	R, n= 37	22 (59.5)	15 (40.5)	0.049	0.266	1.009
	S, n= 1597	1180 (73.9)	417 (26.1)			

Values in the parentheses indicate percentages. R, resistant; S, sensitive; CI, confidence interval; CAZ-AVI, ceftazidime-avibactam; COL, colistin; IPM, imipenem; MER, meropenem.

4.2.7 Risk assessment associated with baseline epidemiological variables in SSI samples

Epidemiological data, including age, sex, education level, sanitation status, history of rearing domestic animals, and previous antibiotic use, were collected from the patients with SSI (as detailed in Chapter 3). Among the enrolled 814 patients with SSI, 45.5% (370/814) were female, and 54.5% (444/814) were male. The prevalence of *E. coli* in males was 18. 7% (83/444) and in females was 12.7% (47/370). Among 129 *E. coli* positive isolates from SSI, 64.3% (83/129) were from male patients, which was statistically significant ($p < 0.05$) compared to the isolates found positive in female patients, 35.7% (46/129). Distribution of age ranges and sex among SSI patients is shown in Figure 4.5.

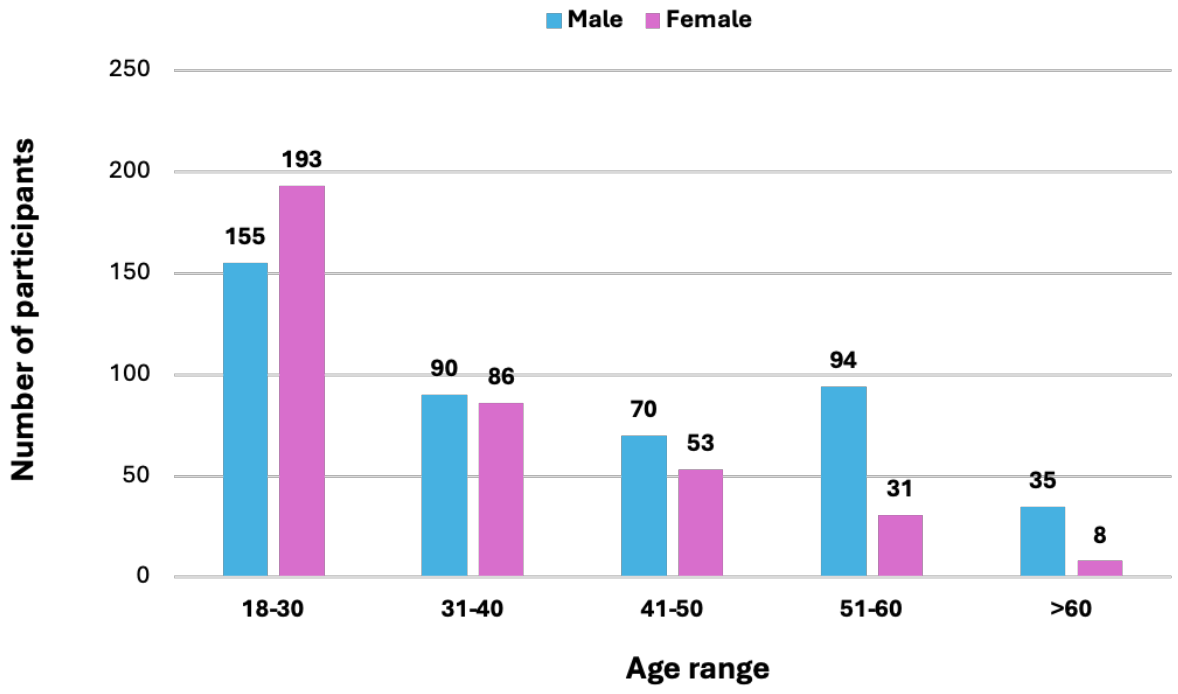


Figure 4.5 Distribution of age and sex among SSI patients enrolled in this study.

For SSI isolates, univariable associations between epidemiological variables and resistance to individual antibiotics were assessed using chi-square tests. The risk association analysis was performed for all 17 antibiotics using baseline epidemiological variables collected from patients. Only the specific antibiotics linked to the attributes are listed and shown in Table 4.9. Only antibiotics showing statistically significant associations with specific attributes are presented in Table 4.9. Resistance to GEN and TZP was significantly more frequent among *E. coli* isolates obtained from female patients compared with male patients ($p < 0.05$). In addition, resistance to GEN and TZP was more commonly observed among isolates from individuals reporting the use of domestic toilet facilities connected to septic systems. Resistance to CAZ was significantly more frequent among isolates from patients aged 31–40 years (Table 4.9). These findings represent statistical associations identified through univariable analysis and do not imply direct causal relationships between epidemiological characteristics and antimicrobial resistance.

Table 4.9 Descriptive statistics for the risk assessment of surgical site isolates and antibiotic resistance

Attributes		CAZ, n=129			GEN, n=129			TZP, n=129		
		R	**S**	***p* value**	**R**	**S**	***p* value**	**R**	**S**	***p* value**
Access to water & soap in toilet	No	13	1	0.764	2	12	0.207	4	10	0.172
	Yes	109	6		35	80		55	60	
Antibiotics Use within 3 months	No	114	5	0.034	37	82	0.037	59	60	0.003
	Yes	8	2		0	10		0	10	
Drinking water	Tap water	33	3	0.365	12	24	0.467	19	17	0.318
	Tube well water	89	4		25	68		40	53	
Raising domestic animals	No	63	2	0.235	22	43	0.191	33	32	0.248
	Yes	59	5		15	49		26	38	
Gender	Female	45	1	0.225	19	27	0.018	28	18	0.010
	Male	77	6		18	65		31	52	
Age	18-30	41	2	0.783	17	26	0.054	23	20	0.211
	31-40	21	5	0.001	9	17	0.454	11	15	0.695
	41-50	23	0	0.205	6	17	0.761	13	10	0.252
	51-60	27	0	1.162	4	23	0.073	9	18	0.146
	>60	10	0	0.430	1	9	0.174	3	7	0.298
Education	Primary	24	2	0.568	4	22	0.093	9	17	0.203
	Secondary	48	3	0.853	19	32	0.082	23	28	0.906
	Tertiary	23	2	0.527	9	16	0.368	15	10	0.111
Toilet facilities	Simple pit latrines with slabs	57	2	0.349	11	48	0.021	21	38	0.034
	Toilets connected to septic systems	65	5		26	44		38	32	

n, number of *E. coli* isolates from surgical site infection, CAZ, ceftazidime; GEN, gentamicin; TZP, piperacillin-tazobactam. Highlighted cells indicate the significant association ($p < 0.05$).

No significant association was found between previous antibiotic usage and resistance of *E. coli* to antibiotics. However, previous antibiotic usage by patients with SSI before hospitalisation is demonstrated in Table 4.10.

Table 4.10 List of antibiotics taken by patients with SSI in the three months prior to sampling

Antibiotics	**Number of patients (n= 11)**
Penicillins	4 (36.4)
Quinolones	2 (18.1)
Macrolides	0 (0)
Metronidazole	1 (9.1)
1st and 2nd generation cephalosporin	0 (0)
3rd generation cephalosporin	3 (27.3)
Nitrofurantoin	0 (0)
Aminoglycosides	1 (9.1)
Unknown	0 (0)

Values in parentheses indicate the column percentage. n, represents the number of patients with *E. coli* infection who have a history of previous antibiotic use before hospitalisation.

4.2.8 Risk assessment associated with baseline epidemiological variables in UTI samples

Epidemiological data, including age, sex, education level, sanitation status, history of rearing domestic animals, and previous antibiotic use, were collected from the patients with UTI (as detailed in Chapter 3). A total of 1,170 urine samples were collected from outpatients suspected of having a UTI. The number of urine samples collected from females was significantly higher, 87.0% (1018/1170), compared to the samples collected from males, 13% (152/1170). The prevalence of *E. coli* from UTI samples in females was higher, 9.8% (100/1018), compared to the prevalence in males, which was 7.8% (12/152). Distribution of age ranges and sex among the participants who provided the urine samples is shown in Figure 4.6.

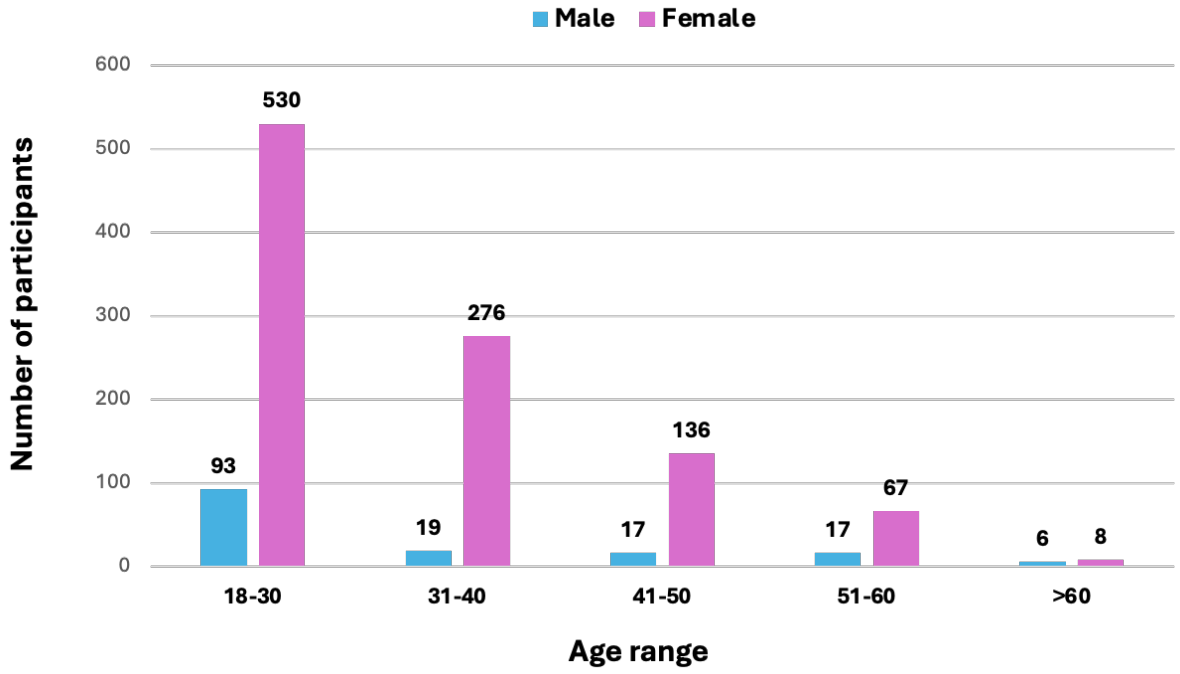


Figure 4.6 Distribution of age and sex among the participants who provided urine samples.

For UTI isolates, univariable associations between epidemiological variables and resistance to individual antibiotics were assessed using chi-square tests. Association analysis was performed for all 17 antibiotics using baseline epidemiological variables collected from participants who provided urine samples, with only antibiotics showing statistically significant associations presented in Table 4.11. Among urinary tract infection isolates, resistance to AMK was more frequently observed in *E. coli* isolates obtained from patients aged over 60 years. Resistance to CTX was significantly more frequent among isolates from patients aged 41–50 years and among female patients compared with other age groups and male patients, respectively ($p < 0.05$). In addition, resistance to CIP was significantly associated with isolates from patients aged 18–30 years and with a reported history of antibiotic use within the preceding three months ($p < 0.05$) (Table 4.11). These findings represent statistical associations identified through univariable analysis and do not imply direct causal relationships between epidemiological characteristics and antimicrobial resistance.

Table 4.11 Descriptive statistics for the risk assessment of urinary tract infection isolates and antibiotic resistance

Attributes		AMK, n=112			CTX, n=112			CIP, n=112		
		R	**S**	***p* value**	**R**	**S**	***p* value**	**R**	**S**	***p* value**
Access to water soap in toilet	No	0	4	0.524	2	2	0.855	2	2	0.942
	Yes	10	98		49	59		52	56	
Antibiotics Use within 3 months	No	9	83	0.497	39	53	0.152	39	53	0.008
	Yes	1	19		12	8		15	5	
Drinking water	Tap water	6	49	0.470	23	32	0.438	24	31	0.341
	Tube well water	4	53		28	29		30	27	
Raising domestic animals	No	9	58	0.041	31	36	0.849	33	34	0.788
	Yes	1	44		20	25		21	24	
Gender	Female	8	92	0.320	42	58	0.030	46	54	0.176
	Male	2	10		9	3		8	4	
Age	18-30	2	51	0.070	20	33	0.116	20	33	0.035
	31-40	2	21	0.965	7	16	0.103	14	9	0.173
	41-50	1	20	0.458	14	7	0.031	9	12	0.586
	51-60	4	9	0.003	8	5	0.218	9	4	0.107
	>60	1	1	0.040	2	0	0.119	2	0	0.139
Education	Primary	5	29	0.157	13	21	0.306	18	16	0.509
	Secondary	2	46	0.126	23	25	0.661	21	27	0.413
	Tertiary	2	20	0.976	10	12	0.993	10	12	0.773
Toilet facilities	Simple pit latrines with slabs	2	34	0.389	19	17	0.290	17	19	0.885
	Toilets connected to septic systems	8	68		32	44		37	39	

n, number of *E. coli* isolates from urinary tract infection; AMK, amikacin; CTX, cefotaxime; CIP, ciprofloxacin. Highlighted cells indicate the significant association ($p < 0.05$).

No significant association was found between the use of antibiotics and the development of resistance in *E. coli* specific to antibiotics in patients with UTI. Previous antibiotic usage (within the previous three months from the time of urine sample collection) by the patients with UTI is demonstrated in Table 4.12.

Table 4.12 List of antibiotics taken by patients with UTI in the three months prior to sampling

Antibiotics	**Number of patients (n=20)**
Penicillins	1 (5)
Quinolones	8 (40)
Macrolides	2 (10)
Metronidazole	0 (0)
1st and 2nd generation cephalosporin	6 (30)
3rd generation cephalosporin	1 (5)
Nitrofurantoin	1 (5)
Aminoglycosides	0 (0)
Unknown	1 (5)

Values in parentheses indicate the column percentage. n, represents the number of patients with *E. coli* infection

4.2.9 Risk assessment associated with baseline epidemiological variables in healthy human volunteers providing rectal swabs

Epidemiological data, including age, sex, education level, sanitation status, socio-economic status, history of rearing domestic animals, and previous antibiotic use, were collected from the healthy human volunteers who provided rectal swabs**.** Among 660 healthy human volunteers, 55.8% (368/660) were female, and 44.2% (292/660) were male. The prevalence of *E. coli* in HRS from male participants was 83.9% (245/292), and the prevalence of *E. coli* in HRS from female participants was 78.5% (289/368).

Distribution of age ranges and sex among healthy human volunteers is shown in Figure 4.7.

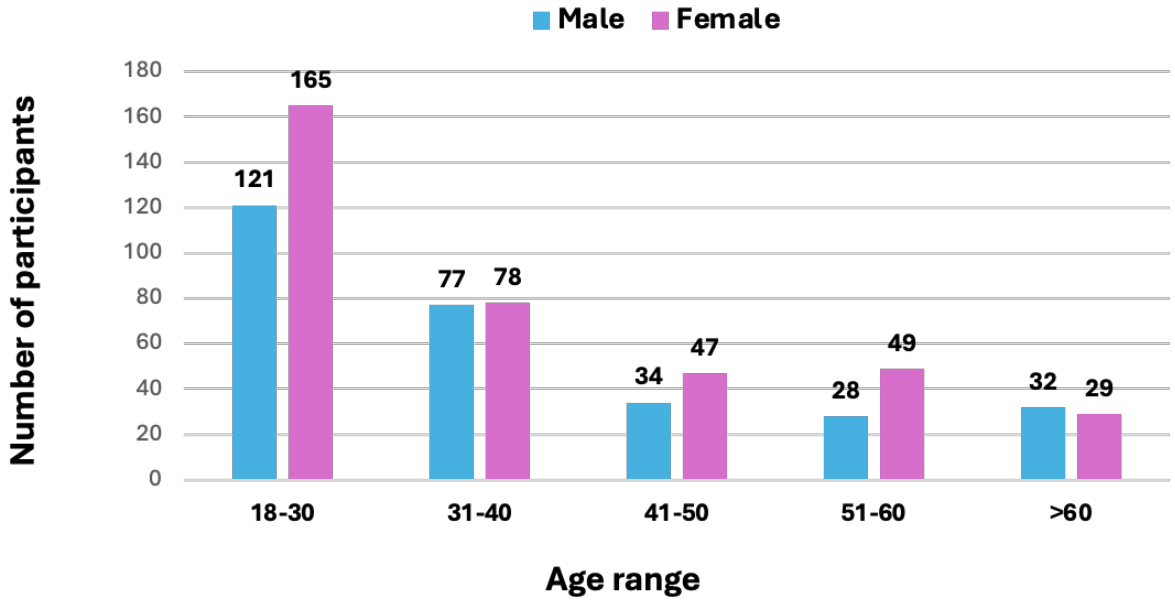


Figure 4.7 Distribution of age and sex among the healthy human volunteers.

For HRS isolates, univariable associations between epidemiological variables and resistance to individual antibiotics were assessed using chi-square tests. Exploratory association analysis was performed for all 17 antibiotics using baseline epidemiological variables collected from healthy human volunteers, with only antibiotics showing statistically significant associations presented in Tables 4.13a and 4.13b. Resistance to AMK and CEF was more frequently observed among *E. coli* isolates obtained from individuals aged 41–50 years compared with other age groups ($p < 0.05$). In addition, resistance to several antibiotics, including AMK, CIP, TZP, IPM, and MER, was significantly more frequent among isolates from individuals reporting antibiotic use within the preceding three months ($p < 0.05$) (Table 4.13a, Table 4.13b). These findings represent statistical associations identified through univariable analysis and do not imply direct causal relationships between epidemiological characteristics and antimicrobial resistance.

Table 4.13a Descriptive statistics for the risk assessment of human rectal swab isolates and antibiotic resistance

Attributes		AMK, n=534			CEF, n=534			CIP, n=534		
		R	**S**	***p* value**	**R**	**S**	***p* value**	**R**	**S**	***p* value**
Access to water soap in toilet	No	1	211	0.821	12	200	0.587	46	166	0.792
	Yes	2	320		22	300		73	249	
Antibiotics Use within 3 months	No	1	488	<0.001	29	460	0.173	103	386	0.025
	Yes	2	43		5	40		16	29	
Drinking water	Tap water	1	76	0.350	3	74	0.337	14	63	0.350
	Tube well water	2	455		31	426		105	352	
Raising domestic animals	No	0	82	0.459	3	79	0.275	16	66	0.512
	Yes	3	449		31	421		103	349	
Gender	Female	3	286	0.110	24	265	0.046	63	226	0.770
	Male	0	245		10	235		56	189	
Age	18-30	1	234	0.709	14	221	0.731	52	183	0.938
	31-40	0	128	0.329	7	121	0.633	31	97	0.546
	41-50	2	67	0.005	9	60	0.015	19	50	0.261
	51-60	0	58	0.544	4	54	0.861	11	47	0.520
	>60	0	44	0.603	0	44	0.071	6	38	0.150
Education	Primary	0	192	0.193	11	181	0.651	48	144	0.259
	Secondary	2	159	0.167	12	149	0.499	33	128	0.514
	Tertiary	0	48	0.585	4	44	0.559	13	35	0.402
Toilet facilities	Simple pit latrines with slabs	1	317	0.353	17	301	0.241	62	256	0.060
	Toilets connected to septic systems	2	214		17	199		57	159	

n, number of *E. coli* isolates from urinary tract infection; AMK, amikacin; CEF, cefepime; CIP, ciprofloxacin. Highlighted cells indicate the significant association ($p < 0.05$).

Table 4.13b Descriptive statistics for the risk assessment of human rectal swab isolates and antibiotic resistance

Attributes		IPM, n=534			MER, n=534			TZP, n=534		
		R	**S**	***p* value**	**R**	**S**	***p* value**	**R**	**S**	***p* value**
Access to water soap in toilet	No	1	211	0.217	1	211	0.217	1	211	0.366
	Yes	0	322		0	322		4	318	
Antibiotics Use within 3 months	No	0	489	0.001	0	489	0.001	3	486	0.011
	Yes	1	44		1	44		2	43	
Drinking water	Tap water	0	77	0.681	0	77	0.681	2	75	0.102
	Tube well water	1	456		1	456		3	454	
Raising domestic animals	No	0	82	0.670	0	82	0.670	0	82	0.339
	Yes	1	451		1	451		5	447	
Gender	Female	1	288	0.357	1	288	0.357	5	284	0.039
	Male	0	245		0	245		0	245	
Age	18-30	0	235	0.375	0	235	0.375	1	234	0.277
	31-40	0	128	0.574	0	128	0.574	1	127	0.835
	41-50	1	68	0.009	1	68	0.009	2	67	0.070
	51-60	0	58	0.727	0	58	0.727	1	57	0.509
	>60	0	44	0.764	0	44	0.764	0	44	0.501
Education	Primary	0	192	0.453	0	192	0.453	1	191	0.455
	Secondary	0	161	0.511	0	161	0.511	2	159	0.630
	Tertiary	0	48	0.753	0	48	0.753	0	48	0.480
Toilet facilities	Simple pit latrines with slabs	0	318	0.225	0	318	0.225	1	317	0.070
	Toilets connected to septic systems	1	215		1	215		4	212	

n, number of *E. coli* isolates from urinary tract infection; IPM, imipenem; MER, meropenem; TZP, piperacillin-tazobactam. Highlighted cells indicate the significant association ($p < 0.05$).

These findings highlight that the prior antibiotic exposure may be a driver of resistance to some antibiotics. Table 4.14 denotes the antibiotics taken by the human participants who were positive for *E. coli*. Human participants predominantly used the fluoroquinolone group of antibiotics.

However, no association was found between antibiotic resistance and demographic/domestic variables such as drinking water sources, raising domestic animals, education level, or home toilet facilities.

Table 4.14 List of antibiotics taken by patients with UTI in the three months prior to sampling

Antibiotics	**Number of human volunteers (n= 45)**
Penicillins	3 (6.7)
Quinolones	18 (40)
Macrolides	6 (13.3
Metronidazole	1 (2.2)
1st and 2nd generation cephalosporin	2 (4.4)
3rd generation cephalosporin	3 (6.6)
Nitrofurantoin	0 (0)
Aminoglycosides	0 (0)
Unknown	12 (26.7)

Values in parentheses indicate the column percentage. n, represents the number of participants with *E. coli* fecal carriage.

4.3 Discussion

The Second UN High-Level Meeting on AMR in September 2024 set ambitious targets to reduce AMR-related deaths by 2030, while also highlighting persistent challenges such as sector-specific gaps, weak implementation, and limited resources in low- and LMICs. The meeting emphasized the need for stronger surveillance systems, innovative and sustainable financing, and a coordinated One Health approach (WHO, 2024). Building on this, discussions at a 2025 G7-aligned High-Level Meetings on AMR further underscored that inadequate surveillance, unequal access to effective antibiotics, and a weak antibiotic development pipeline continue to drive global AMR risk. Together, these global discussions reinforce the importance of strengthening surveillance, improving equitable access, and integrating stewardship, innovation, and One Health principles into AMR risk assessment and control strategies (Canadian Antimicrobial Innovation Coalition, 2025).

Although various studies have reported the prevalence of MDR *E. coli* across different sectors, there is a lack of systematic and aligned data on prevalence, resistance patterns, and associated risk factors from a single unified One Health perspective (Ahmed *et al*., 2022). Present study aimed to fill this gap by deploying a systematic sampling strategy across seasons and data collection from healthy human populations, associated farms, and surrounding environments, to investigate the risk factors and interconnections contributing to *E. coli* AMR.

This study shows the widespread distribution of *E. coli* strains across human, animal, and environmental sectors. The overall prevalence of *E. coli* identified in this study was 43.47% (1634/3759). *E. coli* is considered a ubiquitous organism, including being isolated from the gastrointestinal tract of warm-blooded animals, where they exist as a commensal organism. Therefore, the prevalence of *E. coli* is comparatively high, especially in faecal samples. In present study, a notably high prevalence of 97% was observed in chicken cloacal swabs. A

systematic review based on *E. coli* prevalence in poultry from Bangladesh reported an average prevalence of 69.3%, with values ranging from 24% to 100%. Similarly, a study carried out in Mymensingh documented a prevalence rate of 76% in poultry (Islam *et al*., 2023; Mandal *et al*., 2022). The higher prevalence observed in this study may be attributed to differences in sampling location and time, as this study was conducted more recently and in areas characterized by intensive poultry farming, high stocking density, and limited biosecurity practices. In addition, variations in farm management, hygiene standards, antimicrobial use, and environmental contamination may contribute to the observed differences across studies. The prevalence of *E. coli* in chicken meat swabs was 70.5%. Another research group from Bangladesh detected *E. coli* at a prevalence of 63.5% in chicken meat samples (Rahman *et al*., 2020), and a much lower prevalence (38.4%) of *E. coli* in chicken meat samples was reported by a study from Tanzania (Omar *et al*., 2024). These differences may reflect variations in slaughtering practices, meat handling hygiene, regulatory enforcement, and cold-chain management between countries. In many low-resource settings, inadequate sanitation during slaughter and processing, as well as poor personal hygiene of meat handlers, have been shown to increase *E. coli* contamination (Hussain *et al*., 2017). This study found a very high prevalence (95%) of *E. coli* in rectal swabs collected from domestic animals around areas surrounding farms. A similar isolation rate (86.2%) in cattle faeces was found in Ethiopia (Gemeda *et al*., 2023) suggesting that livestock-associated *E. coli* carriage is common in settings with close human–animal–environment interactions. The limited availability of published data from Bangladesh on *E. coli* prevalence in domestic animal faeces highlights an important knowledge gap.

In contrast, the prevalence of *E. coli* in flying birds around farms from other studies varied significantly. A study in Bangladesh found that the prevalence of *E. coli* from pigeons rearing around the farm and human locality was 52.5% (Karim *et al*., 2020). In contrast, the

prevalence of *E. coli* in this study was 73.3% though in that study they collected swab samples from the bird reared by the owner of the birds. Still, in this study, bird faeces samples were collected from freshly voided faeces of the free-flying birds near the farms. These birds were not consistently living in the same place and roam around different places, which might increase the possibility of carrying more organisms. A study that examined faecal samples from wild birds found that 34.6% of the samples harboured extended-spectrum β-lactamase (ESBL)-producing bacteria, with *E. coli* constituting 74.2% of these isolates (Saeed *et al.*, 2023) which is the consistent of the results of this study.

From the environmental samples, flies showed a prevalence of 83.2%, which was significantly higher than the 60% prevalence reported in another Bangladesh study, but focused only on households (Doza *et al*., 2018) while fly samples in this study were collected from various locations, including poultry and animal farms, nearby households, and hospital areas. The broader range of collection sites in present study contributes to the higher levels of *E. coli* detection and isolation rates. This high prevalence of flies indicates environmental contamination and possible reservoirs, which may contribute to their spread and potential transmission to humans and animals. (Cook *et al.*, 2025; Tufa *et al*., 2020).

The prevalence of *E. coli* in water samples in this study was found to be 43.6% which was lower than the prevalence of *E. coli* reported by Asaduzzman for wastewater, pond and river water with levels of 90%, 68%, and 85%, respectively (Asaduzzaman *et al*., 2022). Another study in Bangladesh showed 76% prevalence of *E. coli* from farm wastewater samples (Mandal *et al*., 2022). In this study, the water samples were collected from various sources, including drains, community sewer lines, ponds, and rivers, as well as lakes near farms and the hospital. The higher prevalence of *E. coli* in water samples from Mymensingh Sadar (61.66%) compared with Tarakanda (22.5%) and other subdistricts may be attributed to differences in

surrounding anthropogenic activities. In Mymensingh Sadar, sampling sites were located near a tertiary hospital as well as adjacent farms, increasing the likelihood of contamination from both human wastewater and animal sources. In contrast, water samples from the other subdistricts were collected primarily around farm-associated water bodies, with less influence from healthcare facilities or dense urban activities, which may explain their comparatively lower *E. coli* prevalence. The specific sample sites and the methods used for sampling may have contributed to variations in the *E. coli* isolation rates compared to other studies.

Over 90% of healthy humans carry *E. coli* as part of their normal intestinal flora (Martinson and Walk, 2020). In present study involving 660 human volunteers, 534 (80.9%) tested positive for *E. coli*, which is lower than the expected prevalence of *E. coli* in rectal samples. It is possible that the sampling process of rectal swabs may contribute to some samples being culture-negative. Healthy human volunteers took their own rectal samples, having been instructed earlier on the proper sampling procedure. However, due to a lack of confidence or other factors (such as embarrassment), some individuals may not have collected the samples correctly. As a result, the expected number of culture-positive samples was not achieved, and therefore, caution must be exercised when considering this data.

The prevalence of *E. coli* in surgical site infections was 15.8%, which is slightly higher than the 12.18% isolated from wound swabs in a recent study conducted in Bangladesh at a tertiary care hospital However, a higher prevalence 24.6% (Jahan *et al*., 2024) and 27.1% (Rahman *et al*., 2024) were reported in studies conducted in Bangladesh (Hasan *et al*., 2024a). The exclusion criteria for this study participants may have led to a lower prevalence, as patients with chronic diseases and those who were immunocompromised were not included. It was ensured in this study ensured that samples were collected only from obvious and active surgical site infections and not from the colonisation. Samples from the surgical site were only collected

from the wounds which showed the signs and symptoms of infections, redness, swelling, and pus (Patel, 2007). In this study, male patients exhibited a higher susceptibility to *E. coli* infections. This aligns with findings from several recent studies in Bangladesh. One study reported that 64.7% of patients with surgical site infections were male (Khan *et al*., 2022). Additionally, another study demonstrated that male patients were more likely to experience post-surgical infections, with a rate of 68.4% compared to 31.6% for female patients (Rahman *et al*., 2024). Furthermore, Jahan *et al*. (2024) found that 75% of patients suffering from post-surgical infections were male, while 25% were female (Jahan *et al*., 2024). Biological differences in skin between men and women may contribute to the higher rates of surgical site infections observed in men. Several studies suggest that men have greater bacterial colonisation at catheter insertion sites. Additionally, factors such as thicker hair and shaving can disrupt the adhesion of wound dressings, which further increases the risk of infection (Cohen *et al*., 2013; Luft *et al*., 2010). Smoking is recognised as a risk factor for increased SSI infections in males (Islam *et al*., 2023), and in Bangladesh, males smoke at significantly higher rates than females (Rahman *et al*., 2021).

The lowest prevalence of *E. coli* in this study (9.6%) was found in patients with UTIs. Studies on UTIs show a wide range of *E. coli* prevalence across Bangladesh. One study showed a prevalence of 51. 6% (Islam *et al*., 2022) whereas another study showed 11.3% (Nobel *et al*., 2021) and in both studies, females were significantly more susceptible to UTIs caused by *E. coli*. In present study, the urine samples collected from females (87%) were significantly higher than those from males (13%). The growth positive rate of *E. coli* from UTI samples in females, (9.8%) is higher than that of *E. coli* isolates from males (7.8%). This study did not include pregnant women or individuals under 18 years old which may account for sex not being a significant factor in case of rate of *E. coli* isolation, as pregnant women and young girls make up a significant portion of those who are susceptible to urinary tract infections (Lee *et al*., 2020;

Islam *et al*., 2010). Although the first and fourth quarters encompassed similar months, the higher prevalence of *E. coli* observed in the first quarter cannot be attributed to a greater number of clinical samples, as both SSI and UTI sample numbers were higher in the fourth quarter. This suggests that the observed difference more likely reflects inter-annual variation in environmental and epidemiological conditions rather than sampling intensity. Differences in post-monsoon environmental contamination, water quality, and sanitation stress between late 2021 and late 2022 may have contributed to elevated *E. coli* persistence during the first sampling year (Levy *et al*., 2018). Such inter-annual variability has been documented in environmental surveillance studies in Bangladesh and similar settings, highlighting the dynamic nature of bacterial prevalence across time (Islam *et al*., 2007).

In this study, the phenotypic resistance patterns of *E. coli* isolates obtained from surgical site infections indicate significantly higher resistance compared to other samples. This resistance is notable for the penicillin group (including amoxicillin-clavulanic acid and ampicillin), as well as cefotaxime, ceftazidime, cefepime, ciprofloxacin, and levofloxacin (Table 4.3). The history of antibiotic use indicates that the highest percentages of patients were treated with third-generation cephalosporins, penicillin, and quinolones (Table 4.10). A recent study conducted in Bangladesh reported high resistance rates to ceftazidime (92.4%) and amoxicillin–clavulanic acid (73.5%) among post-SSI patients, while amikacin and gentamicin remained relatively more effective (Rahman *et al*., 2024), which is consistent with the resistance pattern observed in the study. Similarly, Nobel *et al*. (2022) documented 100% resistance to penicillin and third-generation cephalosporins, findings that are also consistent with this results (Nobel *et al*., 2022). The elevated prevalence of resistance in these studies may be attributed to the extensive and often empirical use of broad-spectrum antibiotics in surgical patients prior to hospital admission, coupled with limited stewardship practices and inadequate infection control measures in many healthcare settings.

A study by Das *et al.* (2023) reported that resistance to ciprofloxacin in chicken cloacal swabs was 77.6%, while in hospitalised patients, was 89%, which closely aligns with findings from this study (Table 4.3) (Das *et al*., 2023). In Bangladeshi poultry sectors, doxycycline, ciprofloxacin, and amoxicillin are the most commonly used antibiotics (Chowdhury *et al*., 2022) which confirms the documented evidence collected from local farmers in this study (Table 4.7). Higher fluoroquinolone resistance in flies, water, CCS, and CMS likely reflects environmental contamination from extensive ciprofloxacin use in farm settings, rather than direct antibiotic exposure of the sampled hosts. Antibiotic residues and resistant bacteria in manure, wastewater, and runoff create reservoirs that facilitate resistance persistence and dissemination through vectors such as flies and water.

MDR *E. coli* was most prevalent in surgical site infections with a 97.6% prevalence. In addition to clinical and poultry isolates, environmental samples such as water and flies were also found to be MDR at a high rate, possessing a risk of transmitting MDR *E. coli* to humans and animals (Caderhoussin *et al*., 2024). In Bangladesh, antibiotic usage patterns differ significantly between poultry and large domestic animals such as cattle and goats. In poultry farming, antibiotics are extensively employed for therapeutic purposes, disease prevention, and growth promotion (Imam *et al*., 2020). One study reported that approximately 94.2% of poultry farmers use antibiotics in their farms to control disease and enhance egg production (Hosain *et al*., 2021). In this study, the use of antibiotics in the farms was observed and reported (Table 4.7).

The epidemiological risk assessment analyses presented in this chapter provide additional context for understanding the observed patterns of antimicrobial resistance. Several baseline variables, including recent antibiotic use, age, gender, and sanitation-related factors, were statistically associated with resistance to specific antibiotics across surgical site infection, urinary tract infection, and healthy human rectal swab isolates. These associations should be

interpreted cautiously, as the analyses were exploratory and univariable in nature and do not imply direct causal relationships. Variables such as toilet facilities and access to sanitation are unlikely to directly influence resistance development; rather, they may act as indicators for broader socio-environmental conditions, including hygiene practices, environmental contamination, population density, and healthcare access. Similarly, age- and gender-related associations may reflect differences in healthcare utilisation or antimicrobial exposure rather than intrinsic susceptibility. The consistent association between recent antibiotic use and resistance to multiple antibiotic classes reinforces the role of antimicrobial exposure as a key driver of resistance selection which supports the statement made by Tacconelli and Pezzani in 2019. While these findings are hypothesis-generating and subject to confounding and sampling limitations, they highlight the importance of integrating epidemiological data with microbiological and genomic analyses to better understand the complex drivers of AMR.

Chapter 5

Clonal diversity, antimicrobial resistance and virulence genotypes of *Escherichia coli* at the human-animal-environmental interface

5.1 Introduction

E. coli is a genetically highly adaptable and geographically widespread organism (Lagerstrom and Hadly, 2023). To better understand its genetic diversity, *E. coli* strains have been classified into phylogenetic groups and further characterised using molecular typing methods such as multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), and WGS, which enable the characterisation of *E. coli* strain relatedness, resistance profiles, and evolutionary dynamics (Didelot *et al*., 2012; Ribot *et al*., 2006; Maiden *et al*., 1998). Recent developments, such as allele-based cg/wgMLST and automated WGS workflows (e.g., RapidONT), have enhanced typing precision, speed, and accessibility, making these techniques more scalable for real-time surveillance in clinical and environmental settings (Shropshire *et al*., 2025; Wu *et al*., 2025).

The phylogeny of *E. coli* consists of eight major phylogroups: A, B1, B2, C, D, E, F, G and cryptic Escherichia clades I to V (Clermont *et al*., 2019; Clermont *et al*., 2013; Clermont *et al*., 2011). These phylogroups vary in their phenotypic and genotypic characteristics, disease-causing abilities, and exhibit diversity, with specific phylogroups showing distinct host and ecological associations. Phylogroups A, B2, and D are predominantly found in human populations. In contrast, phylogroup B1 is commonly isolated from animal and environmental sources, possessing genomic features that enhance survival in soil, water, and plant-associated habitats. While B2, D, and F encompass the majority of ExPEC, phylogroups A, B1, and C primarily include intestinal pathogenic strains (Lagerstrom and Hadly, 2023). There is a strong link between the virulence and phylogeny in *E. coli* infections. Phylogroups B2 and D are associated with virulent ExPEC, whereas *E. coli* strains under phylogroups A and B1 are mainly commensal and less virulent (Lemlem *et al*., 2023; Pakbin *et al*., 2021a). Phylogroups F and G are often found to harbour virulence and AMR determinants (Vangchhia *et al*., 2016). Investigating *E. coli* phylogroups is important in understanding its virulence and resistance

factors. Monitoring phylogroups within a One Health context is essential to mitigate the intersectoral spread of MDR *E. coli*, especially in regions where antibiotics are widely used in both humans and animals.

Characterisation of *E. coli* STs plays a vital role in understanding population structure, transmission dynamics, and AMR trends. Certain STs are associated with specific resistance profiles and virulence factors; for example, ST69 and ST95 are widely distributed and associated with MDR phenotypes, making them critical for public health monitoring. The pandemic clone ST131, is strongly linked with ESBL genes such as $bla_{\text{CTX-M-15}}$, making it one of the most clinically important MDR lineages worldwide (Jauneikaite *et al*., 2022; Nicolas-Chanoine *et al*., 2014), contributing significantly to the global burden of AMR (Soncini *et al*., 2022). ST410 has emerged as high-risk clones associated with carbapenemase genes, including $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$, which contribute to global carbapenem resistance (Ludden *et al*., 2019). Additionally, ST10 and ST69 possess multiple resistance genes against β-lactams, tetracyclines, and aminoglycosides, especially in livestock, where the use of antibiotics creates selective pressure that promotes MDR (Zhou *et al*., 2022a).

There are some clones of *E. coli* that are regarded as global MDR high-risk clones. They are globally distributed, possess diverse AMR genes, persist in hosts long-term, transition effectively among different hosts, and exhibit increased virulence (Pitout *et al*., 2022). A study in Bangladesh reported the presence of high-risk clones, such as ST131, ST405, ST1193, ST410, and ST38, which harbour various AMR genes and are often located on highly mobile plasmids (Mazumder *et al*., 2021). The widespread use of antibiotics in both humans and animals, exacerbated by limited regulatory oversight, together with challenges in sanitation infrastructure, creates an ideal environment for the emergence, persistence and dissemination of these high-risk clones. Therefore, understanding the genomic structure and clonal groups of

E. coli will help guide infection control in both healthcare settings and the community, thereby mitigating the spread of new clonal STs, preventing environmental transmission, and contributing overall to AMR surveillance (Walas *et al*., 2023).

In this chapter, I have explored-

1. The clonal distribution and dissemination of *E. coli* across various sources and varied sampling locations in Bangladesh
2. The identification of high-risk clones focusing on their associations with AMR and virulence

5.2 Results

5.2.1 Distribution of *E. coli* phylogroups among different sampling sources

Clermont typing was used to identify the *E. coli* phylogroups from different sources in this study. Nine phylogroups: phylogroup A, phylogroup B1, phylogroup B2, phylogroup C, phylogroup clade I, phylogroup D, phylogroup E, phylogroup F, phylogroup G were identified. The most frequent phylogroup found was phylogroup B1 (39.22%, 641/1634), followed by phylogroup A (37.02%, 605/1634) (Figure 5.1a). Figure 5.1b shows the frequency of isolates from different sources assigned to phylogroups.

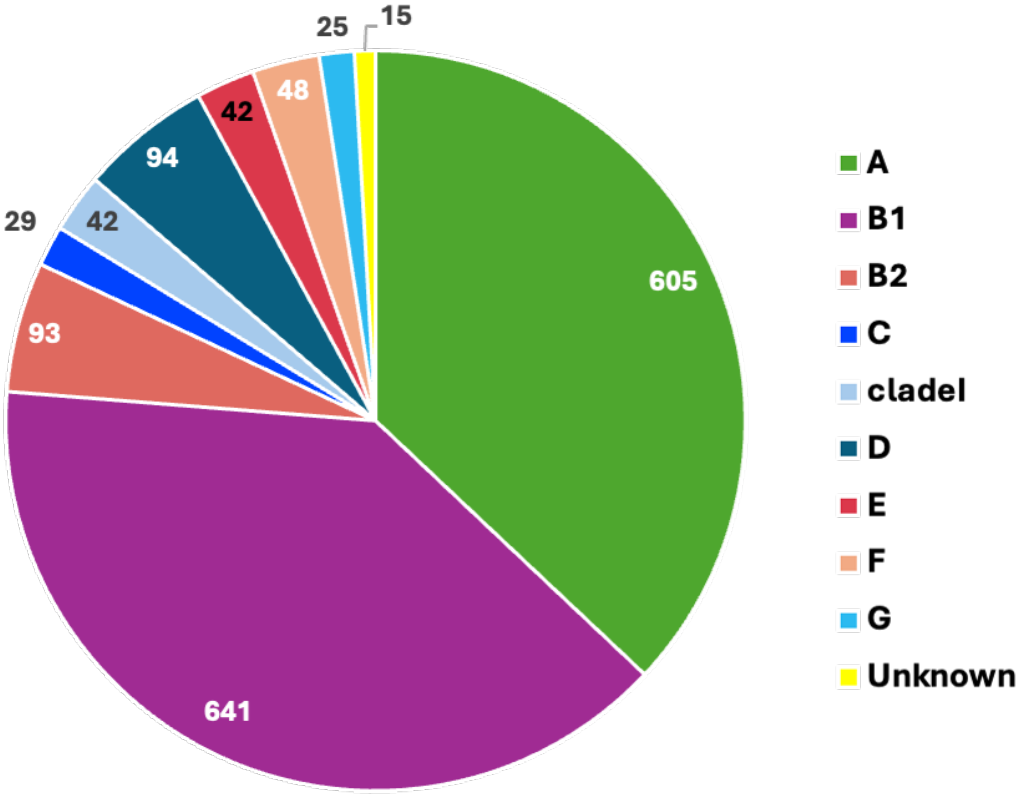


Figure 5.1a Distribution of *E. coli* phylogroups among isolates. The pie chart shows the frequency of isolates assigned to phylogroups. Numbers within each slice indicate the count of isolates

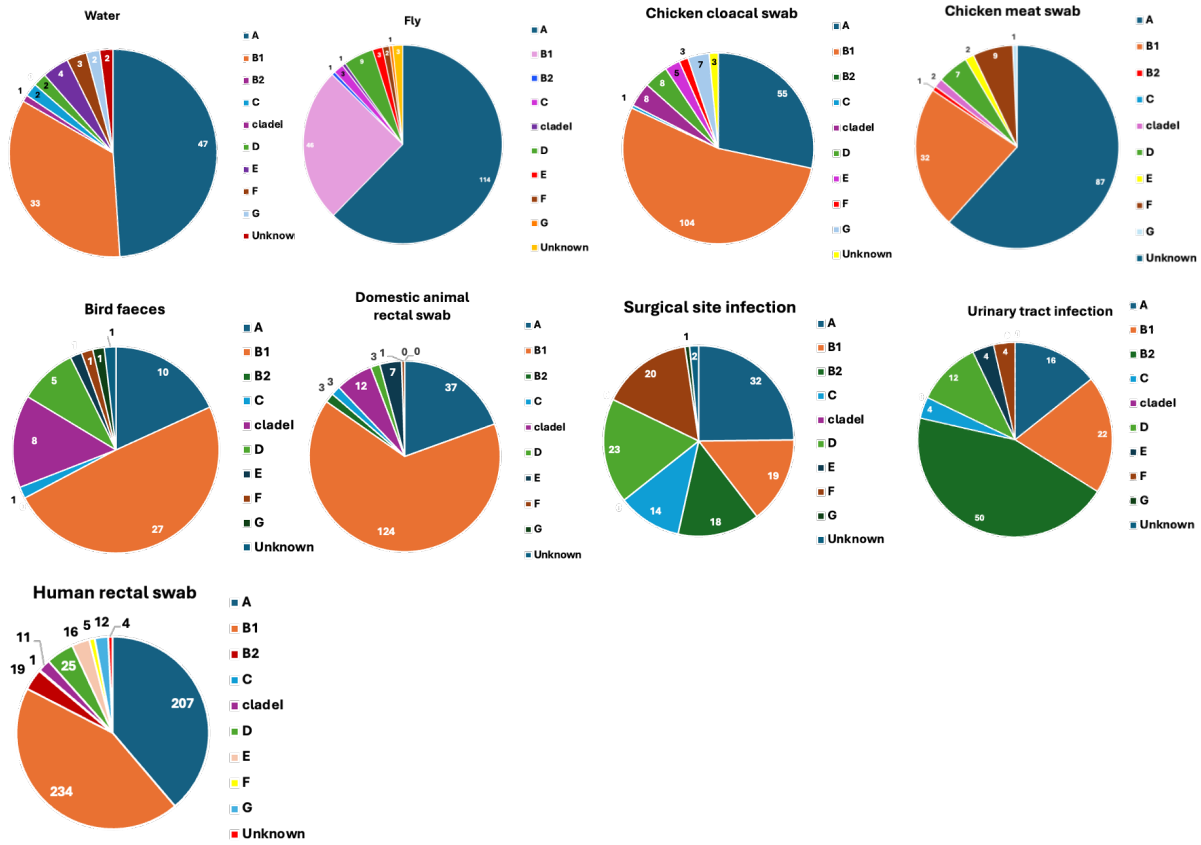


Figure 5.1b Distribution of *E. coli* phylogroups among isolates from different sources. The pie chart shows the frequency of isolates assigned to phylogroups. Numbers within each slice indicate the count of isolates.

E. coli isolates from flies were dominated by phylogroup A (62.3%), followed by phylogroup B1 (25.1%), while other groups were less frequent. Water isolates also showed predominance of phylogroup A (48.9%) and phylogroup B1 (34.4%), with minor representation of phylogroups E, F, and G (2–4%)**.** Similarly, isolates from CCS and CMS were dominated by phylogroup A (57.3% and 61.7%, respectively) with phylogroup B1 (53.6% and 22.7%, respectively), and minor representation for phylogroups D, E, and F. In contrast, DARS and BF showed higher representation of B1 (65.3% and 49.1%, respectively), with lower numbers for phylogroup A (19.5% and 18.2%, respectively) and phylogroup D (14.5% in BF). HRS isolates mainly belonged to phylogroup B1 (43.8%) and phylogroup A (38.8%), with smaller

numbers for phylogroups B2, D, E, and F. SSI isolates showed a higher prevalence of phylogroups D (17.8%), F (15.5%), and B2 (13.9%), whereas UTI isolates predominantly belonged to phylogroup B2 (44.6%), followed by phylogroups B1 (19.6%), A (14.3%), and D (10.7%). The distribution of phylogroups among the sources is listed in Table 5.1.

Table 5.1 Distribution of *E. coli* phylogroups among different sources

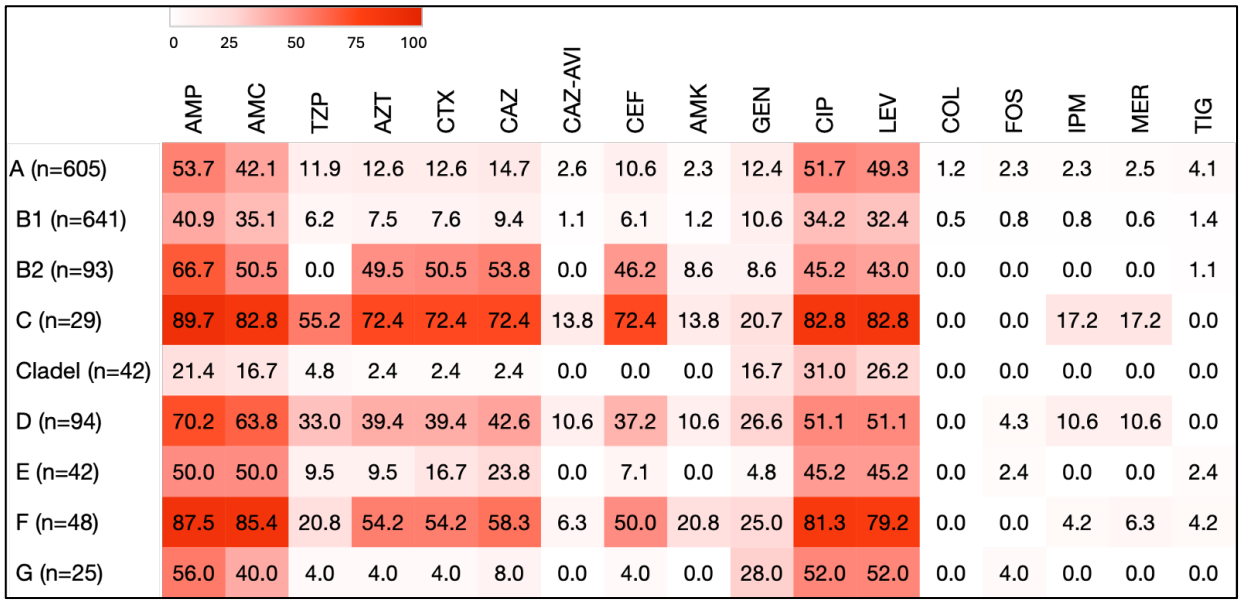
Sources	**Phylogroups**									
	A (n=605)	B1 (n=641)	B2 (n=93)	C (n=29)	Clade I (n=42)	D (n=94)	E (n=42)	F (n=48)	G (n=25)	Unknown (n=15)
Fly (n=183)	114 (62.3%)	46 (25.1%)	1 (0.5%)	3 (1.6%)	1 (0.5%)	9 (4.9%)	3 (1.6%)	2 (1.1%)	1 (0.5%)	3 (1.6%)
Water (n=96)	47 (48.9%)	33 (34.4%)	1 (1.1%)	2 (2.1%)	0 (0.0%)	2 (2.1%)	4 (4.1%)	3 (3.1%)	2 (2.1%)	2 (2.1%)
CCS (n=194)	55 (57.3%)	104 (53.6%)	0 (0.0%)	1 (0.5%)	8 (4.1%)	8 (4.1%)	5 (2.6%)	3 (1.5%)	7 (3.6%)	3 (1.5%)
CMS (n=141)	87 (61.7%)	32 (22.7%)	1 (0.7%)	0 (0.0%)	2 (1.4%)	7 (4.9%)	2 (1.4%)	9 (6.4%)	1 (0.7%)	0 (0.0%)
DARS (n=190)	37 (19.5%)	124 (65.3%)	3 (1.6)	3 (1.6%)	12 (6.3%)	3 (1.6%)	7 (3.7%)	1 (0.5%)	0 (0.0%)	0 (0.0%)
BF (n=55)	10 (18.2%)	27 (49.1%)	0 (0.0%)	1 (1.8%)	8 (14.5%)	5 (9.1%)	1 (1.8%)	1 (1.8%)	1 (1.8%)	1 (1.8%)

Sources	Phylogroups									
	A (n=605)	B1 (n=641)	B2 (n=93)	C (n=29)	cladeI (n=42)	D (n=94)	E (n=42)	F (n=48)	G (n=25)	Unknown (n=15)
HRS (n=534)	207 (38.8%)	234 (43.8%)	19 (3.6%)	1 (0.2%)	11 (2.1%)	25 (4.7%)	16 (2.9%)	5 (0.9%)	12 (2.2%)	4 (0.7%)
SSI (n=129)	32 (24.8%)	19 (14.7%)	18 (13.9%)	14 (10.9%)	0 (0.0%)	23 (17.8%)	0 (0.0%)	20 (15.5%)	1 (0.8%)	2 (1.6%)
UTI (n=112)	16 (14.3%)	22 (19.6%)	50 (44.6%)	4 (3.6%)	0 (0.0%)	12 (10.7%)	4 (3.6%)	4 (3.6%)	0 (0.0%)	0 (0.0%)

Values in the parentheses indicate the row percentages. BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection

5.2.2 Association of phylogroups with antibiotic resistance

A diversified resistance pattern was observed among *E. coli* isolates from various phylogenetic groups (Figure 5.2).



	AMP	AMC	TZP	AZT	CTX	CAZ	CAZ-AVI	CEF	AMK	GEN	CIP	LEV	COL	FOS	IPM	MER	TIG
A (n=605)	53.7	42.1	11.9	12.6	12.6	14.7	2.6	10.6	2.3	12.4	51.7	49.3	1.2	2.3	2.3	2.5	4.1
B1 (n=641)	40.9	35.1	6.2	7.5	7.6	9.4	1.1	6.1	1.2	10.6	34.2	32.4	0.5	0.8	0.8	0.6	1.4
B2 (n=93)	66.7	50.5	0.0	49.5	50.5	53.8	0.0	46.2	8.6	8.6	45.2	43.0	0.0	0.0	0.0	0.0	1.1
C (n=29)	89.7	82.8	55.2	72.4	72.4	72.4	13.8	72.4	13.8	20.7	82.8	82.8	0.0	0.0	17.2	17.2	0.0
Cladel (n=42)	21.4	16.7	4.8	2.4	2.4	2.4	0.0	0.0	0.0	16.7	31.0	26.2	0.0	0.0	0.0	0.0	0.0
D (n=94)	70.2	63.8	33.0	39.4	39.4	42.6	10.6	37.2	10.6	26.6	51.1	51.1	0.0	4.3	10.6	10.6	0.0
E (n=42)	50.0	50.0	9.5	9.5	16.7	23.8	0.0	7.1	0.0	4.8	45.2	45.2	0.0	2.4	0.0	0.0	2.4
F (n=48)	87.5	85.4	20.8	54.2	54.2	58.3	6.3	50.0	20.8	25.0	81.3	79.2	0.0	0.0	4.2	6.3	4.2
G (n=25)	56.0	40.0	4.0	4.0	4.0	8.0	0.0	4.0	0.0	28.0	52.0	52.0	0.0	4.0	0.0	0.0	0.0

Figure 5.2 Heatmap showing antibiotic resistance levels in *E. coli* from different phylogenetic groups.

Figure 5.2 shows the percentage of resistance to antibiotics among *E. coli* phylogroups. Each cell represents the percentage (%) of isolates within a phylogroup that exhibited resistance to a specific antibiotic. Darker shades of red indicate higher resistance rates, while lighter shades or white indicate lower or no resistance. The distribution of antibiotic resistance across different phylogenetic groups reveals potential associations between phylogeny and antimicrobial susceptibility.

A chi-square test was performed to assess associations between *E. coli* phylogroups and resistance to individual antibiotics, with statistical significance determined at $p < 0.05$. Table 5.2 summarises antibiotics for which resistance was significantly enriched within specific phylogroups. For each antibiotic, the proportion of resistant isolates within a given phylogroup was compared with that in all other phylogroups combined, and only statistically significant differences are reported. The analysis demonstrated that resistance to several antibiotics was significantly enriched across more than one phylogroup. Resistance to ampicillin was significantly enriched in phylogroups B2, C, D, and F, indicating that resistance determinants are widely distributed across diverse *E. coli* lineages rather than confined to a single phylogenetic group. No statistically significant association was observed between antibiotic resistance and phylogroups B1, E, or clade I. Resistance to tigecycline was significantly higher in phylogroup A compared with other phylogroups ($p < 0.001$), while resistance to imipenem and meropenem was significantly higher among isolates belonging to phylogroups C and D (Table 5.2).

Table 5.2 Significant association of antibiotic resistance in *E. coli* with various phylogroups

Antibiotics	**Phylogroups**		
	Phylogroup A (n= 605)	**Others* (n= 1029)**	***p* value**
CIP (n= 737)	313 (51.7%)	424 (41.2%)	<0.001
LEV (n= 706)	298 (49.3%)	408 (39.7%)	<0.001
COL (n=10)	7 (1.2%)	3 (0.3%)	0.03
FOS (n= 25)	14 (2.3%)	11 (1.1%)	0.048
TIG (n= 38)	25 (4.1%)	13 (1.3%)	<0.001
Antibiotics	**Phylogroup B2 (n= 93)**	**Others* (n= 1541)**	***p* value**
AMP (n= 834)	62 (66.7%)	772 (50.1%)	0.002
AZT (n= 262)	46 (49.5%)	216 (14%)	<0.001
CTX (n= 267)	47 (50.5%)	220 (14.3%)	<0.001
CAZ (n= 303)	50 (53.8%)	253 (16.4%)	<0.001
CEF (n= 232)	43 (46.2%)	189 (12.3%)	<0.001
AMK (n= 54)	8 (8.6%)	46 (3%)	0.003
Antibiotics	**Phylogroup C (n= 29)**	**Others* (n= 1605)**	***p* value**
AMP (n= 834)	26 (89.7%)	808 (50.3%)	<0.001
AMC (n= 695)	24 (82.8%)	671 (41.8%)	<0.001
TZP (n= 178)	16 (55.2%)	162 (10.1%)	<0.001
AZT (n= 262)	21 (72.4%)	241 (15%)	<0.001
CTX (n= 267)	21 (72.4%)	246 (15.3%)	<0.001
CAZ (n= 303)	21 (72.4%)	282 (17.6%)	<0.001
CAZ-AVI (n=40)	4 (13.8%)	36 (2.2%)	<0.001
CEF (n= 232)	21 (72.4%)	211 (13.1%)	<0.001

AMK (n= 54)	4 (13.8%)	50 (3.1%)	0.001
CIP (n= 737)	24 (82.8%)	713 (44.4%)	<0.001
LEV (n= 706)	24 (82.8%)	706 (43.2%)	<0.001
IPM (n= 36)	5 (17.2%)	31 (1.9%)	<0.001
MER (n= 37)	5 (17.2%)	32 (2.0%)	<0.001
Antibiotics	**Phylogroup D (n= 94)**	**Others* (n= 1540)**	***p* value**
AMP (n= 834)	66 (70.2%)	768 (49.9%)	<0.001
AMC (n= 695)	60 (63.8%)	435 (41.2%)	<0.001
TZP (n= 178)	31 (33%)	147 (9.5%)	<0.001
AZT (n= 262)	37 (39.4%)	225 (14.6%)	<0.001
CTX (n= 267)	37 (39.4%)	230 (14.9%)	<0.001
CAZ (n= 303)	40 (42.6%)	263 (17.1%)	<0.001
CAZ-AVI (n=40)	10 (10.6%)	30 (1.9%)	<0.001
CEF (n= 232)	35 (37.2%)	197 (12.8%)	<0.001
AMK (n= 54)	10 (10.6%)	44 (2.9%)	<0.001
FOS (n= 25)	4 (4.3%)	21 (1.4%)	0.027
IPM (n= 36)	10 (10.6%)	26 (1.7%)	<0.001
MER (n= 37)	10 (10.6%)	27 (1.8%)	<0.001
Antibiotics	**Phylogroup F (n= 48)**	**Others* (n= 1586)**	***p* value**
AMP (n= 834)	42 (87.5%)	792 (49.9%)	<0.001
AMC (n= 695)	41 (85.4%)	654 (41.2%)	<0.001
TZP (n= 178)	10 (20.8%)	168 (10.6%)	0.025
AZT (n= 262)	26 (54.2%)	236 (14.9)	<0.001
CTX (n= 267)	26 (54.2%)	22 (45.8%)	<0.001

CAZ (n= 303)	28 (58.3%)	275 (17.3%)	<0.001
CEF (n= 232)	24 (50%)	208 (13.1%)	<0.001
AMK (n= 54)	10 (20.8%)	44 (2.8%)	<0.001
GEN (n= 212)	12 (25%)	200 (12.6%)	0.012
CIP (n= 737)	39 (81.3%)	698 (44%)	<0.001
LEV (n= 706)	38 (79.2%)	668 (42.1%)	<0.001
Antibiotics	**Phylogroup G (n= 25)**	**Others* (n= 1609**	***p* value**
GEN (n= 212)	7 (28%)	205 (12.7%)	0.024

n, number of isolates. Values in parentheses indicate column percentage. Others* indicates the total of the remaining phylogroups. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; AMK, amikacin; AZT, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; CEF, cefepime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MER, meropenem; TZP, piperacillin-tazobactam; TIG, tigecycline.

To investigate the relationship between antibiotic resistance patterns among different phylogroups of *E coli*, a pairwise Pearson correlation matrix was constructed using resistance percentages for 17 antibiotics across nine phylogroups. This matrix facilitates the identification of antibiotics that exhibit similar resistance trends, potentially signifying co-selection or shared resistance mechanisms. Strong positive correlations among antibiotics suggest that resistance to those antibiotics may be acquired or selected together, and interconnected resistance mechanisms may exist. In contrast, strong negative correlations could indicate divergent resistance trends. Figure 5.3 illustrates a strong positive correlation between the resistance patterns of TZP and CAZ-AVI, IPM, and MER. The resistance pattern of AZT exhibits a strong

correlation with CTX, CAZ, and CEF. The resistance patterns of IPM and MER exhibit a strong correlation with those of TZP and CAZ-AVI. In addition to these β-lactam and β-lactam correlations, Figure 5.3 also reveals strong positive correlations between resistance to β-lactams and non-β-lactam antibiotics, including fluoroquinolones (CIP and LEV) and the aminoglycoside AMK.

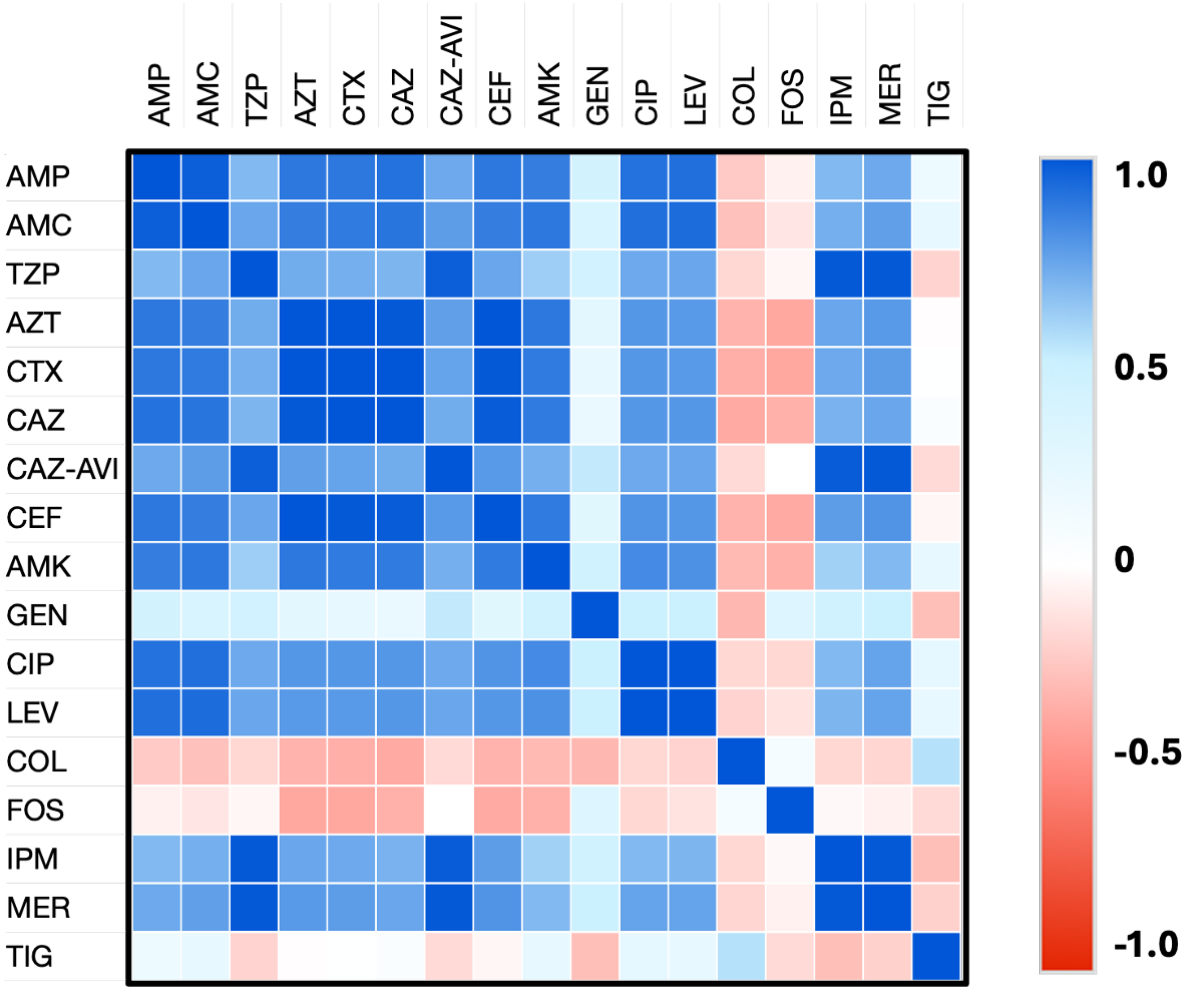


Figure 5.3 Correlation analysis of antibiotic resistance patterns across phylogroups. A 17×17 correlation matrix showing pairwise Pearson correlation coefficients (r) between resistance percentages of 17 antibiotics tested against nine phylogroups. Correlation coefficients were calculated using resistance percentages of each antibiotic across the nine phylogroups (n=9 paired observations). Dark blue cells indicate strong positive correlation; Dark red cells indicate strong negative correlation; white cells indicate no correlation. AMP,

ampicillin; AMC, amoxicillin-clavulanic acid; AMK, amikacin; AZT, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; CEF, cefepime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MER, meropenem; TZP, piperacillin-tazobactam; TIG, tigecycline.

5.2.2.1 Distribution of Antimicrobial Resistance Genes

While the correlation analysis provided insights into phenotypic resistance patterns across phylogroups, further investigation was performed at the genotypic level to identify the underlying ARGs. A comprehensive analysis of resistance genes using ResFinder revealed the presence of diverse resistance determinants across multiple classes of antibiotics. The distribution and frequency of key ARGs, particularly those associated with clinically significant resistance mechanisms, are summarised in Figure 5.4.

For β-lactam antibiotics, *bla*$_{\text{TEM-1B}}$ was the most frequently detected gene, identified in 33.84% (553/1634) of all isolates. Among the ESBLs, *bla*$_{\text{CTX-M-15}}$ was the predominant variant, present in 208 isolates (12.72%). For carbapenem resistance, *bla*$_{\text{NDM-5}}$ was the most common gene, found in 27 isolates (1.65%), while *bla*$_{\text{OXA-181}}$ and *bla*$_{\text{NDM-1}}$ were identified in a smaller number of isolates. *bla*$_{\text{NDM-20}}$ was identified in one isolate which also carried *bla*$_{\text{NDM-5}}$. Resistance to aminoglycosides emerged as the most widespread mechanism of resistance (98.5%,1611/1634). The predominant genes included *aph(6)-Id*, *ant(3'')-Ia*, *aph(3'')-Ib, and aph(3')-Ia.* For quinolone resistance, *qnrS1* was the most frequently detected gene, present in 35.9% (587/1634) of isolates, followed by *qnrB4* (4.3%), 71/1634 and *qnrS4* (1%, 16/1634). Fosfomycin resistance genes were detected at lower frequencies, with *fosA3* and *fosA7* identified in 15 and 9 isolates, respectively. Three isolates carried the colistin resistance gene, *mcr-1.1*. Resistance to trimethoprim was primarily mediated by *dfrA14* (248/1634), *dfrA17* (190/1634), and *dfrA12* (141/1634), while sulfonamide

resistance was associated with the prevalence of *sul2* (353/1634), *sul3* (288/1634) and *sul1* (270/1634). The most frequently detected gene, associated with tetracycline resistance was *tet*(A), found in 46.9% (767/1634) of the isolates. Additionally, tigecycline resistance was observed in eight isolates harbouring the *tet*(X4) gene.

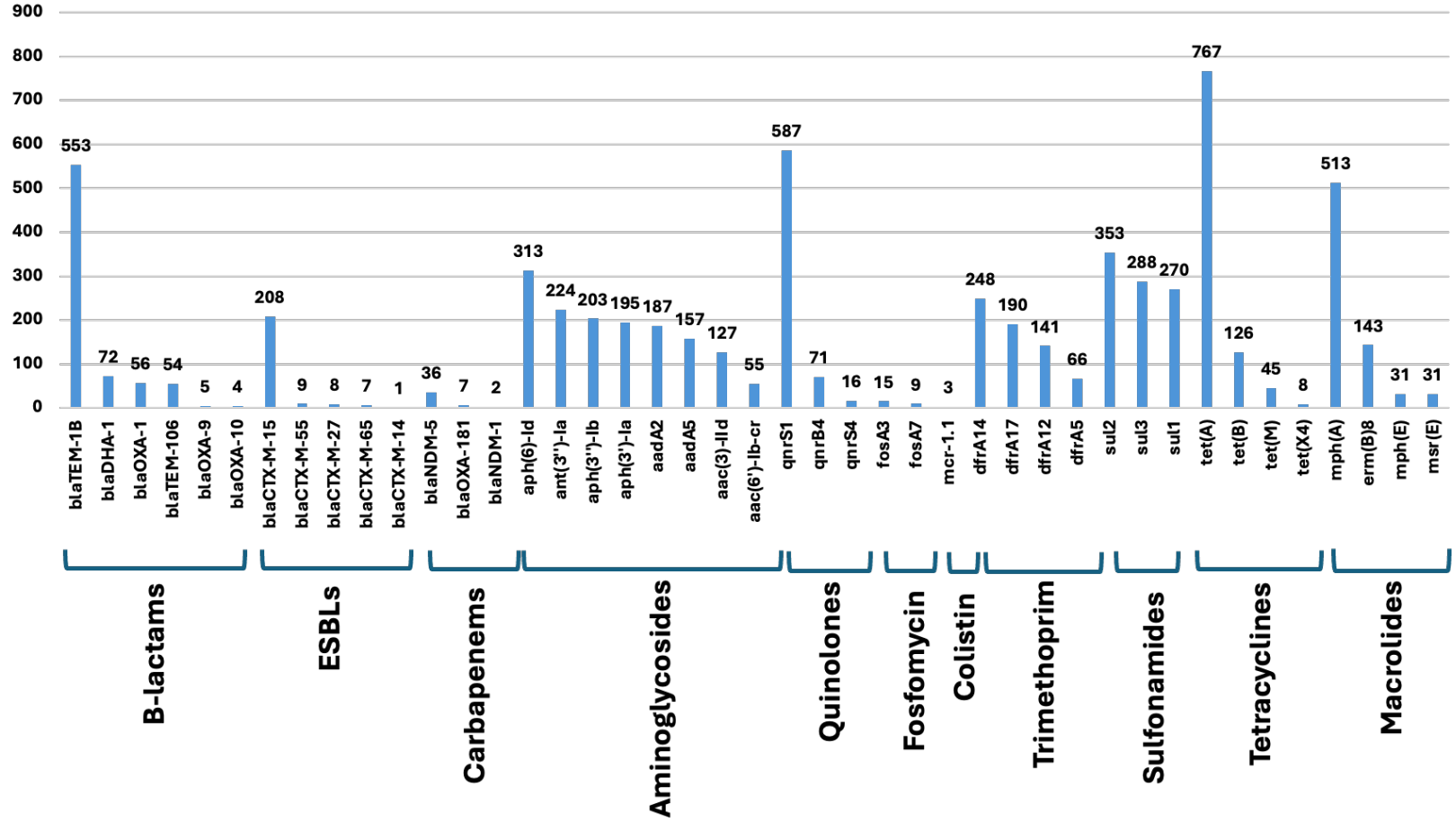


Figure 5.4 Frequency of dominant ARGs found in *E. coli*. The genes have been organised according to their mechanism.

Among the wide range of ARGs, *bla*$_{\text{NDM-5}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA-181}}$, *bla*$_{\text{CTX-M-15}}$, *mcr-1.1*, *tet*(X4), and *aac(6')-Ib-cr* genes were focused due to their clinical importance and distinct resistance mechanisms. These genes were selected to investigate their distribution across various sources and *E. coli* phylogroups, and to assess their contribution to the overall AMR burden. *bla*$_{\text{CTX-M-15}}$, the most globally common extended-spectrum β-lactamase, confers resistance to third-generation cephalosporins and is often found on mobile genetic elements, which supports its rapid dissemination, especially in both hospital-acquired and community-onset infections. The carbapenemase genes *bla*$_{\text{NDM-1}}$, *bla*$_{\text{NDM-5}}$, and *bla*$_{\text{OXA-181}}$ mediate resistance to carbapenems, regarded as last-resort therapeutic agents for treating MDR Gram-negative infections. The *mcr-1.1* genes are responsible for resistance to colistin, another last-resort antibiotic used against carbapenem-resistant infections, and are plasmid-borne, raising significant concerns regarding their potential for horizontal transfer. Furthermore, *tet*(X4) contributes to resistance against tigecycline, an advanced tetracycline derivative used to treat severe MDR infections (Fan *et al*., 2024; Castanheira *et al*., 2021). The *aac(6')-Ib-cr* gene is especially notable for conferring resistance to both aminoglycosides and fluoroquinolones (such as ciprofloxacin), thereby limiting treatment options. The combined presence of these genes indicates a high-risk AMR profile.

Table 5.3 Distribution of important ARGs among various sampling sources

ARGs	Water n= 96	Fly n= 183	CCS n= 194	CMS n= 141	DARS n= 190	BF n= 55	UTI n= 112	SSI n= 129	HRS n= 534
aac(6')-Ib-cr	3 (3.1)	4 (2.2)	0 (0)	1 (0.7)	0 (0)	0 (0)	12 (10.7)	32 (24.8)	3 (0.60
bla$_{\text{CTX-M-15}}$	10 (10.4)	15 (8.2)	0 (0)	0 (0)	4 (2.1)	0 (0)	41 (36.6)	103 (79.8)	35 (6.6)
bla$_{\text{NDM-1}}$	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.8)	0 (0)
bla$_{\text{NDM-5}}$	4 (4.2)	2 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	4 (3.6)	16 (12.4)	1 (0.2)
bla$_{\text{NDM-20}}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.8)	
bla$_{\text{OXA-181}}$	2 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (3.1)	1 (0.2)
mcr-1.1	0 (0)	0 (0)	2 (1)	0 (0.0)	0 (0)	1 (1.8)	0 (0)	0 (0)	0 (0)
tet(X4)	1 (1)	4 (2.2)	0 (0)	3 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Values in the parentheses indicate the column percentage. n, number of isolates. Highlighted cells indicate a statistically significant association between the respective ARGs and sampling sources ($p < 0.05$). BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection.

Table 5.3 illustrates the distribution of key ARGs across clinical, environmental, and animal-associated *E. coli* isolates. The *aac(6')-Ib-cr* gene conferring resistance to aminoglycosides and fluoroquinolones, was found in both water (3.1%) and fly samples (2.2%) but also found at higher frequencies in SSI (24.8%) and UTI isolates (10.7%). Importantly, $bla_{\text{CTX-M-15}}$, the globally dominant ESBL gene, was present in 76.8% of SSI isolates and 36.6% of UTI isolates, while also being detected in water, flies, and HRS isolates.

Among carbapenem resistance determinants, $bla_{\text{NDM-1}}$ was detected in flies and SSI isolates (one in each sector), and , $bla_{\text{NDM-20}}$ was detected in only one isolate from SSI, whereas $bla_{\text{NDM-5}}$ was the predominant carbapenemase gene. This gene showed a notably high prevalence in SSI isolates (12.4%), followed by water (4.2%), and was also found in flies, UTI, and HRS samples. The $bla_{\text{OXA-181}}$ gene was observed in water and SSI isolates. Co-occurrence of $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ was observed in three isolates from water and HRS samples.

mcr genes showed limited spread: *mcr-1.1* was confined to farm-associated samples (CCS and BF). Importantly, no *mcr* genes were detected in clinical or healthy human isolates. The Tigecycline resistance gene, *tet*(X4) was most prevalent in flies (n = 4), followed by CMS (n = 3) and water (n = 1).

5.2.2.2 Phylogroup-specific patterns of resistance genes

To further understand the relationship between resistance genes and the genetic background of *E. coli*, I analysed the distribution of key ARGs across different phylogroups. This enables the identification of specific phylogenetic lineages that may act as reservoirs or vectors for clinically significant resistance mechanisms (Petitjean *et al*., 2021). Table 5.4 lists the prevalence of selected ARGs among *E. coli* phylogroups.

The distribution of key ARGs differed significantly across *E. coli* phylogroups (Table 5.4). The ESBL *bla*$_{\text{CTX-M-15}}$ showed the highest prevalence in phylogroups C (55.2%), followed by phylogroup F (47.9%), and phylogroup B2 (47.3%). In contrast, *aac(6')-Ib-cr*, which confers resistance to both aminoglycosides and fluoroquinolones, was most common in phylogroups F (27.1%), C (24.1%), and B2 (12.9%). *bla*$_{\text{NDM-5}}$, the main mechanism of carbapenem resistance in this study, was found across multiple phylogroups, with the highest frequencies in C (13.8%), D (8.5%), and A (1.5%). *bla*$_{\text{NDM-1}}$ was rarely detected, identified in only two isolates from phylogroups A and B1. The *bla*$_{\text{OXA-181}}$ gene was sporadically present across several phylogroups, including cladeI and C. *mcr-1.1* was rarely observed and was limited to phylogroups B1 and G. The tigecycline resistance gene *tet*(X4) was only detected in phylogroup A.

Table 5.4 Distribution of important ARGs among various phylogroups of *E. coli*

ARGs	n, (%) isolates by phylogroups								
	A n= 605	B1 n= 641	B2 n= 93	C n= 29	cladeI n= 42	D n= 94	E n= 42	F n= 48	G n= 25
aac(6')-Ib-cr	7 (1.2)	5 (0.8)	12 (12.9)	7 (24.1)	0 (0)	9 (9.6)	0 (0)	13 (27.1)	0 (0)
$bla_{\text{CTX-M-15}}$	63 (10.4)	32 (5)	44 (47.3)	16 (55.2)	0 (0)	29 (30.0)	1 (2.4)	23 (47.9)	0 (0)
$bla_{\text{NDM-1}}$	1 (0.2)	1 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
$bla_{\text{NDM-5}}$	9 (1.5)	4 (0.6)	0 (0)	4 (13.8)	0 (0)	8 (8.5)	0 (0)	2 (4.2)	0 (0)
$bla_{\text{OXA-181}}$	1 (0.2)	2 (0.3)	0 (0)	2 (6.9)	1 (1.1)	1 (2.1)	0 (0)	0 (0)	0 (0)
mcr-1.1	0 (0)	2 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)
tet(X4)	8 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Values in the parentheses indicate the column percentage. n, number of isolates. Highlighted cells indicate the statistically significant association between the respective ARGs and *E. coli* phylogroups ($p < 0.05$).

5.2.3 Characterisation of virulence factors and their association with sampling sources and phylogroups of *E. coli*

To characterise the virulence potential of *E. coli* strains in this study, a comprehensive set of virulence-associated genes (VAGs) was identified using whole-genome sequencing. The details of VAGs identified in this study are summarised in Table 5.5, and the genes are categorised according to their functions (Pakbin *et al*., 2021b; Sora *et al*., 2021; Buckles *et al*., 2015).

Table 5.5 Categorisation of virulence genes identified in *E. coli* isolates, grouped by functional class

Category	Gene(s)	Function
Adhesins & Fimbriae	*fimA–fimI*, *papA–papX*, *sfaA–sfaY*	Epithelial adhesion
	afaA–afaF, *daaF*, *draA–draE*	Afimbrial adhesins- diffuse adherence in DAEC
	aggA–aggD, *agg3A–agg3D*, *aafA–aafD*	Aggregative adherence fimbriae (AAF types I and III)
	bfpA–bfpU, *ecpA–ecpE*	Bundle-forming pilus (EPEC) and *E. coli* common pilus (biofilms)
	focA–focH, *faeC–faeJ*, *f17d-A–f17d-G*	Fimbriae adherence in various pathotypes
Toxins	*hlyA–hlyD*, *cnf1*, *cdtA–cdtC*	Hemolysin, cytotoxic necrotizing factor
	astA, *eltA*, *eltB*, *estIa*	Heat-stable/-labile enterotoxins- ETEC-related
	pet, *pic*, *sat*, *vat*	SPATE family- serine proteases with mucosal damage
	stx1B, *stx2A*, *stx2B*, *stxA*	Shiga toxins—ribosomal inactivation (EHEC)
	senA, *senB*, *tcpC*, *sigA*, *pla*	Inflammation-related or immune evasion toxins

Iron Acquisition Systems	*entA–entF*, *fepA–fepG*, *fes*, *entS*	Enterobactin synthesis and uptake-core siderophore system
	iucA–iucD, *iutA*	Aerobactin system-plasmid-encoded, ExPEC associated
	iroB–iroN	Salmochelin system-iron acquisition resistant to host defenses
	irp1, *irp2*, *fyuA*, *ybtA*, *ybtE*, *ybtP–ybtX*	Yersiniabactin system-PAI-encoded siderophore
	chuA–chuY, *shuA–shuY*	Heme acquisition systems-iron from host hemoglobin
Secretion Systems (T3SS)	*escC–escV*, *sepD*, *sepL*, *sepQ*, *sepZ*	Type III secretion system machinery-EHEC/EPEC
	cesAB–cesT, *cesD–cesF*, *cesL*	Chaperones stabilizing T3SS effectors
	espA–espX6, *tir*, *map*, *nleA–nleH2*, *cif*	Effectors that hijack host cytoskeleton and signalling
Other Secretion Systems	*gspC–gspM*, *ompA*	Type II secretion components and outer membrane functions
Invasion & Survival	*ibeA*	Invasion of brain endothelium
	ipaA–ipaH, *ipgA–ipgF*, *mxiA–mxiN*, *spa9–spa47*	Shigella-like invasion systems
	icsA–icsP, *icsB*, *sopA*	Intracellular motility
	ospB–ospI, *senA*, *senB*	Host immune interference

Capsule & Resistance	*kpsM*, *kpsT*, *kpsD*	Capsule biosynthesis- immune evasion
	traT	Serum resistance- complement inhibition
	csgA–csgG	Curli fimbriae- biofilm matrix production
	ompA, *ykgK*, *ecpR*, *cfaD'*, *fdeC*	Adhesion, regulation, envelope structure

DAEC, diffusely adhering *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli;* EHEC, enterohemorrhagic *E. coli*

5.2.3.1 Distribution of VAGs among various sources

The source-wise distribution of the critical VAGs is presented in Table 5.6. *fimH* was the most frequently detected gene, present in high levels across all sources, reflecting its role as a ubiquitous adhesin. The prevalence of *fimH* was significantly higher in isolates from CCS (95.4%, 185/194) and DARS (94.2%, 179/190) compared to other sources ($p < 0.05$). *iutA* and *fyuA* were also found to be widely distributed among all sources, especially in human clinical isolates and CCS isolates. Among UTI isolates, 60.7% harboured the *iutA* gene. In comparison, 48.1% of SSI isolates and 50% of CCS isolates contained *iutA*, which was statistically significant ($p < 0.05$) compared to other sources. The percentage of *fyuA* in UTI (66.1%) and SSI (57.4%) isolates was found to be statistically significant ($p < 0.05$). *astA*, an enterotoxin gene, was found to be widely distributed among various sources, but a statistically significant association was observed in CCS (32.5%), CMS (34%), and BF (34.5%) isolates ($p < 0.05$). Among other critically important genes, *papG* and *cnf1* were more specific to UTI and SSI isolates, and the prevalence of both genes in UTI isolates was significantly higher compared to

the other isolates (Table 5.5). The distribution of *stx2A*, characteristic of Shiga toxin-producing *E. coli* (STEC), was limited to DARS (15.3%) and BF (1.8%); *nleA*, *bfpA*, and *tir* were found mainly in human or avian isolates. Among environmental isolates (water and flies), the prevalent genes were *fimH*, *iutA*, *fyuA*, *kpsM*, and *astA*. Overall, isolates from clinical sources, especially those associated with UTIs, demonstrated significantly more virulent profiles compared to isolates from other sources.

Table 5.6 Distribution of high-priority VAGs of *E. coli* among sampling sources

Gene	n (%) of isolates by sources								
	Water n= 96	Fly n= 183	CCS n= 194	CMS n= 141	DARS n= 190	BF n= 55	UTI n= 112	SSI n= 129	HRS n= 534
fimH	83 (86.5)	163 (89.1)	185 (95.4)	130 (92.2)	179 (94.2)	51 (92.7)	101 (90.2)	97 (75.2)	485 (90.8)
papG	0 (0)	2 (1.1)	5 (2.6)	2 (1.4)	0 (0)	0 (0)	39 (34.8)	15 (11.6)	7 (1.3)
sfaS	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (2.7)	1 (0.8)	1 (0.2)
hlyA	1 (1)	2 (1.1)	0(0)	0(0)	40 (21.1)	0(0)	28 (25)	9 (7)	13 (2.4)
cnf1	0 (0)	0(0)	0(0)	0(0)	1 (0.5)	0(0)	15 (13.4)	6 (4.7)	7 (1.3)
iutA	11 (11.5)	15 (8.2)	97 (50)	30 (21.3)	2 (1.1)	3 (5.5)	68 (60.7)	62 (48.1)	94 (17.6)
iroN	8 (8.3)	26 (14.2)	52 (26.8)	24 (17)	5 (2.6)	2 (3.6)	23 (20.5)	6 (4.7)	26 (4.9)
fyuA	12 (12.5)	13 (7.1)	49 (25.3)	5 (3.5)	5 (2.6)	2 (3.6)	74 (66.1)	74 (57.4)	234 (21.3)
chuA	2 (2.1)	1 (0.5)	0 (0)	1 (0.7)	0 (0)	0 (0)	52 (46.4)	19 (14.7)	20 (3.7)
kpsM	6 (6.3)	14 (7.7)	28 (14.4)	14 (9.9)	32 (16.8)	14 (25.5)	66 (58.9)	46 (35.7)	67 (12.5)
eae	1 (1)	2 (1.1)	0 (0)	0 (0)	1 (0.5)	3 (5.5)	0 (0)	0 (0)	5 (0.9)
tir	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.2)

Gene	n (%) of isolates by sources								
	Water n= 96	Fly n= 183	CCS n= 194	CMS n= 141	DARS n= 190	BF n= 55	UTI n= 112	SSI n= 129	HRS n= 534
espB/D	1 (1)	2 (1.1)	0 (0)	1 (0.7)	1 (0.5)	3 (5.5)	0 (0)	0 (0)	5 (0.9)
map	1 (1)	2 (1.1)	0 (0)	1 (0.7)	1 (0.5)	3 (5.5)	0 (0)	0 (0)	5 (0.9)
nleA	1 (1)	2 (1.1)	0 (0)	1 (0.7)	0 (0)	2 (3.6)	0 (0)	0 (0)	3 (0.6)
stx2A/B	0 (0)	0 (0)	0 (0)	0 (0)	29 (15.3)	1 (1.8)	0 (0)	0 (0)	1 (0.2)
pet	3 (3.1)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (1.5)
bfpA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.2)
astA	23 (24)	32 (17.5)	63 (32.5)	48 (34)	22 (11.6)	19 (34.5)	14 (12.8)	12 (9.3)	58 (10.9)

Values in the parentheses indicate the column percentage. n, number of isolates. Highlighted cells indicate a statistically significant association between the respective VAGs and sampling sources ($p < 0.05$).

5.2.3.2 Distribution of VAGs across phylogroups

Phylogenetic analysis showed that VAGs clustered non-randomly among phylogroups. ExPEC-associated genes such as *papG*, *iutA*, *iroN*, and *kpsM* were significantly prevalent in phylogroup B2, and *hlyA*, *cnf1*, *chuA*, and *fyuA* were also linked considerably to phylogroup B2 ($p < 0.05$). The gene *fimH* was found at a high frequency across all the phylogroups, with a specific association to phylogroup B1 (94.1%). Enterotoxigenic markers, such as *astA*, were more frequent in phylogroups A, B1, and E, which are generally associated with commensals and enteric pathotypes. Phylogroup D showed a significant presence of genes *papG* (11.7%), *iutA* (31.9%), *fyuA* (37.2%), and *kpsM* (63.8%) ($p < 0.05$) (Table 5.7). Similarly, phylogroup F was significantly associated with genes *iutA* (43.8%), *fyuA* (35.4%), *kpsM* (66.7%), and *tir* (2.1%).

Overall, phylogroup B2 exhibited the highest burden of traditional extraintestinal virulence genes. In contrast, phylogroups A and B1, which accounted for the most significant number of isolates, showed a high prevalence of *fimH*. Toxin genes such as *astA* were widely distributed, while *stx2A/B* were mainly found in phylogroups A and B1. The prevalence of virulence factors typical of enteropathogenic *E. coli* (e.g., *bfpA*, *espB/D, map*, *nleA*, *tir*) was lower and scattered across different phylogroups.

Table 5.7 Distribution of high-priority VAGs of *E. coli* among phylogroups

Gene	n (%) of isolates by phylogroups								
	A n= 605	B1 n= 641	B2 n= 93	C n= 29	CladeI n= 42	D n= 94	E n= 42	F n= 48	G n= 25
fimH	521 (86.1)	603 (94.1)	87 (93.5)	28 (96.6)	37 (88.1)	89 (94.7)	41 (97.6)	30 (62.5)	25 (100)
papG	6 (1)	0 (0)	44 (47.3)	0 (0)	2 (4.8)	11 (11.7)	1 (2.4)	3 (6.3)	3 (12)
sfaS	0 (0)	0 (0)	5 (5.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
hlyA	4 (0.7)	42 (6.6)	39 (41.9)	0 (0)	1 (2.4)	6 (6.4)	0 (0)	1 (2.1)	0 (0)
cnf1	0 (0)	1 (0.2)	27 (29.0)	0 (0)	0 (0)	1 (1.1)	0 (0)	0 (0)	0 (0)
iutA	89 (14.7)	111 (17.3)	72 (77.4)	11 (37.9)	10 (23.8)	30 (31.9)	8 (2.1)	21 (43.8)	23 (92)
iroN	40 (6.6)	76 (11.9)	31 (33.3)	3 (10.3)	2 (4.8)	4 (4.3)	2 (4.8)	6 (12.5)	7 (28)
fyuA	79 (13.1)	77 (12)	87 (93.5)	12 (41.4)	3 (7.1)	35 (37.2)	8 (19)	17 (35.4)	12 (48)
chuA	0 (0)	0 (0)	88 (94.6)	0 (0)	0 (0)	3 (3.2)	0 (0)	4 (8.3)	0 (0)
kpsM	43 (7.1)	32 (5)	88 (94.6)	0 (0)	23 (54.8)	60 (63.8)	4 (9.5)	32 (66.7)	0 (0)
eae	1 (0.2)	9 (1.4)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.1)	0 (0)
tir	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.1)	0 (0)

Gene	n (%) of isolates by phylogroups								
	A n= 605	B1 n= 641	B2 n= 93	C n= 29	CladeI n= 42	D n= 94	E n= 42	F n= 48	G n= 25
espB/D	1 (0.2)	10 (1.6)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.1)	0 (0)
map	1 (0.2)	10 (1.6)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.1)	0 (0)
nleA	2 (0.3)	6 (0.9)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
stx2A/B	6 (1)	24 (3.7)	0 (0)	0 (0)	2 (4.8)	0 (0)	0 (0)	0 (0)	0 (0)
pet	4 (0.7)	7 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
bfpA	1 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
astA	124 (20.5)	100 (15.6)	3 (3.2)	3 (10.3)	17 (40.5)	17 (18.1)	14 (33.3)	6 (12.5)	1 (4)

Values in the parentheses indicate the column percentage. n, number of isolates. Highlighted cells indicate a statistically significant association between the respective VAGs and phylogroups ($p < 0.05$).

5.2.4 Analysis of MLST profiles and their associations with phylogroups of *E. coli*

Multi-Locus Sequence Typing was used to characterise *E. coli* isolates based on the sequences of internal fragments of seven housekeeping genes. A total of 489 STs of *E. coli* were identified in this study. The major STs, in terms of frequency, found here are ST10, ST155, ST1196, ST48, ST131, ST206, and ST296. Figures 5.5 (a) and 5.5 (b) show the frequency of STs identified in this study. The STs with a frequency of 10 and above are displayed in Figure 5.5 (b), which accounts for 45% of the total identified STs. All other STs make up 55% of the total STs.

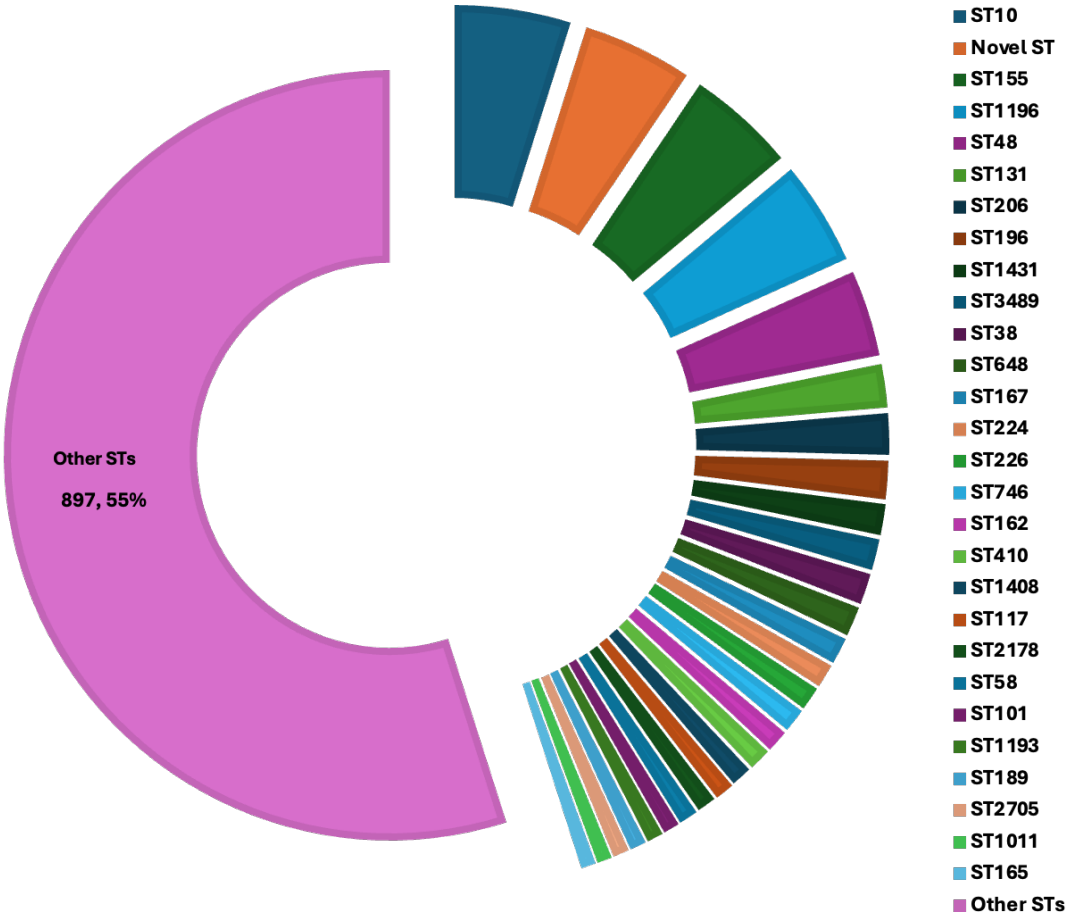


Figure 5.5 (a) Distribution of STs among *E. coli* isolates.

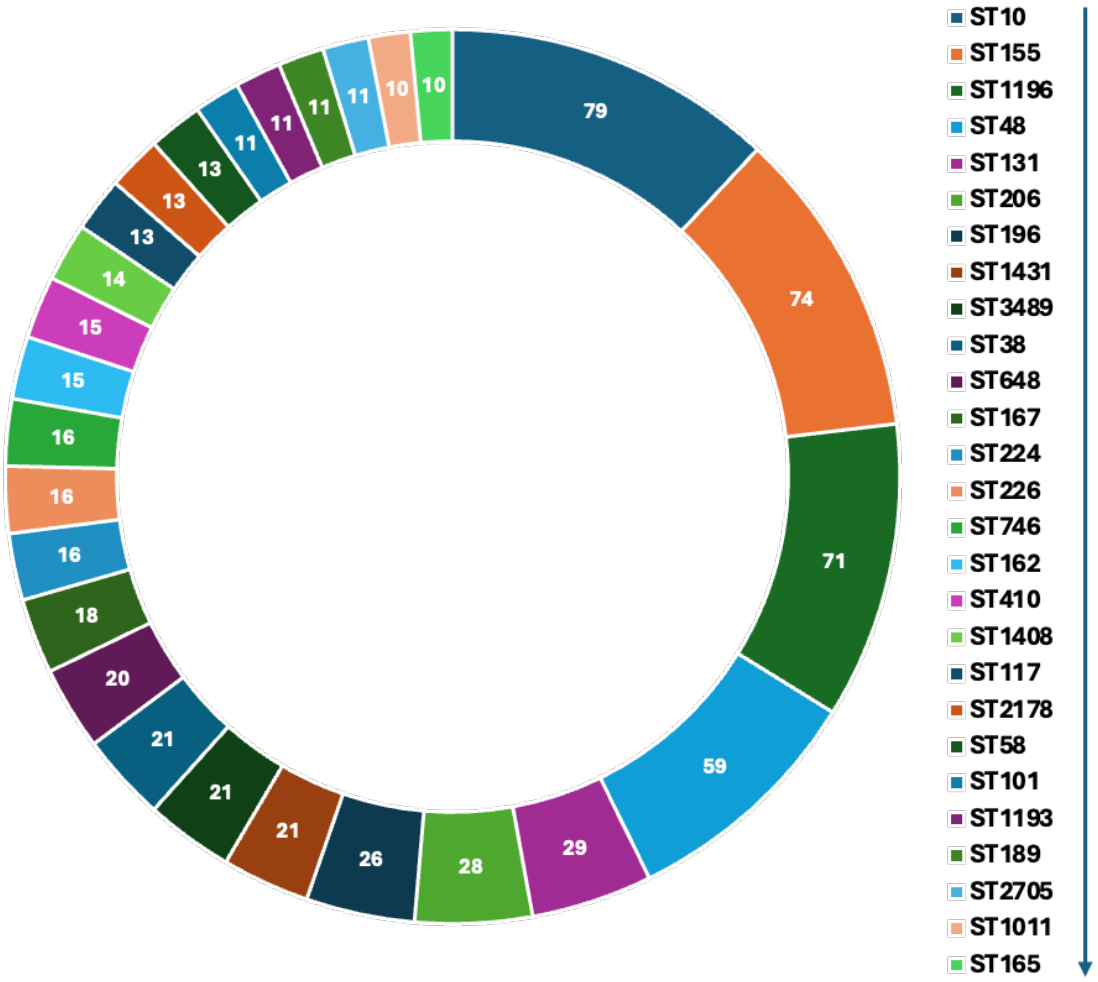


Figure 5.5 (b) Frequency of major STs with at least 10 isolates, arranged in descending order (arrow indicates direction). The numbers within the chart represent the frequency of isolates for each ST.

To explore the distribution of *E. coli* lineages across human, animal, and environmental compartments, MLST profiles were examined in relation to phylogroups. This analysis was undertaken to assess whether shared sequence types occur across sources, thereby providing insight into potential cross-sector dissemination within a One Health framework. The distribution of the most prevalent STs across the sources and respective phylogroups is illustrated in Figure 5.6. ST10 was distributed among all sources, predominantly in HRS (44/79), and most of the ST10 (76/79) belonged to phylogroup A. ST155 was mainly identified in chicken and HRS isolates, and all ST155 belonged to phylogroup B1. ST1196 was primarily found in CCS (44/71), followed by HRS (9/71) and CMS (8/71). All ST1196 isolates belonged to phylogroup B1, except one, which belonged to phylogroup A. ST1196 was absent in clinical isolates. ST48 was notably distributed among water, flies, chicken, and HRS isolates but 58/59 ST48 isolates belonged to phylogroup A. ST206 was distributed primarily in flies (11/28) and all ST206 were found in phylogroup A. Although ST196 was mainly found in HRS isolates, it was distributed across a wide range of sources; water, flies, DARS, BF, UTI and SSI except CCS and CMS. All ST196 were under phylogroup B1. ST131 isolates mostly originated from UTI (15/29), SSI (12/29), and two ST131 isolates were from HRS. All isolates from ST131 belonged to phylogroup B2. ST38 was found in human isolates, including UTI (4/21), SSI (9/21), and HRS (4/21), with a few from flies (1/21), BF (2/21), and CMS (1/21). All ST38 isolates were associated with phylogroup D. ST648 isolates were primarily from UTI (3/20), SSI (12/20), with a few from water (2/20), CMS (1/20) and HRS (2/20), of which 19/20 isolates belonged to phylogroup F, and one isolate to phylogroup D.

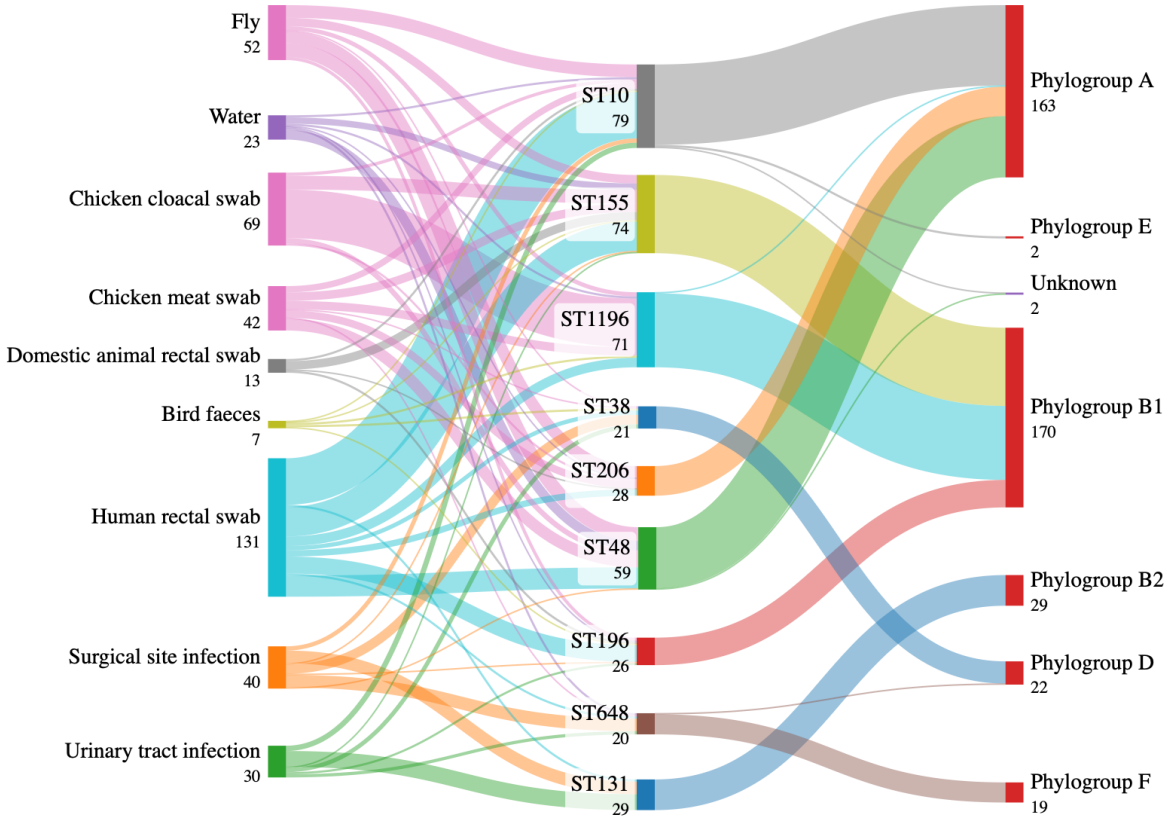


Figure 5.6 Sankey diagram showing the distribution of major *E. coli* STs across different sample sources and their corresponding phylogenetic groups. Only the most frequent STs are presented here.

5.2.5 Investigating the emergence of high-risk clones and their genomic characterisation

E. coli clones, which are widespread globally and associated with variability in resistance and virulence genes, are considered high-risk clones. The top high-risk clones, according to current global surveillance and research, are ST131, ST69, ST10, ST405, ST38, ST95, ST648, ST73, and ST1193 (Chanchaithong *et al*., 2025; Kocsis *et al*., 2022). Given the large size of the dataset (1,634 sequenced isolates), a focused subset of globally recognised high-risk clones was selected for detailed phylogenetic analysis to allow in-depth and interpretable evolutionary investigation within the scope of this thesis. In this study, all the clones mentioned above were found to be present. The frequency of these high-risk clones is illustrated in Figure 5.7.

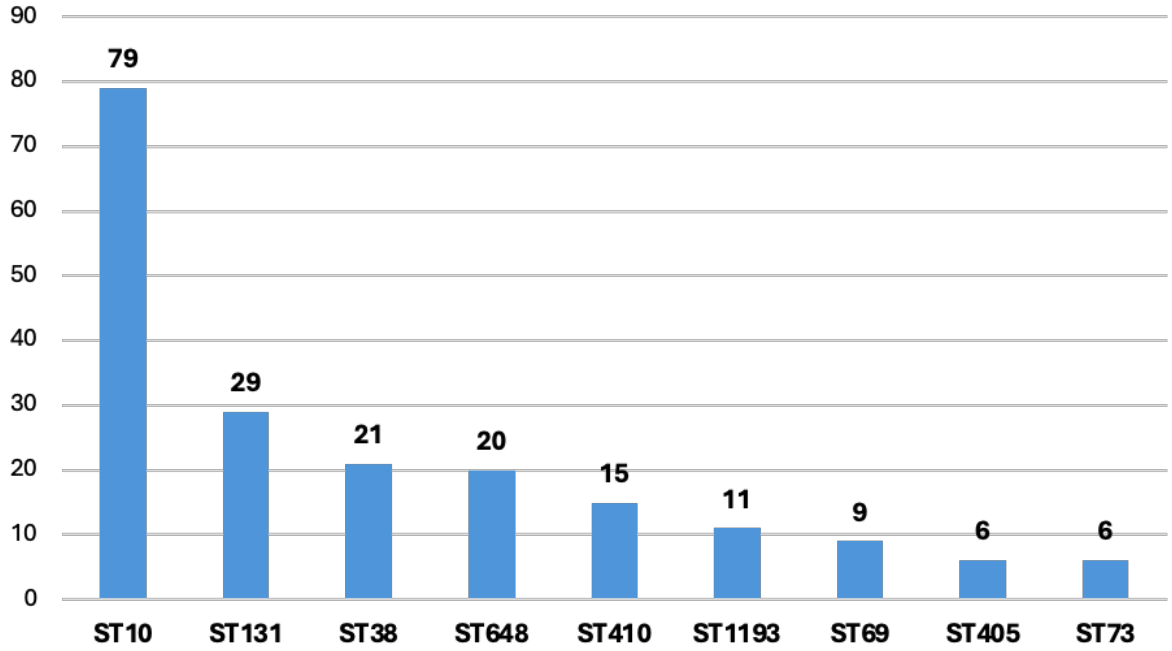
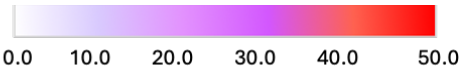


Figure 5.7 Frequency of high-risk *E. coli* clones identified in this study.

The distribution of high-risk clones across the sources is shown in Figure 5.8. ST10 is distributed widely across most sources but is prevalent mainly in HRS isolates. These high-risk

clones are associated with clinical isolates from UTI and SSI, followed by HRS isolates (Figure 5.8).



	Water	Fly	CCS	CMS	DARS	BF	UTI	SSI	HRS
ST10 (79)	2.0	12.0	3.0	6.0	2.0	1.0	5.0	4.0	44.0
ST131 (29)	0.0	0.0	0.0	0.0	0.0	0.0	15.0	12.0	2.0
ST38 (21)	0.0	1.0	0.0	1.0	0.0	2.0	4.0	9.0	4.0
ST648 (20)	2.0	0.0	0.0	1.0	0.0	0.0	3.0	12.0	2.0
ST410 (15)	2.0	1.0	0.0	0.0	0.0	0.0	4.0	8.0	0.0
ST1193 (11)	0.0	0.0	0.0	0.0	0.0	0.0	7.0	2.0	2.0
ST69 (9)	0.0	0.0	1.0	1.0	0.0	0.0	2.0	2.0	3.0
ST405 (6)	1.0	0.0	0.0	0.0	0.0	0.0	1.0	4.0	0.0
ST73 (6)	0.0	0.0	0.0	0.0	0.0	0.0	4.0	1.0	1.0

Figure 5.8 Distribution of high-risk clones of *E. coli* among various sources.

The heatmap illustrates the distribution of high-risk *E. coli* clones across various sources. Each cell represents the frequency (number) of STs within respective sources. Darker shades of red indicate higher frequency, while lighter shades or white indicate lower or absence of isolates from respective sources. BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab; DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection.

Figure 5.9 illustrates the distribution and interconnectedness of high-risk *E. coli* clones across diverse sources and geographic locations. Among the clones, ST10, ST131, ST38, and ST648 emerged as dominant clades, collectively identified from both clinical and environmental origins.

ST10, the most widespread clone, was detected in nearly all sampling sources, including flies, water, CCS, CMS, HRS, and clinical infections (UTI and SSI). ST10 was most prevalent in Mymensingh Sadar but also showed a notable presence in other sub-districts. ST131, a globally recognised extraintestinal pathogenic clone (Nicolas-Chanoine *et al.*, 2014) was confined only to human sources, (UTI, SSI and HRS) and predominantly found in Mymensingh Sadar, with two isolates from HRS in Tarakanda. ST38 and ST69 were distributed across various locations, including Mymensingh Sadar. ST38 was found in Gauripur, Muktagachha, and Tarakanda, while ST69 was found in Gauripur, Muktagachha, and Trishal. ST405 was confined solely to Mymensingh Sadar (Figure 5.9).

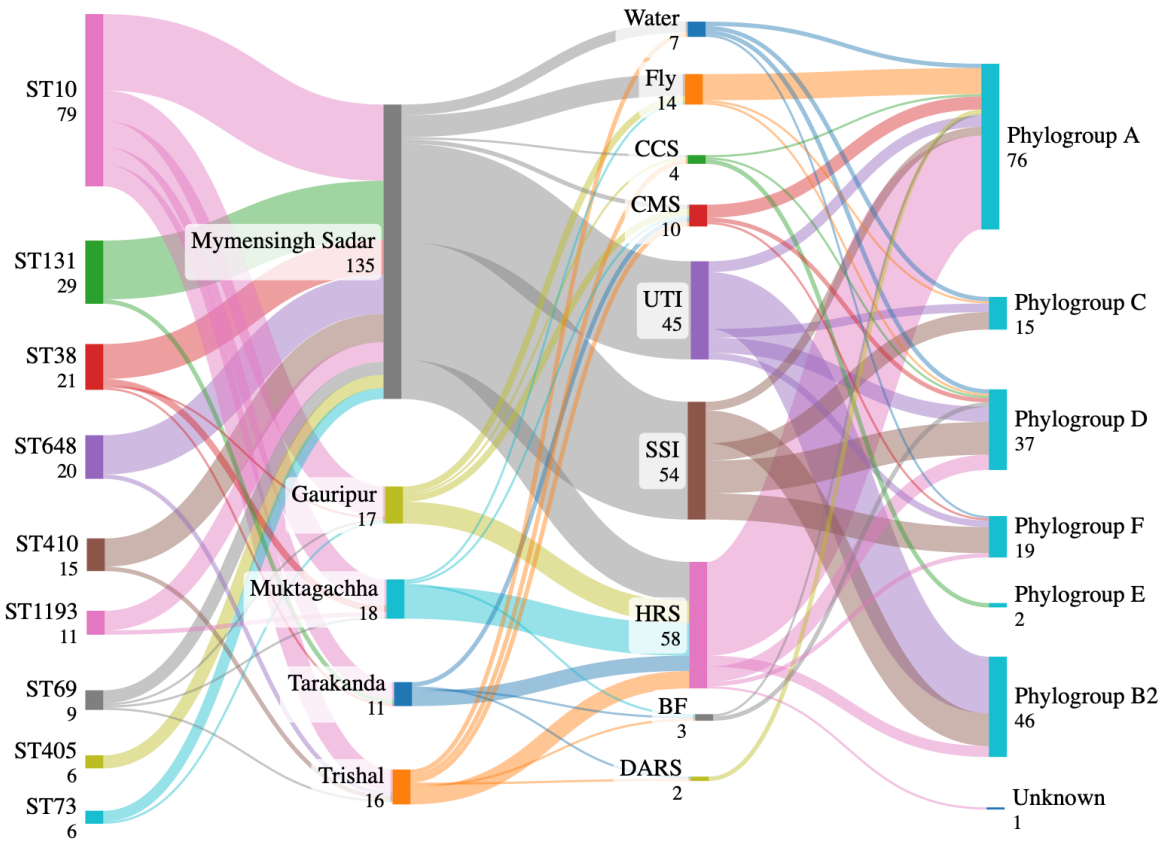


Figure 5.9 Sankey diagram illustrating the distribution of high-risk *E. coli* STs across different sampling locations in the Mymensingh district, their sources, and respective phylogroups. Each flow represents the number of isolates connecting a specific ST with its sampling location, source, and phylogroup. BF, bird faeces; CCS, chicken cloacal swab; CMS, chicken meat swab;

DARS, domestic animal rectal swab; HRS, human rectal swab; SSI, surgical site infection; UTI, urinary tract infection.

5.2.6 Antibiotic resistance profiling of high-risk clone of *E. coli*

A heatmap analysis was conducted to visualise the resistance patterns of high-risk *E. coli* STs to specific antibiotics (Figure 5.10). The figure shows a diverse pattern of resistance prevalence among the STs. ST131, ST405, ST648, and ST410 displayed consistently high rates across multiple antibiotics. ST405 and ST648 each showed 100% resistance to several antibiotics. ST405 exhibited the highest resistance rates to AMK (66.7%), CAZ-AVI (66.7%), IPM (66.7%), and MER (66.7%). Most of the STs were found to be sensitive to colistin, fosfomycin, and tigecycline.

	AMP	AMC	TZP	AZT	CTX	CAZ	CAZ-AVI	CEF	AMK	GEN	CIP	LEV	COL	FOS	IPM	MER	TIG
ST10 (n=79)	40.5	34.2	6.3	16.5	16.5	19.0	0.0	15.2	1.3	6.3	49.4	48.1	0.0	3.8	0.0	0.0	1.3
ST131 (n=29)	96.6	72.4	0.0	86.2	89.7	93.1	0.0	75.9	27.6	24.1	82.8	79.3	0.0	0.0	0.0	0.0	0.0
ST38 (n= 21)	85.7	81.0	33.3	61.9	61.9	61.9	3.8	61.9	23.8	33.3	71.4	66.7	0.0	4.8	23.8	23.8	0.0
ST648 (20)	95.0	95.0	50.0	90.0	90.0	90.0	15.0	85.0	45.0	40.0	100.0	100.0	0.0	0.0	10.0	15.0	0.0
ST410 (15)	100.0	100.0	80.0	93.3	93.3	93.3	13.3	93.3	20.0	20.0	100.0	100.0	0.0	0.0	20.0	20.0	0.0
ST1193 (11)	90.9	81.8	0.0	72.7	72.7	81.8	0.0	72.7	0.0	9.1	100.0	100.0	0.0	0.0	0.0	0.0	0.0
ST69 (9)	44.4	44.4	0.0	33.3	33.3	22.2	0.0	22.2	0.0	11.1	33.3	11.1	0.0	0.0	0.0	0.0	0.0
ST405 (6)	100.0	100.0	100.0	100.0	100.0	100.0	66.7	100.0	66.7	50.0	100.0	100.0	0.0	0.0	66.7	66.7	0.0
ST73 (6)	66.7	50.0	0.0	66.7	66.7	66.7	0.0	66.7	0.0	0.0	0.0	16.7	0.0	0.0	0.0	0.0	0.0

Figure 5.10 Heatmap of antibiotic resistance among high-risk *E. coli* clones.

The heatmap illustrates the percentage of resistance to different antibiotics across high-risk *E. coli* STs. Each cell represents the proportion of isolates within a specific ST that were resistant to a particular antibiotic. Darker shades of red indicate higher resistance rates, whereas lighter shades or white reflect lower or no resistance. This visualisation highlights distinct resistance profiles and patterns among the major high-risk clones. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; AMK, amikacin; AZT, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; CAZ-AVI,

ceftazidime-avibactam; CEF, cefepime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MER, meropenem; TZP, piperacillin-tazobactam; TIG, tigecycline.

5.2.6.1 ARG associations with high-risk *E. coli* clones

The distribution of key ARGs among high-risk STs is summarised in Table 5.8. The gene *aac(6')-Ib-cr,* which confers resistance to aminoglycosides, showed a statistically significant association with ST131, ST648, and ST410 ($p < 0.05$). In contrast, another aminoglycoside resistance gene, *aph(6)-Id***,** was widely distributed across all STs but did not exhibit a significant association with any particular clone. The ESBL gene $bla_{\text{CTX-M-15}}$, a major contributor to third-generation cephalosporin (3GC) resistance, was detected in over 75% of isolates belonging to ST131, ST648, and ST1193, a significantly higher prevalence compared to other STs. Additionally, among the 36 isolates positive for $bla_{\text{NDM-5}}$, 14 were found within high-risk clones, with a significant association observed specifically with ST38 and ST405 ($p < 0.05$). $bla_{\text{NDM-5}}$ was found across diverse STs, with eight positive isolates identified in ST167. Additional STs carrying $bla_{\text{NDM-5}}$ included ST155, ST2083, ST2659, ST2851, ST361, ST448, ST46, ST5173, ST8346, and ST90. The *fosA* gene was detected in ST10, while *mph*(A) was broadly distributed across multiple STs except ST73. A significant association was observed between *mph*(A) and high-risk clones ST131 and ST648. Among the three isolates positive for *mcr-1.1*, two were from ST1196, while the remaining one was ST657. None of the isolate co-harbour *mcr* and carbapenem or 3GC resistance genes. The prevalence of the *sul1* gene was significantly higher in ST10, ST131 and ST648 compared to the other STs.

Table 5.8 Distribution of major ARGs of *E. coli* among high-risk clones

Resistance genes	**n, (%) isolates by STs**								
	ST10 (79)	ST131 (29)	ST38 (21)	ST648 (20)	ST410 (15)	ST1193 (11)	ST69 (9)	ST405 (6)	ST73 (6)
aac(6')-Ib-cr	0 (0)	11 (37.9)	5 (23.8)	11 (55.0)	7 (46.7)	1 (9.1)	0 (0)	2 (33.3)	0 (0)
aph(6)-Id	14 (17.7)	4 (13.8)	5 (23.8)	4 (20)	1 (6.7)	4 (36.4)	4 (44.4)	1 (16.7)	3 (50)
bla$_{\text{CTX-M-15}}$	10 (12.7)	22 (75.9)	11 (52.4)	16 (80)	10 (66.7)	9 (81.8)	3 (33.3)	4 (66.7)	4 (66.7)
bla$_{\text{DHA-1}}$	6 (7.6)	2 (6.9)	1 (4.8)	4 (20)	1 (6.7)	1 (9.1)	0 (0)	0 (0)	0 (0)
bla$_{\text{NDM-5}}$	0 (0)	0 (0)	5 (23.8)	3 (15)	2 (13.3)	0 (0)	0 (0)	4 (66.7)	0 (0)
bla$_{\text{OXA-181}}$	0 (0)	0 (0)	1 (4.8)	1 (5)	2 (13.3)	0 (0)	0 (0)	0 (0)	0 (0)
bla$_{\text{TEM-1B}}$	20 (25.3)	1 (3.4)	9 (42.9)	9 (45)	2 (13.3)	7 (63.6)	5 (55.6)	3 (500	2 (33.3)
dfrA14	8 (10.1)	0 (0)	0 (0)	3 (15)	0 (0)	0 (0)	2 (22.2)	0 (0)	2 (33.3)
fosA3	3 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
mph(A)	35 (44.4)	25 (86.2)	15 (71.4)	17 (85.0)	11 (73.3)	8 (72.7)	2 (22.2)	5 (83.3)	0 (0)
qnrS1	33 (41.8)	0 (0)	4 (19)	6 (30)	3 (20)	0 (0)	3 (33.3)	0 (0)	0 (0)

Resistant genes	**n, (%) isolates by STs**								
	ST10 (79)	ST131 (29)	ST38 (21)	ST648 (20)	ST410 (15)	ST1193 (11)	ST69 (9)	ST405 (6)	ST73 (6)
sul1	26 (32.9)	22 (75.9)	10 (47.6)	15 (75)	8 (53.3)	1 (9.1)	2 (22.2)	4 (66.7)	0 (0)
sul2	17 (21.5)	4 (13.8)	5 (23.8)	4 (20)	2 (13.3)	3 (27.3)	4 (44.4)	1 (16.7)	3 (50)
sul3	16 (20.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)
tet(A)	38 (48.1)	6 (20.7)	8 (38.1)	7 (35)	9 (60)	1 (9.1)	4 (44.4)	1 (16.7)	2 (33.3)
tet(X4)	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Values in the parentheses indicate the column percentage. n, number of isolates. Highlighted cells indicate a statistically significant association between the respective ARGs and high-risk STs ($p < 0.05$).

5.2.6.2 Core genome phylogeny and clonal relationships

A phylogenetic tree was constructed based on core genome alignment of 196 *E. coli* isolates and annotated with STs, phylogroups, sampling locations, and the presence or absence of clinically important ARGs (Figure 5.11). The inner ring represents the distribution of major STs. ST131, ST10, ST38, ST648, and ST410 were more prevalent. These STs form distinct clusters. ST131 forms a well-defined clade. The middle ring indicates phylogroups, with most isolates belonging to groups B2, A, and D. Phylogroup B2 is notably associated with ST131, ST1193 and ST73. In contrast, phylogroup A is associated with ST10. The outer ring shows the geographic origin of the isolates, indicating sampling location. Isolates from different locations are interspersed throughout the tree. Resistance genes are widely distributed across multiple clades, with a high prevalence among isolates in ST131, ST648, and ST405.

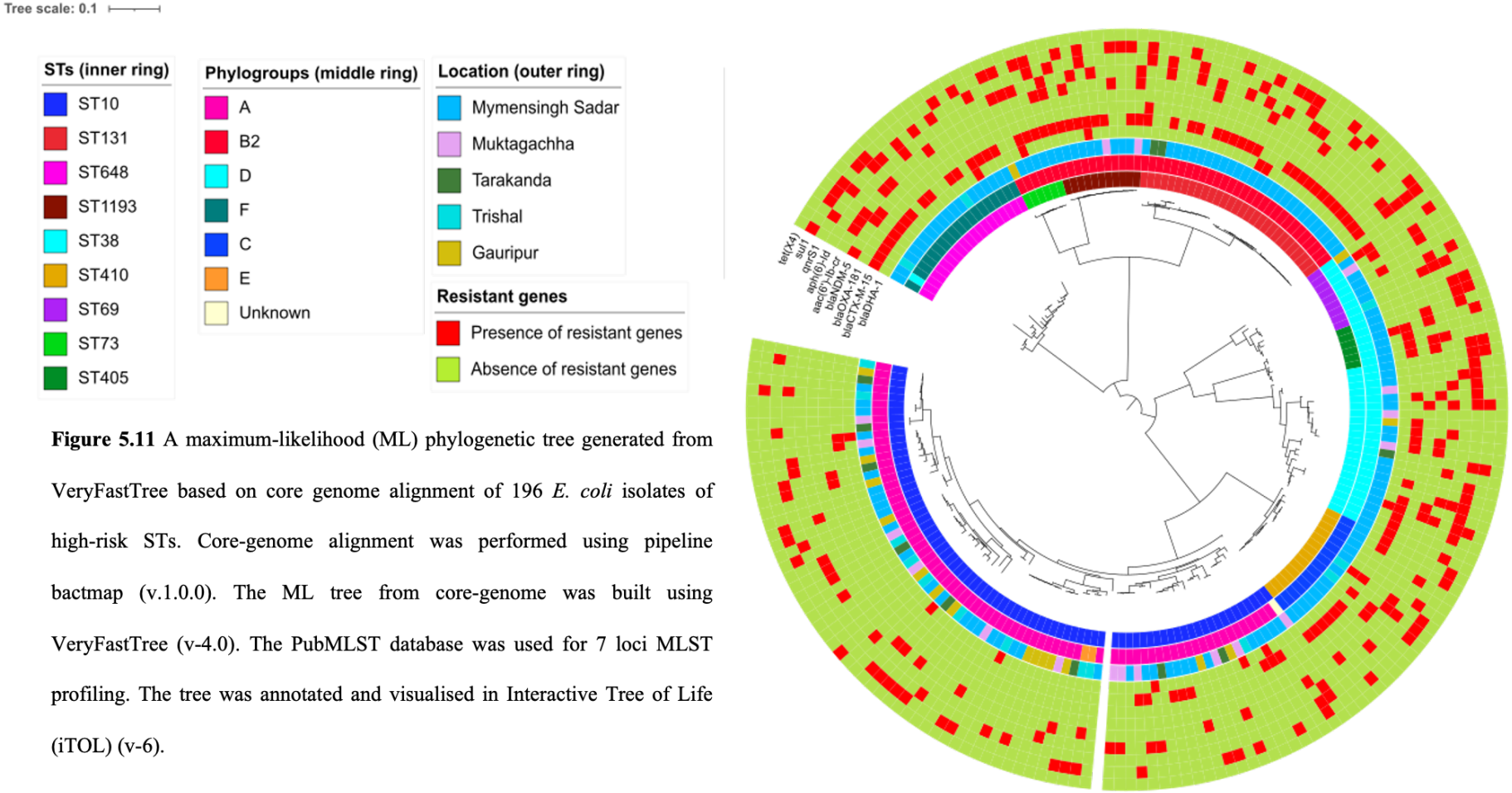


Figure 5.11 A maximum-likelihood (ML) phylogenetic tree generated from VeryFastTree based on core genome alignment of 196 *E. coli* isolates of high-risk STs. Core-genome alignment was performed using pipeline bactmap (v.1.0.0). The ML tree from core-genome was built using VeryFastTree (v-4.0). The PubMLST database was used for 7 loci MLST profiling. The tree was annotated and visualised in Interactive Tree of Life (iTOL) (v-6).

A core-genome single-nucleotide polymorphism (SNP)-based phylogenetic tree was constructed to illustrate the genetic distance among the isolates of the high-risk clones within a cluster and their dissemination across sources and locations (Figure 5.12). A 100-SNP threshold was used to define clusters of related isolates, consistent with recent One Health genomic surveillance studies that apply broader SNP cutoffs to detect cross-source linkages across human, animal, and environmental reservoirs, rather than strict outbreak-specific relationships (Watt *et al*., 2025). This analysis revealed nine distinct SNP clusters, each comprising at least three isolates that differ by 100 SNPs or fewer within the clusters. These clusters represent closely related bacterial lineages.

Clusters A and C were exclusively composed of ST131 isolates derived from SSI and UTI. Cluster B, representing ST1193, included isolates from HRS, UTI and SSI, all collected from Mymensingh Sadar and Muktagachha. Cluster D, consisting of ST10 isolates, was isolated from HRS and UTI and showed geographic spread across Mymensingh Sadar, Gauripur and Trishal.

Interestingly, Clusters E and H, both belonging to ST410, appeared closely related. While Cluster E included isolates from UTI and SSI, Cluster H comprised isolates from SSI and water. Notably, the water isolates from Trishal clustered with clinical isolates from Mymensingh Sadar.

Cluster F grouped isolates from SSI and fly samples, with distribution across Mymensingh Sadar and Gauripur. Similarly, Cluster G included three isolates from distinct sources: SSI, UTI, and HRS, with the HRS isolate from Trishal geographically separate from the clinical isolates.

Cluster I, consisting of ST38 isolates, demonstrated multisectoral and spatial distribution, including samples from SSI, HRS, and flies. These were collected from Mymensingh Sadar, Muktagachha, and Gauripur, with HRS isolates appearing in both clinical and environmental settings.

The presence of specific STs in multiple SNP clusters reflects their genetic diversity and widespread distribution, rather than a single recent transmission event. ST131, found in Clusters A and C, is a globally disseminated high-risk clone known for causing extraintestinal infections and harbouring ESBLs, particularly bla_{CTXM} genes (Figure 5.11). Similarly, ST1193 (Cluster B) is an emerging MDR clone that has rapidly spread worldwide. It was detected across both colonisation (HRS) and infection sources (UTI and SSI. ST10 (Cluster D) is a widespread commensal and opportunistic clone, often linked to both animal and human reservoirs.

The identification of ST410 in clusters E and H is notable, as it is a globally expanding clone associated with fluoroquinolone and carbapenem resistance, often found in both clinical and environmental samples. ST38 (Cluster I) is an emerging clone associated with both community-acquired and healthcare-associated infections. It was present in clinical, colonisation, and fly samples across multiple subdistricts.

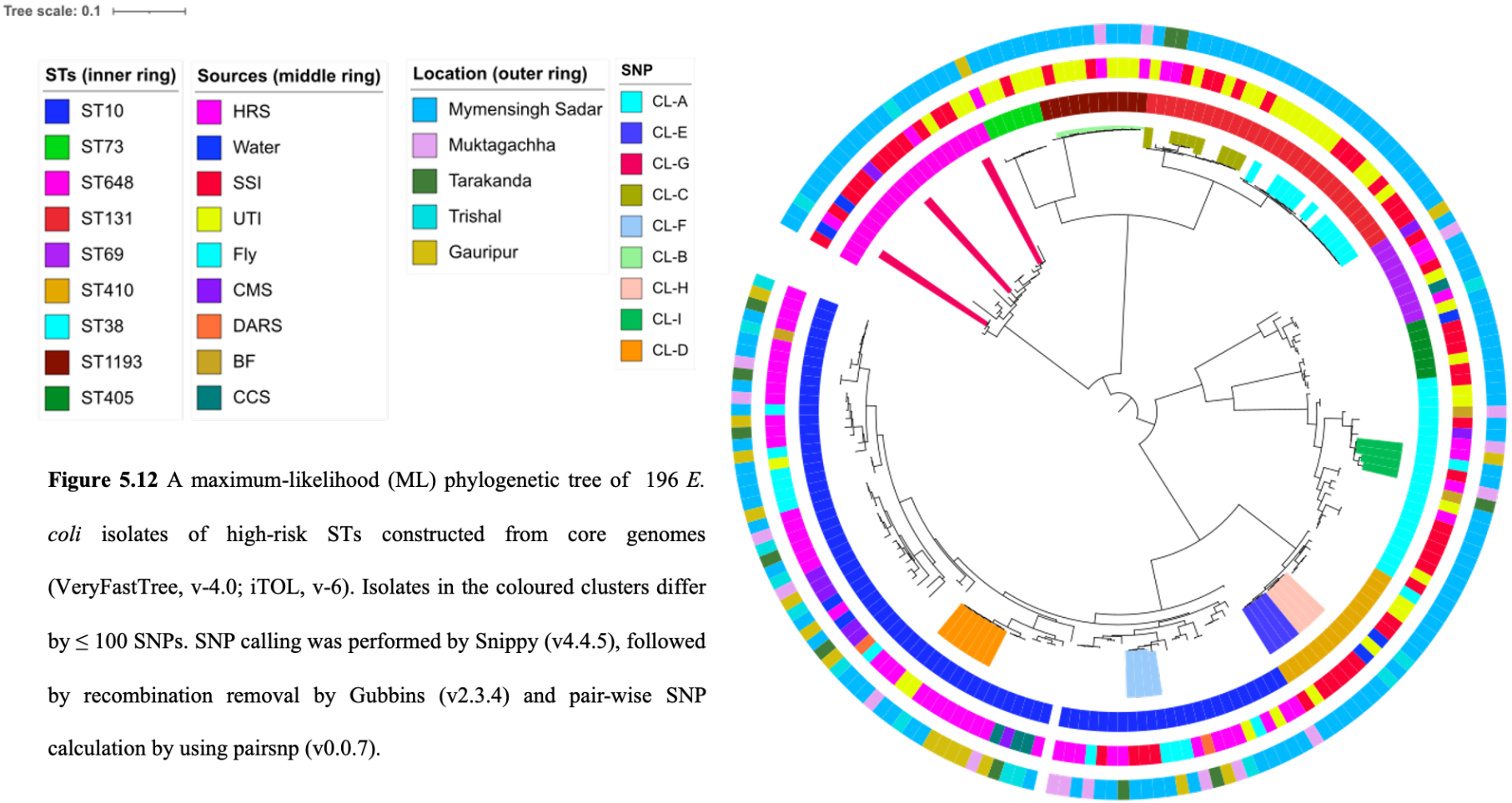


Figure 5.12 A maximum-likelihood (ML) phylogenetic tree of 196 *E. coli* isolates of high-risk STs constructed from core genomes (VeryFastTree, v-4.0; iTOL, v-6). Isolates in the coloured clusters differ by ≤ 100 SNPs. SNP calling was performed by Snippy (v4.4.5), followed by recombination removal by Gubbins (v2.3.4) and pair-wise SNP calculation by using pairsnp (v0.0.7).

5.2.7 Virulence profiling of high-risk clone of *E. coli*

The distribution of virulence genes among different *E. coli* high-risk STs reveals significant differences in their pathogenic potential (Table 5.9). The *fimH* gene, which encodes a type 1 fimbria adhesin, was found in all STs. Classical uropathogenic *E. coli* virulence markers, such as *papG*, *hlyA*, and *cnf1*, showed strong links with ST131, ST73, and, to a lesser extent, with ST69. ST131 exhibited a broad spectrum of virulence factors, with 100% carriage of *papG*, *iutA*, *fyuA*, *chuA*, and *kpsM*. Similarly, ST73 carried multiple virulence genes, including *hlyA*, *cnf1*, *iutA*, and *iron*, at 100%. In contrast, ST10 lacked most classical uropathogenic *E. coli* genes but still contained *fimH* (97.5%) and *iutA* (45.6%). Notably, ST648, an emerging lineage, carried *iutA* in all isolates but showed relatively lower frequencies of other virulence factors. Genes such as *sfaS*, *pet*, *stx2A/B*, *map*, and *tir* were absent across all these STs, while *bfpA*, often linked with enteropathogenic *E. coli*, was found in a single ST10 isolate. These findings highlight the genetic diversity of *E. coli* STs for virulence content, with ST131 and ST73 emerging as virulence-rich lineages.

Table 5.9 Distribution of high-priority VAGs of *E. coli* among high-risk clones

Virulence gene	**n, (%) isolates by STs**								
	ST10	ST131	ST38	ST648	ST410	ST1193	ST69	ST405	ST73
fimH	77 (97.5)	29 (100)	20 (95.2)	4 (20)	15 (100)	11 (100)	9 (100)	6 (100)	5 (100)
papG	1 (1.3)	17 (58.6)	3 (14.3)	2 (10)	0 (0)	0 (0)	5 (55.6)	2 (33.3)	5 (100)
sfaS	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
hlyA	1 (1.3)	9 (31.0)	3 (14.3)	1 (5)	0 (0)	0 (0)	2 (22.2)	0 (0)	5 (100)
cnf1	0 (0)	7 (24.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (100)
iutA	36 (45.6)	26 (89.7)	11 (52.4)	10 (100)	10 (66.7)	11 (100)	5 (55.6)	4 (66.7)	5 (100)
iroN	8 (10.8)	0 (0)	0 (0)	0 (0)	1 (6.7)	0 (0)	3 (33.3)	0 (0)	5 (100)
fyuA	28 (35)	29 (100)	10 (47.6)	14 (70)	10 (66.7)	11 (100)	6 (66.7)	6 (100)	5 (100)
chuA	0 (0)	29 (100)	0 (0)	0 (0)	0 (0)	11 (24.4)	0 (0)	0 (0)	5 (100)
kpsM	4 (5.1)	29 (100)	17 (81)	11 (55)	0 (0)	11 (100)	6 (66.7)	3 (50)	5 (100)
pet	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
bfpA	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
astA	16 (23.3)	1 (3.4)	3 (14.3)	1 (5)	1 (6.7)	0 (0)	1 (11.1)	1 (16.7)	0 (0)

Values in the parentheses indicate the column percentage. n, number of isolates. Highlighted cells indicate a statistically significant association between the respective VAGs and high-risk STs ($p < 0.05$).

5.2.7.1 Phylogenetic relationships of high-risk clones of *E. coli* and virulence profiles

Figure 5.13 illustrates the phylogenetic relationships among 196 *E. coli* isolates belonging to selected high-risk STs, annotated with STs, phylogroups, sampling locations, and the presence or absence of virulence-associated genes. The phylogeny is based on core-genome alignments, with branch lengths representing evolutionary distances between isolates.

The inner ring denotes STs, with prominent representation of ST131, ST38, ST648, ST10, ST1193, and ST73. These STs form well-defined clades, consistent with their recognised clonal structure. In particular, ST131, ST1193, and ST73 appear as distinct and closely related clusters.

The middle ring indicates phylogroups, with phylogroups B2, A, D, and F being the most frequently observed. Phylogroup B2 is closely associated with ST131, ST1193, and ST73, which cluster together within the same major clade and well-known for harbouring numerous virulence factors. The outer ring shows sampling locations, with isolates from different subdistricts interspersed throughout the tree.

The outer heatmap layer displays the presence (purple) or absence (light purple) of virulence-associated genes. A high density of virulence genes is observed among isolates belonging to ST131 and phylogroup B2. Several isolates within ST73 and ST38 also exhibit substantial carriage of virulence genes. In contrast, isolates belonging to ST10 and ST648 show more variable and generally lower virulence gene content.

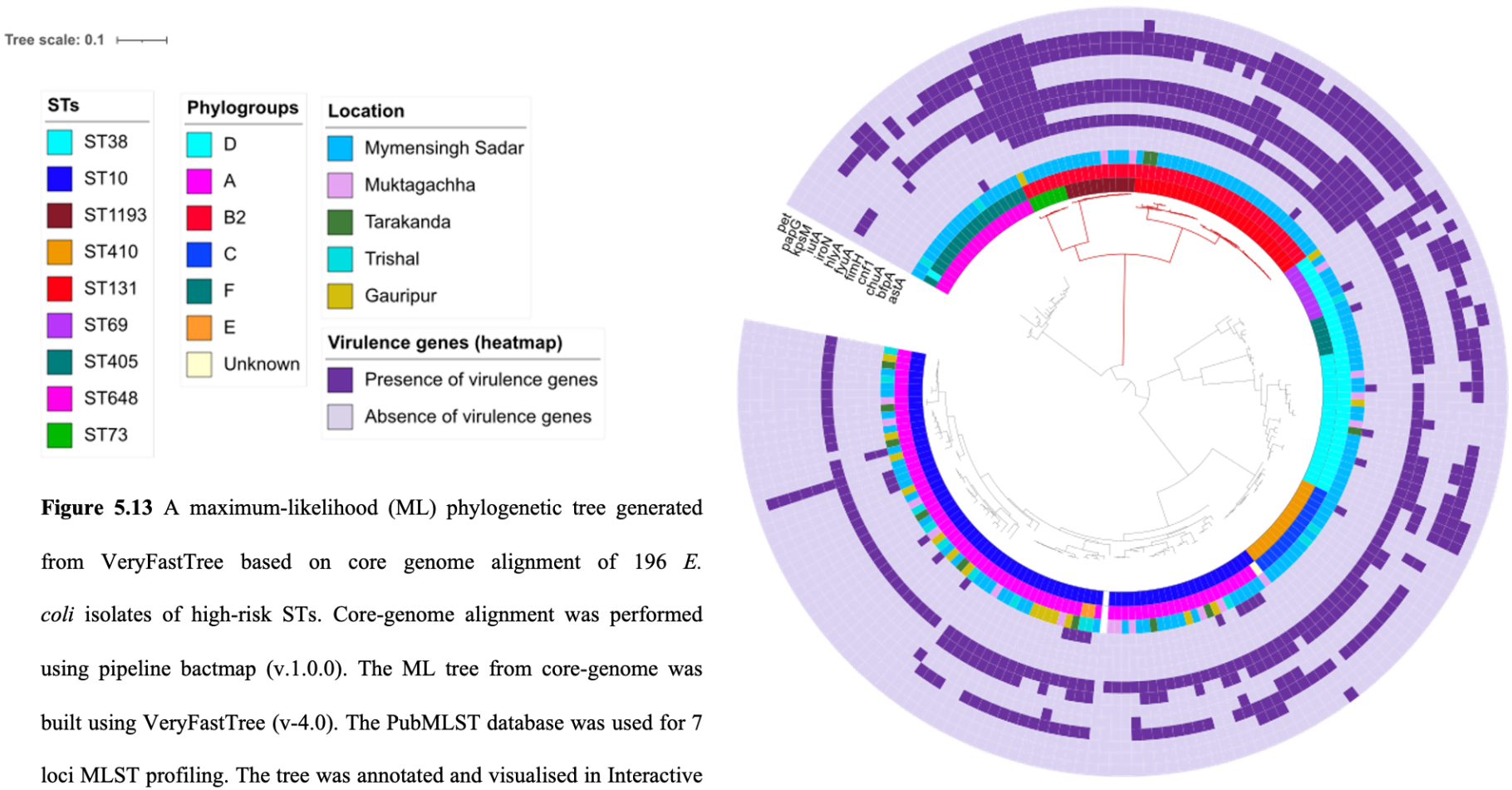


Figure 5.13 A maximum-likelihood (ML) phylogenetic tree generated from VeryFastTree based on core genome alignment of 196 *E. coli* isolates of high-risk STs. Core-genome alignment was performed using pipeline bactmap (v.1.0.0). The ML tree from core-genome was built using VeryFastTree (v-4.0). The PubMLST database was used for 7 loci MLST profiling. The tree was annotated and visualised in Interactive Tree of Life (iTOL, v-6).

This chapter shows a complex relationship between AMR, phylogenetic background, and virulence in *E. coli* isolates from Bangladesh. High-risk clones like ST131, ST648, and ST410 are linked to clinically important resistance genes such as $bla_{\text{CTX-M-15}}$, *aac(6')-Ib-cr*, and *mph*(A*)*. Conversely, $bla_{\text{NDM-5}}$ is widely distributed across various STs (Table 5.8). Dominant phylogroups, particularly B2 and D, are associated with both MDR and critical virulence genes (Table 5.4; Table 5.7). Diverse STs among the isolates from various sampling sources harboured different resistance genes. The co-occurrence of resistance and virulence traits in specific clones was observed in *E. coli* isolates.

5.3 Discussion

In this chapter, I have analysed the *E. coli* isolates identified in this study for their phylogenetic group distributions, major STs, resistance and virulence genes and their genetic relatedness. The distribution and ecological structure of *E. coli* phylogroups are influenced by multiple factors, including environmental conditions, bacterial characteristics in different regions under antibiotic usage and host genetic variability. In this study, the phylogenetic classification revealed that phylogroup A and phylogroup B1 were the predominant phylogroups, based on their frequency. In terms of source, environmental samples (water and flies) were predominantly classified within these two phylogroups. In a recent study in Bangladesh, they also observed the predominance of phylogroups A and B1 among the environmental isolates (Bhowmik *et al*., 2023). This also aligns with previous studies that have shown phylogroup B1 commonly persist in the environment, while phylogroup A is also considered well-adapted to diverse environmental conditions, contributing to their widespread distribution (Anastasi *et al*., 2012; Walk *et al*., 2007). In the case of CCS, DARS, and BF isolates, phylogroup B1 was the most prevalent, followed by phylogroup A. Conversely, in CMS, phylogroup A accounted for the highest proportion, followed by phylogroup B1. This finding is supported by a recent study in Bangladesh by Ali *et al*. (2023), who showed that 66.04% of their isolates from chicken meat samples belonged to phylogroup A (Ali *et al*., 2023).

Previous research has indicated that extraintestinal pathogenic *E. coli* strains in humans are predominantly associated with phylogroup B2, with a smaller proportion linked to group D, while intestinal pathogenic and commensal strains are commonly found in phylogroups A and B1 (Halaji *et al*., 2022; Lee *et al*., 2016). In the present study, commensal *E. coli* isolated from HRS were primarily classified into phylogroups A and B1, consistent with their typical distribution. In contrast, uropathogenic *E. coli* (UPEC) isolates were mainly assigned to phylogroup B2, followed by B1 and A. Additionally, strains isolated from SSI were

distributed across phylogroups A, D, B1, and B2 (Table 5.1), a pattern that closely aligns with findings from a study conducted in Bangladesh, which reported similar phylogroup prevalence among extraintestinal *E. coli* (Mazumder *et al*., 2020; Khan *et al*., 2018).

Diverse STs were identified in this study, with ST10 being the most frequent, followed by ST155. ExPEC *E. coli* is responsible for a vast majority of human extraintestinal infections, such as UTI and SSI. ST131 is the most common ST for ExPEC from approximately 2000 onwards and encompasses an expansive geographic region, as identified in previous studies (Mazumder *et al*., 2020; Manges *et al*., 2019). In present study, ST131 was exclusively detected in human-derived isolates, predominantly from clinical sources, UTIs and SSIs, and to a lesser extent, from HRS isolates. It was the most frequently occurring ST among UTI and SSI isolates, in agreement with previous studies conducted in Bangladesh and other regions globally (Feng *et al*., 2025; Mazumder *et al*., 2022; Jain *et al*., 2021). The distribution of sequence types across sources further reinforces the One Health relevance of this study. ST10 was the only clone detected across all sectors, consistent with its recognised role as a ubiquitous commensal and opportunistic lineage capable of persisting in humans, animals, and the environment. In contrast, ST131 was exclusively associated with human clinical and colonisation samples, reflecting its adaptation to the human host and its strong association with extraintestinal infections. This contrast illustrates how some lineages act as cross-sectoral generalists, while others remain host-adapted specialists, shaping the routes by which resistance and virulence traits may disseminate within One Health systems.

All ST131 isolates identified in this study belonged to phylogroup B2, which is recognised for its high virulence potential. Although phylogroup B2 is widely associated with extraintestinal pathogenic *E. coli* and clinical antimicrobial resistance (Clermont *et al*., 2013), no carbapenemase-producing isolates were identified within this phylogroup. Instead,

carbapenemase genes such as *bla*$_{\text{NDM-5}}$ were detected predominantly in other phylogroups, reflecting previous observations that carbapenem resistance is often driven by plasmid-mediated acquisition in diverse genetic backgrounds rather than being confined to traditionally virulent lineages.

In addition to ST131, other notable high-risk clones such as ST648, ST38, and ST410 were also common among SSI isolates. These STs have been implicated in global ExPEC transmission and are known for their environmental resilience. Interestingly, these same STs were also detected in environmental samples in this study, suggesting their potential for cross-host transmission and environmental persistence. Meanwhile, sequence types ST405, ST1193, and ST73 were also exclusively found in human isolates. ST1193 and ST73 belonged to the virulent phylogroup B2, while ST405 was part of phylogroup D, both groups being associated with extraintestinal virulence (Kocsis *et al*., 2022). ST10 was also detected in human clinical and rectal swab samples, as well as in water, flies, CCS, CMS, DARS, and BF isolates, indicating its wide host range and clonal dissemination among hosts. The detection of these clones in non-human and environmental sources in the current study underscores a broader pattern of dissemination beyond healthcare settings, supporting global concerns about the environmental persistence and zoonotic potential of ExPEC.

In this study, *bla*$_{\text{CTX-M-15}}$ was identified as the predominant genetic determinant responsible for ESBL production. Among SSI isolates, 79.8% carried *bla*$_{\text{CTX-M-15}}$, whereas 36.6% of UTI isolates were positive for the same gene. In contrast, the prevalence was notably lower in environmental sources, with 10.4% detected in water samples, 8.2% in flies, and 6.6% in samples from HRS. The high prevalence of *bla*$_{\text{CTX-M-15}}$ in clinical isolates is consistent with previous reports from Bangladesh (Jain *et al*., 2021; Mazumder *et al*., 2020; Khan *et al*., 2018), as well as global studies (Runcharoon *et al*., 2025; Sumbana *et al*., 2021), which similarly

identified *bla*$_{\text{CTX-M-15}}$ as the dominant ESBL gene. These findings highlight the continued dominance of *bla*$_{\text{CTX-M-15}}$ in clinical *E. coli* isolates. *bla*$_{\text{CTX-M-15}}$ was detected in water isolates (10.4%) and isolates from flies (8.2%), indicating the possible transmission of resistance genes between humans and the environment (Hu *et al*., 2013). No *bla*$_{\text{CTX-M-15}}$ was detected in chicken samples, but *bla*$_{\text{CTX-M-55}}$ was detected in chicken cloacal swab isolates, aligning with findings from a study in China (Liu *et al*., 2022), where *bla*$_{\text{CTX-M-55}}$ was reported as the predominant ESBL gene in poultry. This variant is widely distributed in Asia and Southeast Asia and is now emerging in global surveillance reports**,** underscoring its growing significance. The detection of ESBL-producing *E. coli* in chickens poses a potential public health risk, as these resistance genes can be transmitted to humans through multiple pathways, including environmental contamination**,** direct animal contact**,** and the food supply chain**.** Such transmission is particularly concerning because it can contribute to MDR infections in humans, limiting treatment options for conditions such as UTIs and SSIs.

The *bla*$_{\text{CTX-M-15}}$ genes detected in isolates of diverse genetic backgrounds, affiliated with seven different phylogroups and diverse STs, including the high-risk clones ST131, ST10, ST38, ST69, ST410, ST1193, ST648, ST73, and ST405, harboured a significantly higher number of *bla*$_{\text{CTX-M-15}}$ than other STs and 75% of ST131 isolates harboured *bla*$_{\text{CTX-M-15}}$ which is aligned with a study in Bangladesh by Mazumder (Mazumder *et al*., 2021; Mazumder *et al*., 2020).

In the present study, *bla*$_{\text{NDM-5}}$ emerged as the predominant carbapenem resistance gene**,** detected across both clinical and environmental isolates. Notably, more than half of the *bla*$_{\text{NDM-5}}$-positive isolates originated from SSI samples, with only a single isolate identified from an HRS isolate. The gene was distributed among multiple sequence types, including ST38, ST167, ST648, ST10, ST405, and ST155**.** These findings are consistent with those reported by Khan *et al*., 2018, who documented the presence of *bla*$_{\text{NDM-5}}$ in *E. coli* clinical isolates from Mymensingh, the same district as the current study. In both studies, ST167 and

ST38 were prominent carriers of *bla*$_{NDM-5}$, suggesting the ongoing persistence and local circulation of this resistance determinant in the region. Other reports from Bangladesh have also highlighted the emergence of *bla*$_{NDM-5}$ in clinical *E. coli* isolates, underscoring its growing epidemiological significance. In this study, the significant prevalence of *bla*$_{NDM-5}$ in SSI isolates reflects its direct involvement in hospital-acquired infections. The detection of *bla*$_{NDM-5}$ in water indicates the potential for environmental transmission to human infection or bidirectional gene flow.

Among the widespread distribution of *bla*$_{NDM-5}$, *bla*$_{NDM-1}$ was identified in a limited number of isolates, specifically those originating from flies and SSI samples. Meanwhile, *bla*$_{OXA-181}$ was detected in isolates from water, HRS and SSI. Notably, three isolates exhibited co-occurrence of *bla*$_{NDM-5}$ and *bla*$_{OXA-181}$, all of which were recovered from water and HRS samples. One isolate presented co-occurrence of *bla*$_{NDM-5}$ and *bla*$_{NDM-20}$. This co-presence highlights the possibility of horizontal gene transfer and suggests the emergence of multi-resistance traits within environmental reservoirs. Although previous studies in Bangladesh have documented *bla*$_{NDM-1}$ and *bla*$_{OXA-181}$ primarily in clinical settings (Farzana *et al*., 2023; Khatun & Shamsuzzaman, 2016), a notable gap remains in the literature regarding their occurrence in environmental sources. Only a few recent investigations, such as the study by Nisa *et al*. (2024), have begun to address the environmental dimension of carbapenem resistance genes (Nisa *et al*., 2024). The absence of carbapenemase genes such as *bla*$_{NDM}$ in animal and poultry isolates, alongside the limited detection of third-generation cephalosporin resistance genes (e.g. *bla*$_{CTX-M}$), likely reflects differences in antimicrobial usage between sectors in Bangladesh. Carbapenems and third-generation cephalosporins are mainly used in human clinical settings but are not commonly administered in livestock or poultry production (Ahmed *et al*., 2019; WHO, 2017). This sector-specific selective pressure suggests that dissemination

of these resistance determinants is currently driven primarily by human healthcare-associated antibiotic use rather than animal reservoirs (Imam *et al*., 2020; Hosain *et al*., 2021).

Colistin resistance was primarily attributed to the presence of *mcr1.1*, which was detected in only three isolates originating from CCS and BF samples in this study. Compared to previous reports from Bangladesh, where *mcr1* prevalence ranged from 25% to 58% in chicken meat, gut, and faeces (Ali *et al*., 2023; Ahmed *et al*., 2020; Islam *et al*., 2020), the prevalence observed in this study is considerably lower. Although *mcr1.1* has not yet been reported from free-flying bird faeces in Bangladesh, global evidence suggests its presence in migratory and wild birds, indicating the potential for avian dissemination across regions (Munir *et al*., 2025; Zhang *et al*., 2021).

In comparison to the previous studies from Bangladesh, which reported relatively high rates of *mcr* gene carriage and colistin resistance, particularly in poultry and retail meat samples, present study observed a notably low prevalence of both *mcr* genes and phenotypic resistance to colistin. This discrepancy may be attributed to several factors. Firstly, the sample types and sources used in present study, such as clinical or environmental isolates, differ from prior investigations that predominantly focused on poultry gut flora or livestock-associated isolates, where colistin use was more common (Ahmed *et al*., 2020). Secondly, geographic variation in antimicrobial usage practices may have contributed to the lower resistance rates observed; the farms selected for this study did not use colistin during and before sample collection (Table 4.7), while previous studies reported the use of colistin in the sampling farms (Ahmed *et al*., 2020; Islam *et al*., 2020). Additionally, the sampling in this study was conducted more recently, at a time when regulatory measures to limit colistin use, such as the 2022 ban imposed by the Directorate General of Drug Administration (DGDA) on its veterinary sector, were likely just beginning to take effect. These measures may have contributed to a reduction in selective

pressure for colistin resistance. Taken together, these findings suggest that, although colistin resistance remains a public health concern, its lower prevalence in this study may indicate early signs of progress resulting from antimicrobial stewardship efforts and the implementation of national policy.

In this study, *tet*(X4) genes were detected in eight isolates from diverse sources, including flies, water, and CMS, which underscores the widespread environmental dissemination of resistance to tetracyclines, particularly the newer-generation antibiotic tigecycline. The presence of *tet*(X4), a gene capable of degrading tigecycline, raises concerns due to its plasmid-mediated mobility, which facilitates horizontal gene transfer across ecological boundaries. The occurrence of this gene in flies and water suggests possible contamination routes through poor waste management. Its detection in chicken meat swabs indicates that foodborne transmission could be a potential public health risk. Although studies on *tet*(X4) in Bangladesh remain limited, recent genomic surveillance has highlighted the emergence of *tet*(X4) in poultry-associated *E. coli* (Davies *et al*., 2024). The presence of *tet*(X4) in water and fly samples has not been reported from Bangladesh, emphasising the need for integrated One Health surveillance to monitor and control the spread of this critical resistance determinant across human, animal, and environmental sectors. The detection of *tet*(X) exclusively in poultry-associated samples, water and flies, but not in human isolates, likely reflects indirect selection driven by widespread tetracycline use in poultry farming rather than tigecycline exposure itself. Tigecycline is a last-resort antibiotic primarily used in severe human infections and is not approved for use in animal production, whereas older tetracyclines are extensively used in poultry (Pan *et al*., 2020).

The comprehensive analysis of virulence gene distribution across different sources, phylogroups, and STs of *E. coli* highlights the complex interplay between genetic

backgrounds, ecological niches, and pathogenic potential. In this study, *fimH, papG, sfaS, hlyA, cnf1, astA, kpsM, iutA, fyuA, and iroN* were the most frequently identified across the sampling sources. However, most of these virulent genes were significantly more prevalent in the clinical isolates than in the other sources. Environmental and animal-associated *E. coli* isolates, particularly those obtained from water, flies, CCS and CMS, demonstrated limited virulence profiles, with a consistent presence of basic colonization factors like *fimH*. The high prevalence of *fimH* among CCS (95.4%) and CMS (92.2%) isolates aligns with findings from a study conducted in Bangladesh, where *fimH* was detected in 100% of *E. coli* isolates derived from chicken samples (Azim *et al*., 2021; Saha *et al*., 2020a), reinforcing its role as a ubiquitous adhesin across both commensal and pathogenic strains. Additionally, the relatively high prevalence of iron acquisition genes such as *iutA* and *iroN*, along with the enterotoxin gene *astA*, in chicken isolates in the current study mirrors observations from previous reports in Bangladesh and global studies. These studies also reported notably high frequencies of *astA* among avian *E. coli* strains (Islam *et al*., 2024; Hussain *et al*., 2022; Ahmed *et al*., 2020 suggesting that while avian and environmental isolates may lack many classical ExPEC virulence factors, they often harbour genes that facilitate persistence and colonisation in host-associated environments.

The Shigatoxin-producing gene *stx2A/B* was found exclusively in DARS isolates, with a significantly higher percentage than in other sources. This finding is supported by a study in Bangladesh, which reported that 40% of *E. coli* from domestic animal rectal swabs carried the *stx2* gene (Parvej *et al*., 2020). In contrast, present study found a percentage of 15.3%. The difference in percentages may be due to the sampling methods. In the referenced study, a wider area was included, and the authors did not specify whether the animals were healthy or diseased. In contrast, this research focused on aparently healthy animals.

E. coli isolates recovered from flies and water sources predominantly carried virulence genes, including *fimH*, *iutA*, *iroN*, *kpsM*, and *astA*. These genes are commonly associated with adhesion, iron acquisition, and enterotoxin production, suggesting a baseline level of virulence potential even in environmental reservoirs. Similar findings were reported in a recent study from India, where waterborne *E. coli* isolates exhibited a comparable virulence gene profile (Saini *et al*., 2024). The parallel patterns observed between the two countries may be influenced by shared environmental and infrastructural factors, particularly related to sanitation and waste disposal practices. In both Bangladesh and India, inefficient waste management systems likely contribute to the contamination of surface water and the proliferation of *E. coli* strains harbouring such virulence traits.

Isolates from human clinical infections, particularly from UTIs, SSIs, and HRS, harboured a broader array of virulence factors, including iron acquisition systems (*iutA*, *fyuA*), toxins (*hlyA*, *cnf1*), and capsular genes (*kpsM*, *chuA*), reflecting their higher pathogenic potential. This study showed that isolates from UTI harboured most of the virulence genes, and these were significantly higher in percentage than those of other isolates. The high virulence potential of UPEC is also supported by global studies (Chagneau *et al*., 2023; Kim *et al*., 2022; Mazumder *et al*., 2020).

Phylogenetic analysis revealed that these virulence traits were not randomly distributed but strongly associated with specific phylogroups. Phylogroup B2 was found harbouring multiple virulence genes, including *papG*, *hlyA*, *cnf1*, *fyuA*, *iutA*, *chuA*, and *kpsM*, aligning with previous literature that identifies B2 and D as dominant ExPEC lineages (Qasemi *et al*., 2022; Hyun *et al*., 2021; Mazumder *et al*., 2020). Phylogroups A and B1, often associated with commensal or environmental isolates, lacked most of these genes. Interestingly, phylogroup G, which makes up 48% of isolates from HRS, despite being smaller in number, showed a unique

virulence gene pattern, with 100% *fimH* positivity and relatively high carriage of *iutA* and *fyuA*, indicating an emerging pathogenic potential in healthy individuals that may serve as carriers of these virulence genes. These findings emphasise the One Health relevance of this study, demonstrating that environmental and animal reservoirs can harbour *E. coli* with virulence potential that intersects with human clinical populations. Such interconnected reservoirs may facilitate the circulation and evolution of pathogenic *E. coli* at the human–animal–environment interface.

At the sequence type level, ST131 and ST73 were identified as the most virulent lineages, carrying a variety of ExPEC-associated genes and representing classic high-risk clones. ST131, in particular, showed high frequencies of *papG*, *iutA*, *fyuA*, *chuA*, and *kpsM*, supporting its global dissemination and success in clinical settings, which aligns with the studies in Bangladesh as well as internationally (Sung *et al*., 2024; Zou *et al*., 2023; Kim *et al*., 2022; Jain *et al*., 2021). ST10, despite being among the most prevalent lineages, showed low levels of virulence genes, aligning with its typical commensal or environmental backgrounds. The detection of virulence factors in lineages like ST648 and ST405 indicates ongoing genetic diversification.

A limitation of the present analysis is that genomic investigations focused on selected high-risk clones based on global and local studies and existing literature, rather than the full diversity of *E. coli* lineages identified, despite whole-genome sequencing being performed for all isolates. As a result, dominant clones such as ST167, which represented the most prevalent $bla_{\text{NDM-5}}$ carrying lineage in this dataset, were not included in detailed phylogenetic and virulence analyses. Future work should specifically address the genomic context and transmission dynamics of bla_{NDM}-positive ST167 isolates.

Despite the limitations, this chapter demonstrates that the clonal expansion of specific *E. coli* lineages strongly drives the dissemination of AMR in Bangladesh. A limited number of STs, including ST10, ST155, ST1193, ST38, ST131, and ST648, accounted for a large share of isolates across human, animal, and environmental sources, highlighting their ecological versatility and capacity for inter-host transmission. Phylogenetic analyses further revealed the clustering of isolates from diverse niches within the same clonal lineages, indicating ongoing clonal spread at the human-animal environment interface. The convergence of globally recognised high-risk clones (e.g., ST131, ST648, ST410) with resistance determinants such as $bla_{\text{CTX-M-15}}$, $bla_{\text{NDM-5}}$, and *mcr-1* underscores the role of clonal dissemination in shaping the AMR landscape. These findings reinforce the importance of monitoring both resistance gene flow and clonal dynamics to understand and contain the spread of AMR in LMICs.

Chapter 6

Horizontal gene transfer of *bla*$_{\text{NDM-5}}$ and *tet*(X4) in *Escherichia coli*: Plasmid characterisation

6.1 Introduction

Plasmids are extrachromosomal, self-replicating DNA molecules that play a central role in bacterial adaptation and evolution. They often carry accessory genes that are not essential for basic survival but confer selective advantages, such as AMR or virulence traits. Plasmids are categorised into replicon types based on their replication initiation and control systems, typically defined by incompatibility (Inc) groups. Each replicon type determines the plasmid's stability, host range, and compatibility with other plasmids within the same bacterial cell (Chen *et al*., 2024; Dewan and Uecker, 2023).

Plasmids serve as key vehicles for the dissemination of ARGs, primarily through conjugation, which is the dominant and most efficient mechanism of plasmid-mediated horizontal gene transfer in bacterial populations. In contrast, plasmid transfer via transformation or transduction occurs only rarely and is considered to play a minor role in the spread of resistance. Broad-host-range plasmids, particularly IncF and IncX, are highly efficient in transferring clinically relevant resistance genes across diverse bacterial hosts. Multi-replicon plasmids, which carry more than one replication system, may further enhance plasmid stability, persistence, and transferability (Chen et al., 2024; Tao et al., 2022). IncF plasmids, which are widely distributed in Enterobacteriaceae, function as conjugative multi-replicon elements that facilitate efficient horizontal gene transfer across species. Their mosaic genetic architecture allows them to replicate in a broad range of hosts and plays a central role in bacterial adaptation and evolution (Ruzickova *et al*., 2025). In contrast, Col-type plasmids are typically small, non-conjugative, high-copy-number elements with a narrow host range. Although they lack complete conjugation machinery, they may carry resistance or virulence genes and can be mobilised in the presence of co-resident conjugative plasmids, contributing to the maintenance and spread of antimicrobial resistance (Partridge *et al*., 2018; Frost *et al*., 2005; Carattoli, 2009). With the

advent of WGS, it has become possible to track this plasmid diversity with high precision, revealing not only their structural complexity but also the resistance genes they mobilize. Oxford Nanopore Technology are the confirmatory approach for plasmid characterisation and tools such as PlasmidFinder are crucial for identifying plasmid replicon types, providing insights into their evolution, mobility, and epidemiological significance (Carattoli and Hasman, 2020; Conlan *et al*., 2014).

Carbapenems are considered last-resort antibiotics, making resistance to them a significant clinical challenge, especially in treating infections caused by ESBL bacteria. (Hossain *et al*., 2020). Resistance to carbapenem has emerged globally due to the acquisition of carbapenemase genes often located on highly mobile plasmids (Logan & Weinstein, 2017). The first New Delhi metallo-β-lactamase (NDM-1) was reported in 2008 from a *Klebsiella pneumoniae* isolate in India, and since then carbapenemase-producing Enterobacterales have disseminated rapidly across regions (Kumarasamy *et al*., 2020; Yong *et al.,* 2009). Carbapenem-resistant *E. coli* has increased rapidly in recent decades, posing a serious global health threat (Huang *et al*., 2024). Resistance is primarily driven by three major carbapenemases: NDM, KPC, and OXA-48. Among these, NDM-producing strains are particularly common in South Asia (Logan & Weinstein, 2017).

Since its first description in 2008, the New Delhi metallo-β-lactamase (NDM) family has diversified rapidly, with 67 variants identified in Enterobacterales and other Gram-negative pathogens (Al-Marzooq *et al*., 2024; Wu *et al*., 2019). These variants differ in their amino acid substitutions, which can influence enzymatic activity, substrate specificity, and dissemination potential. Among identified NDM variants, NDM-1 and NDM-5 are the most widespread, with NDM-5 exhibiting greater carbapenemase activity than NDM-1 (Yin *et al*., 2020). The $bla_{\text{NDM-5}}$ gene, mainly hosted by *E. coli*, has spread extensively across bacterial species and regions,

largely via plasmid-mediated horizontal transfer from diverse environmental and geographical sources (Liu *et al*., 2023).

Carbapenem-resistant *E. coli*, particularly NDM-5-producing strains, represent a critical public health concern in Bangladesh, where their emergence in clinical settings severely restricts therapeutic options and is associated with high mortality rates (Farzana *et al*., 2023). NDM-5 variants are particularly concerning because they often co-exist with additional resistance genes on mobile plasmids, facilitating their rapid spread within hospitals. A study from Bangladesh has reported the occurrence of $bla_{\text{NDM-5}}$ in *E. coli* UTIs, highlighting serious clinical consequences. In one study, *E. coli* harbouring $bla_{\text{NDM-5}}$ led to extensively drug-resistant UTIs with limited therapeutic options (Hossain *et al*., 2020). Another investigation by Farzana *et al* documented neonatal sepsis cases where $bla_{\text{NDM-5}}$-positive *E. coli* infections were associated with prolonged hospital stays and increased treatment failure (Farzana *et al*., 2023).

While clinical isolates have traditionally been considered the primary reservoirs of ARGs, aquatic environments (wastewater, agricultural fields and different water sources) are increasingly recognised as important pathways for their dissemination. (Li *et al*., 2021). In addition to water, flies, especially those associated with human and animal waste, serve as mobile reservoirs and vectors of ARGs, transporting resistant bacteria across environmental, agricultural, and clinical settings (Yin *et al*., 2022). Compared with clinical isolates, relatively few studies have explored the detailed genetic features and mobile genetic elements of carbapenem-resistant *E. coli* from environmental waters or insect vectors. Notably, $bla_{\text{NDM-5}}$ has been reported in environmental reservoirs across Asia, including its detection in wastewater in Bangladesh (Flatgard *et al*., 2024; Nisa *et al*., 2024; Mazumder *et al*., 2021).

Tigecycline and colistin are regarded as the final options for treating infections caused by multidrug-resistant Enterobacterales (Sun *et al*., 2019). However, the ongoing appearance of plasmid-borne resistance genes such as bla_{NDM} for carbapenems and *mcr-1* for colistin has

made these antibiotics less effective in clinical practice. Consequently, tigecycline has emerged as a vital last-resort treatment in such cases (Li *et al*., 2023). In this state, the emergence of plasmid-mediated tigecycline resistance genes, particularly *tet*(X) variants, represents a serious threat to the clinical utility of this drug. Among the identified *tet*(X) family members, *tet*(X4) has drawn global concern due to its ability to confer high-level tigecycline resistance and its rapid dissemination across bacterial hosts, especially *E. coli*, through conjugative plasmids (Sun *et al*., 2019). Since its first report in China in 2019, *tet*(X4) has been increasingly detected in both clinical and agricultural settings, often co-localized with other clinically relevant resistance determinants, which may facilitate multidrug resistance and limit treatment options (Jiang *et al*., 2024).

Relatively few studies have examined the genetic platforms of *tet*(X4)-positive *E. coli* in Bangladesh. In Bangladesh, *tet*(X4) has recently been detected in *E. coli* isolates from poultry meat swabs (Davies *et al*., 2024). In the present study, *tet*(X4) was identified in *E. coli* from multiple non-clinical sources, including environmental water, flies from hospital environments, and chicken meat swabs.

In this chapter, I have focused on determining the mode of transmission of $bla_{\text{NDM-5}}$ and *tet*(X4). Specifically, the following objectives are sought:

1. To investigate the source distribution, phylogenetic groups, sequence types, and antimicrobial resistance profiles of $bla_{\text{NDM-5}}$–positive *E. coli*, and to characterise the plasmids carrying $bla_{\text{NDM-5}}$ and their potential horizontal transmission routes.

2. To investigate the source distribution, phylogenetic groups, sequence types, and antimicrobial resistance profiles of *tet*(X4)–positive *E. coli*, and to characterise the plasmids carrying *tet*(X4) and their potential horizontal transmission routes.

6.2 Results

6.2.1 Plasmid replicon types identified in *E. coli* isolates in this study

Whole-genome sequencing data and *in silico* plasmid replicon typing, using PlasmidFinder, were employed to identify the plasmid replicons in this study from 1634 *E. coli* isolates. In total, 66 types of plasmid replicon were identified, and according to their frequency, the replicon types are shown in Figure 6.1. These included a wide variety of Inc (incompatibility) types and Col types of plasmids

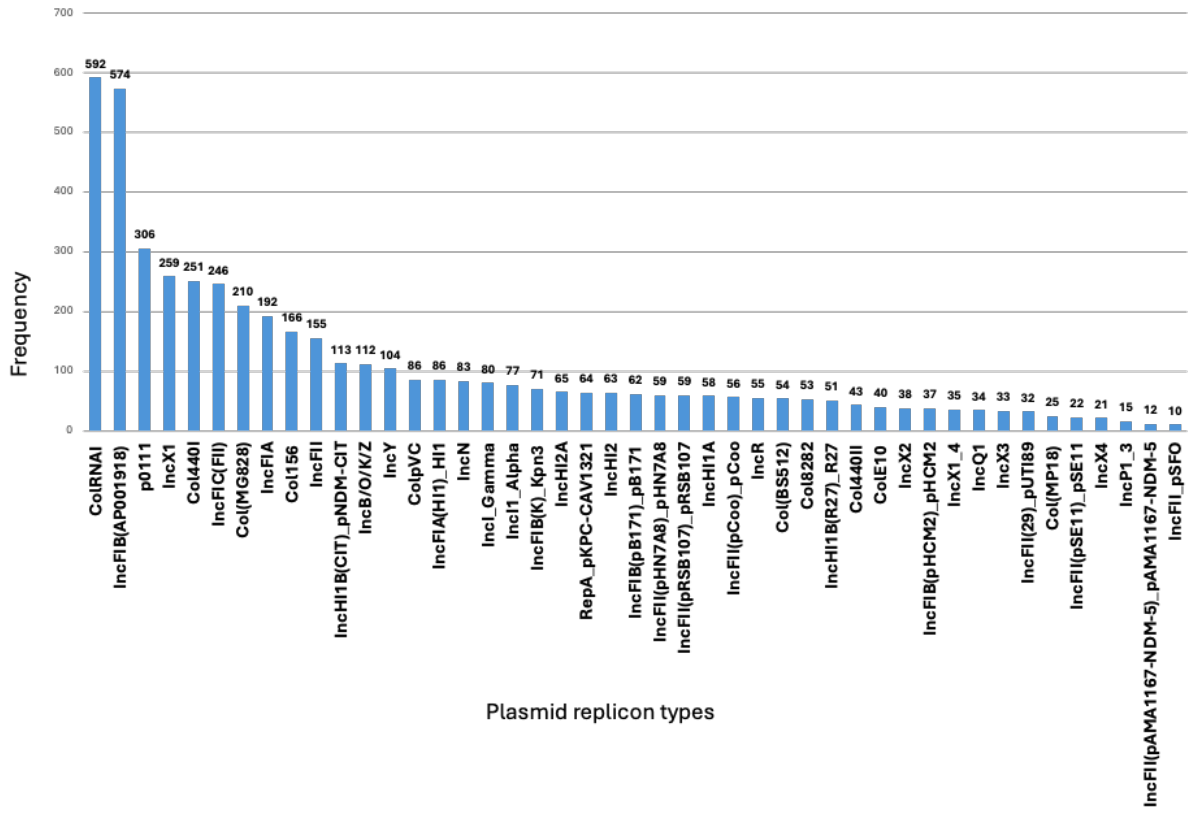
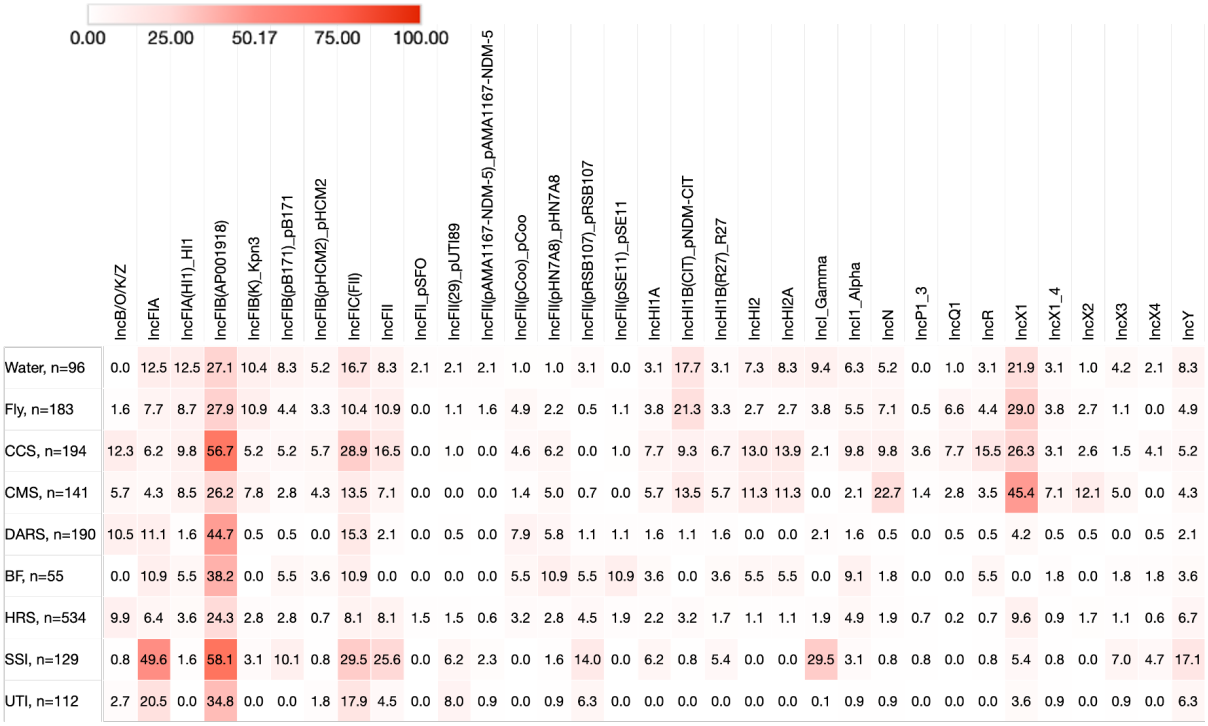


Figure 6.1 Frequency of plasmid replicon types detected among *E. coli* isolates from this study.

The source-wise distribution of the plasmid replicons is illustrated in Figures 6.2 (Inc-type) and 6.3 (Col-type).

IncFIB, IncX1, IncFIA, IncFIC and IncFII were found to be more prevalent and distributed across different sources. IncFIB(AP001918) was found to be the most prevalent distributed across all the sources, being the highest prevalent particularly in CCS (56.7%) and in SSI (58.1%). IncX1 was also prevalent, being found in 45.4% of CMS isolates, and IncFIA was identified in 49.6% of SSI isolates.

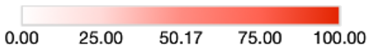


	IncB/O/K/Z	IncFIA	IncFIA(HI1)_HI1	IncFIB(AP001918)	IncFIB(K)_Kpn3	IncFIB(pB171)_pB171	IncFIB(pHCM2)_pHCM2	IncFIC(FII)	IncFII	IncFII_pSFO	IncFII(29)_pUTI89	IncFII(pAMA1167-NDM-5)_pAMA1167-NDM-5	IncFII(pCoo)_pCoo	IncFII(pHN7A8)_pHN7A8	IncFII(pRSB107)_pRSB107	IncFII(pSE11)_pSE11	IncHI1A	IncHI1B(CIT)_pNDM-CIT	IncHI1B(R27)_R27	IncHI2	IncHI2A	IncI_Gamma	IncI1_Alpha	IncN	IncP1_3	IncQ1	IncR	IncX1	IncX1_4	IncX2	IncX3	IncX4	IncY
Water, n=96	0.0	12.5	12.5	27.1	10.4	8.3	5.2	16.7	8.3	2.1	2.1	2.1	1.0	1.0	3.1	0.0	3.1	17.7	3.1	7.3	8.3	9.4	6.3	5.2	0.0	1.0	3.1	21.9	3.1	1.0	4.2	2.1	8.3
Fly, n=183	1.6	7.7	8.7	27.9	10.9	4.4	3.3	10.4	10.9	0.0	1.1	1.6	4.9	2.2	0.5	1.1	3.8	21.3	3.3	2.7	2.7	3.8	5.5	7.1	0.5	6.6	4.4	29.0	3.8	2.7	1.1	0.0	4.9
CCS, n=194	12.3	6.2	9.8	56.7	5.2	5.2	5.7	28.9	16.5	0.0	1.0	0.0	4.6	6.2	0.0	1.0	7.7	9.3	6.7	13.0	13.9	2.1	9.8	9.8	3.6	7.7	15.5	26.3	3.1	2.6	1.5	4.1	5.2
CMS, n=141	5.7	4.3	8.5	26.2	7.8	2.8	4.3	13.5	7.1	0.0	0.0	0.0	1.4	5.0	0.7	0.0	5.7	13.5	5.7	11.3	11.3	0.0	2.1	22.7	1.4	2.8	3.5	45.4	7.1	12.1	5.0	0.0	4.3
DARS, n=190	10.5	11.1	1.6	44.7	0.5	0.5	0.0	15.3	2.1	0.0	0.5	0.0	7.9	5.8	1.1	1.1	1.6	1.1	1.6	0.0	0.0	2.1	1.6	0.5	0.0	0.5	0.5	4.2	0.5	0.5	0.0	0.5	2.1
BF, n=55	0.0	10.9	5.5	38.2	0.0	5.5	3.6	10.9	0.0	0.0	0.0	0.0	5.5	10.9	5.5	10.9	3.6	0.0	3.6	5.5	5.5	0.0	9.1	1.8	0.0	0.0	5.5	0.0	1.8	0.0	1.8	1.8	3.6
HRS, n=534	9.9	6.4	3.6	24.3	2.8	2.8	0.7	8.1	8.1	1.5	1.5	0.6	3.2	2.8	4.5	1.9	2.2	3.2	1.7	1.1	1.1	1.9	4.9	1.9	0.7	0.2	0.7	9.6	0.9	1.7	1.1	0.6	6.7
SSI, n=129	0.8	49.6	1.6	58.1	3.1	10.1	0.8	29.5	25.6	0.0	6.2	2.3	0.0	1.6	14.0	0.0	6.2	0.8	5.4	0.0	0.0	29.5	3.1	0.8	0.8	0.0	0.8	5.4	0.8	0.0	7.0	4.7	17.1
UTI, n=112	2.7	20.5	0.0	34.8	0.0	0.0	1.8	17.9	4.5	0.0	8.0	0.9	0.0	0.9	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.9	0.9	0.0	0.0	0.0	3.6	0.9	0.0	0.9	0.0	6.3

Figure 6.2 Heatmap showing distribution of Inc-type plasmid replicons of *E. coli* across different sources.

Each cell represents the percentage (%) of isolates within a source that carries the respective replicon types. Darker shades of red indicate higher percentage rates, while lighter shades indicate lower rates, and white indicates. Differences in replicon distribution across sources were assessed using the chi-square test. Replicon frequencies of 10 or more are displayed in the figure.

ColRNAI was the most prevalent replicon among the Col-type plasmid replicons and was found to be distributed across all the sources. Col440I was mostly found in environmental and chicken isolates. Col(MG828) was prevalent in water, CCS and SSI isolates.



	Col(BS512)	Col(IRGK)	Col(MG828)	Col(MP18)	Col(Ye4449)	Col156	Col3M	Col440I	Col440II	Col8282	ColE10	ColKP3	ColpVC	ColRNAI
Water, n=96	5.2	4.2	19.8	0.0	0.0	3.1	0.0	28.1	2.1	1.0	6.3	1.0	12.5	46.8
Fly, n=183	0.5	1.6	6.9	0.5	0.0	3.3	1.1	24.6	4.4	3.3	6.0	0.0	10.4	43.1
CCS, n=194	0.0	0.0	32.0	2.1	0.0	21.6	0.0	18.6	6.7	9.8	1.5	0.0	10.3	64.9
CMS, n=141	0.7	0.7	12.1	2.8	0.0	11.3	0.0	34.8	4.3	3.5	9.2	0.0	10.6	47.5
DARS, n=190	0.0	0.0	6.3	4.7	0.5	12.1	0.0	5.8	1.1	1.1	0.0	0.0	0.0	34.7
BF, n=55	0.0	0.0	5.5	0.0	0.0	0.0	0.0	14.5	0.0	1.8	0.0	0.0	0.0	20.0
HRS, n=534	3.2	0.0	6.0	1.1	0.0	6.2	0.0	10.5	1.1	1.9	1.1	0.0	3.2	25.7
SSI, n=129	18.6	0.0	20.2	0.0	0.0	0.9	0.0	12.4	4.7	4.7	0.8	2.3	2.3	38.0
UTI, n=112	5.4	0.0	7.1	0.9	0.0	14.3	0.0	2.7	0.0	2.7	0.0	0.0	0.0	10.7

Figure 6.3 Heatmap showing distribution of Col-type plasmid replicons of *E. coli* across different sources.

Each cell represents the percentage (%) of isolates within a source that carries the respective replicon types. Darker shades of red indicate higher percentage rates, while lighter shades indicate lower rates. Differences in replicon distribution across sources were assessed using the chi-square test.

The diversity of replicon types was observed to be more pronounced in environmental and CCS, CMS isolates than in clinical isolates; however, clinical isolates still carried high frequencies of clinically important plasmids, such as IncFIB and IncFII**.**

While a wide diversity of plasmid replicon types was detected in the *E. coli* isolates, particular attention is warranted for those plasmids that harbour clinically significant AMR genes, especially *bla*$_{\text{NDM-5}}$ and *tet*(X4).

6.2.2 Clonal distribution, resistance profile and plasmid background of *bla*$_{\text{NDM-5}}$

A total of 27 *E. coli* isolates harbouring the *bla*$_{\text{NDM-5}}$ gene were identified from diverse sources, including environmental (n=4 from water, n=2 from flies) and human (n=21 from human infections and colonisation) origins. Among clinical isolates, SSI were the most common source (n=16), followed by UTI (n=4) and one isolate was identified from HRS (Table 6.1).

The isolates represented a wide range of STs**,** with ST167 being the most frequently detected (n=6), followed by ST38 (n=5), ST410 (n=2), and others such as ST648, ST2083, ST361, ST405, and ST2851. Notably, ST167 isolates were present in both environmental (fly) and clinical samples (Table 6.1).

Clermon Typing shows that the majority of isolates belonged to phylogroup A (n=9)**,** followed by group D (n=7)**,** group B1 (n=4), group C (n=4), and group F (n=2) (Table 6.1).

The majority of the *bla*$_{\text{NDM-5}}$-positive plasmids in *E. coli* (51.9% [14/27]) were characterised as hybrid replicons. Hybrid plasmids were defined as plasmids carrying more than one replicon type. For the analyses presented, plasmid replicons were counted on a per-isolate basis, such that hybrid plasmids were counted each time they were detected in an isolate. This approach reflects the prevalence of replicon types across isolates rather than the number of unique plasmids. NDM-5 is predominantly carried by the plasmids of IncF family particularly IncFIA, IncFIB(AP001918), and IncFII. Among those plasmids, IncFII was the most common, found in over half (55.5%) of the isolates (n=15 Three of the *bla*$_{\text{NDM-5}}$-positive plasmids lacked known replicon types (Table 6.1). As the genomic location of *bla*$_{\text{NDM-5}}$ was not experimentally

validated in these isolates, it was not possible to conclusively determine whether *bla*$_{\text{NDM-5}}$ was plasmid-borne or chromosomally integrated. The absence of detectable replicons may reflect chromosomal integration of *bla*$_{\text{NDM-5}}$ or, alternatively, the presence of highly divergent or hybrid plasmids lacking recognisable replicon sequences. The combination of ST167 with IncF-type replicons was repeatedly observed across different sources (n=6, 22.2%).

Table 6.1 Genotypic characteristics and plasmid replicon types of *E. coli* isolates carrying *bla*$_{NDM-5}$

Isolate ID	Source	ST	Phylogroup	MIC value ((µg/mL)		Replicon type of plasmids carrying *bla*$_{NDM-5}$	Plasmid circularised (Yes or no)
				IPM	MER		
W1EC	Water	ST361	A	8	32	IncFIA, IncFII	No
W114EC	Water	ST8346	B1	16	32	IncFIA, IncFII, IncFIB(AP001918)	Yes
W115EC	Water	ST2083	B1	32	32	IncFII, IncFIB(pB171)	No
W193EC	Water	ST410	C	8	8	IncFII	No
F137EC	Fly	ST90	C	16	16	No replicon	No
F140EC	Fly	ST167	A	16	16	IncFIA, IncFIC(FII)	No
U48EC	UTI	ST46	A	8	8	IncFIA, IncFIB(AP001918), IncFII(pRSB107)	Yes
U102EC	UTI	ST167	A	8	32	IncFIA, IncFII, IncX1	Yes
U326EC	UTI	ST2083	B1	8	32	IncFIA, IncFIB(AP001918), IncFII(pAMA1167-NDM-5)	No
U689EC	UTI	ST405	D	8	16	IncFIB(AP001918)	No
S46EC	SSI	ST38	D	4	16	IncFII	Yes

S124EC	SSI	ST167	A	16	32	IncFIA, IncFIC(FII), IncFIB(AP001918)	
S333EC	SSI	ST405	D	16	8	IncFII	Yes
S374EC	SSI	ST648	F	64	64	IncFII	No
S407EC	SSI	ST167	A	8	8	IncFIA, IncFIB(pB171)	No
S416EC	SSI	ST410	C	8	4	IncFIA, IncFIC(FII), IncFIB(AP001918)	No
S504EC	SSI	ST167	A	4	8	IncFIA, IncFIC(FII), IncFIB(AP001918)	Yes
S563EC	SSI	ST2851	C	16	8	IncFII	Yes
S598EC	SSI	ST648	F	8	8	IncFIA, IncFIB(AP001918), IncFII(pRSB107)	Yes
S604EC	SSI	ST38	D	16	16	IncFII	Yes
S605EC	SSI	ST38	D	4	8	IncFIA, IncFIC(FII), IncFIB(AP001918)	No
S608EC	SSI	ST448	B1	16	64	IncR	Yes
S664EC	SSI	ST167	A	2	16	No replicon	No
S665EC	SSI	ST361	A	32	32	No replicon	No
S679EC	SSI	ST38	D	16	16	IncFII	Yes
S708EC	SSI	ST2659	D	32	8	IncFIA, IncFIB(pB171)	Yes
H66EC	HRS	ST38	D	32	16	IncFII	Yes

The prevalence of *bla*$_{NDM-5}$ among different sources is shown in Figure 6.4. A Fisher's exact test was performed to analyse the resistance profiles to antibiotics for *bla*$_{NDM-5}$ positive *E. coli* (NDM-5PEC) and *bla*$_{NDM-5}$ negative *E. coli* (NDM-5NEC) (Table 6.2) to assess whether *bla*$_{NDM-5}$ carriage was associated with broader multidrug resistance beyond its known β-lactamase activity. NDM-5PEC isolates showed significantly higher resistance to nearly all the tested antibiotics (AMP, AMC, TZP, AMK, GEN, AZT, CTX, CAZ, CAZ-AVI, CEF, CIP, LEV, IPM, MER) compared to NDM-5-NEC (Fisher's exact test, p <0.001 for most antibiotics). Notably, 100% of NDM-5PEC were resistant to beta-lactams (AMP, TZP, CTX, CAZ, CAZ-AVI, CEF), carbapenems (IPM, MER), and AZT, but sensitive to COL and TIG. Resistance to amikacin, gentamicin, and fluoroquinolones was also significantly higher in NDM-5PEC (p < 0.001). Figure 6.5 illustrates the MIC distributions of imipenem and meropenem among carbapenem-resistant NDM-5PEC and NDM-5NEC. NDM-5PEC isolates tended to exhibit higher MIC values compared to NDM-5NEC isolates.

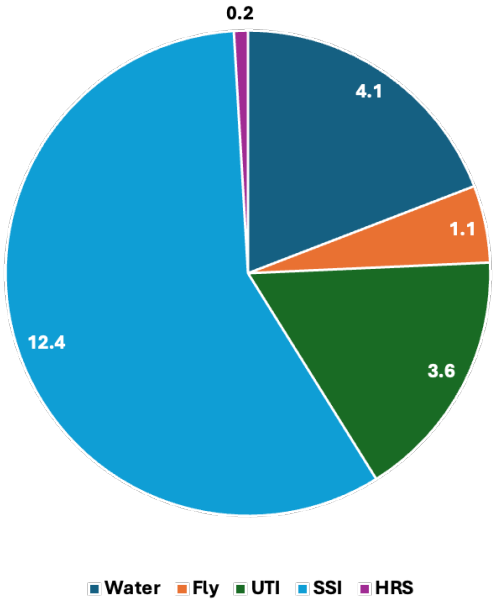


Figure 6.4 Prevalence of *bla*$_{NDM-5}$ among *E. coli* isolates from different sources.
The values indicate the percentage of *E. coli* isolates carrying *bla*$_{NDM-5}$ among different sources. UTI, urinary tract infection; SSI, surgical site infection; HRS, human rectal swab.

Table 6.2 Resistance profiles of NDM-5PEC and NDM-5NEC *E. coli* isolates

Antibiotics	Resistant to respective antibiotics, n (%)		
	NDM-5PEC	NDM-5NEC	*p* value
AMP	27 (100)	807 (50.2)	<0.001
AMC	26 (96.3)	669 (41.6)	<0.001
TZP	27 (100)	151 (9.4)	<0.001
AMK	16 (59.3)	38 (2.4)	<0.001
GEN	18 (66.7)	194 (12.1)	<0.001
AZT	27 (100)	235 (14.6)	<0.001
CTX	27 (100)	240 (14.9)	<0.001
CAZ	27 (100)	276 (17.2)	<0.001
CAZ-AVI	27 (100)	13 (0.8)	<0.001
CEF	27 (100)	205 (12.8)	<0.001
CIP	25 (92.6)	712 (44.3)	<0.001
LEV	25 (92.6)	681 (42.4)	<0.001
COL	0 (0)	10 (0.6)	1
FOS	1 (3.7)	24 (1.5)	0.343
IPM	26 (96.3)	10 (0.6)	<0.001
MER	27 (100)	10 (0.6)	<0.001
TIG	0 (0)	38 (2.4)	1

n, number of isolates. Values indicate the number and percentage of isolates resistant to each antibiotic. Resistance frequencies were compared between NDM-5PEC and NDM-5NEC using Fisher's exact test and *p*-values indicate differences in resistance prevalence between the two groups. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; AMK, amikacin; AZT, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; CEF,

cefepime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MER, meropenem; TZP, piperacillin-tazobactam; TIG, tigecycline.

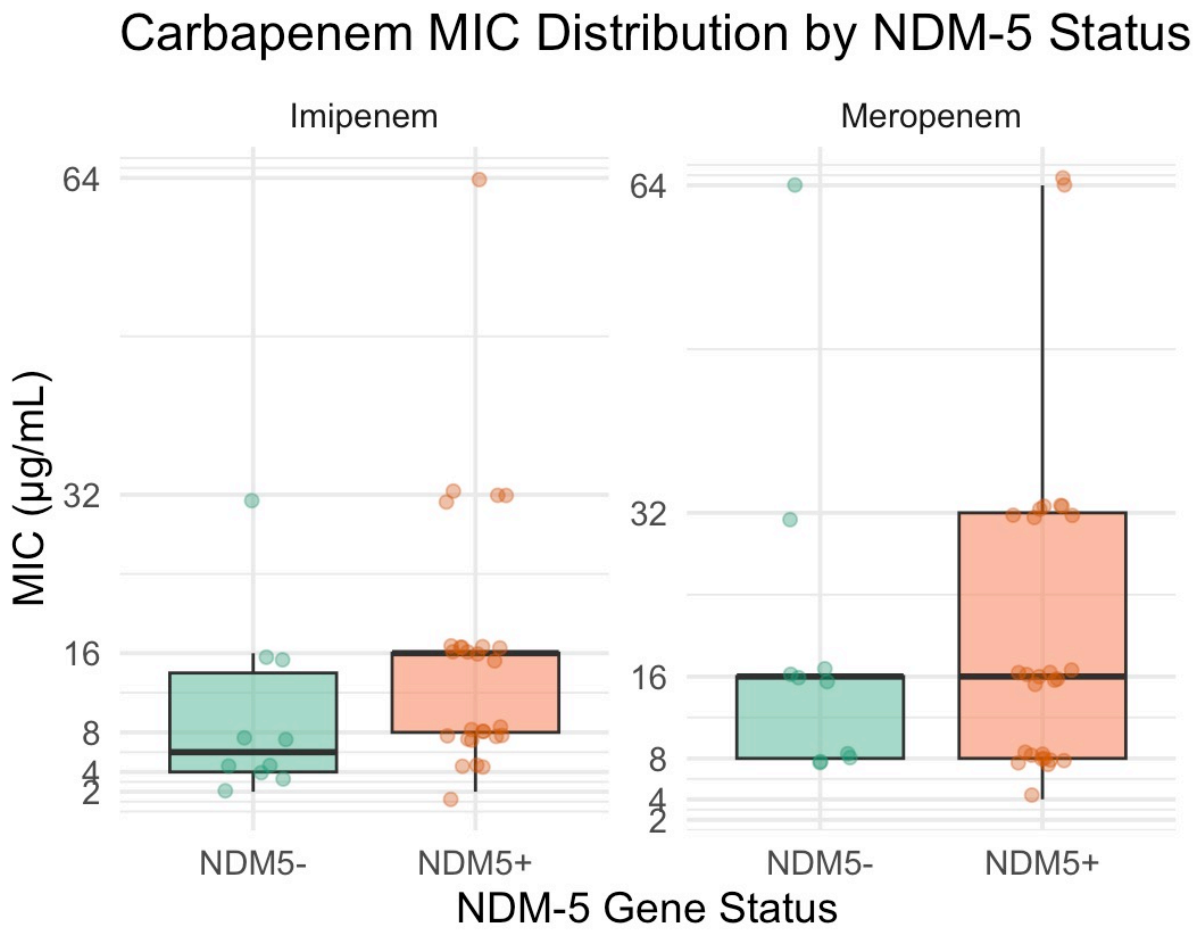


Figure 6.5 Distribution of MICs for imipenem and meropenem among *E. coli* isolates stratified by $bla_{\text{NDM-5}}$ status

Boxplots show median MIC values and interquartile ranges, with individual isolates displayed as jittered points. MIC distributions are shown descriptively to visualise differences between NDM-5PEC ($NDM5^{+}$) and NDM-5NEC ($NDM5^{-}$) isolates. MIC values are expressed in µg/mL. The figure was generated using R (v4.5.1).

The phylogenetic analysis of 27 *E. coli* isolates carrying the *bla*$_{\text{NDM-5}}$ gene (Figure 6.6) revealed substantial genetic diversity, as evidenced by the distribution of isolates across multiple lineages and phylogroups (A, B1, C, D, and F). The presence of *bla*$_{\text{NDM-5}}$ in genetically distinct backgrounds suggests that this resistance gene is not confined to a specific clonal lineage but is disseminated across diverse strains, likely via horizontal gene transfer. Isolates carrying *bla*$_{\text{NDM-5}}$ were from both clinical (UTI and SSI) and HRS, as well as environmental sources (water, flies).

These isolates belonged to three distinct geographical locations, MMCH, two poultry farms in Trishal, and Muktagachha, across four quarterly time points.

The heatmaps further highlight the co-occurrence of multiple ARGs among the isolates, demonstrating a high degree of MDR. Certain resistance profiles were conserved within specific phylogenetic clusters.

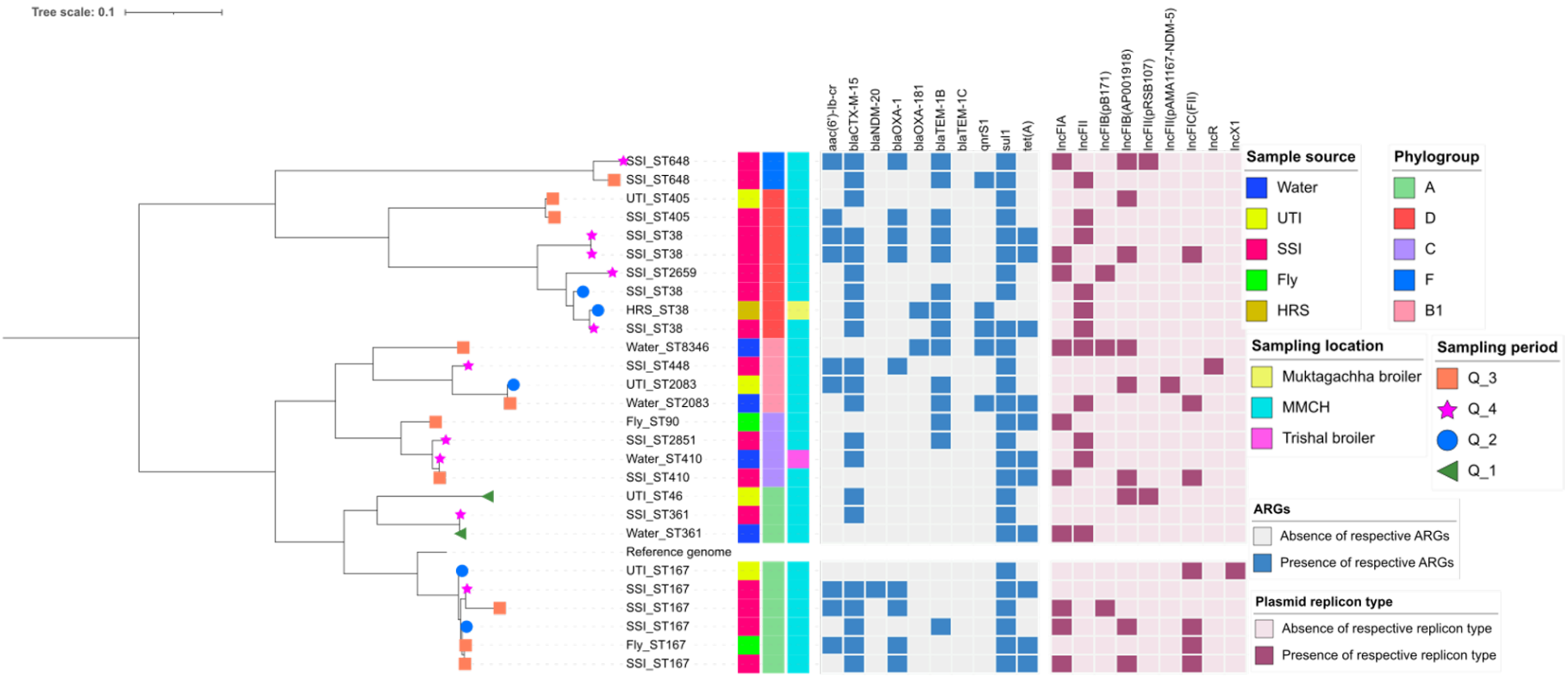


Figure 6.6 Clonal distribution of *bla*$_{\text{NDM-5}}$ positive *E. coli.* The ML tree was generated using VeryFastTree (v-4.0). Core-genome alignment was performed using pipeline bactmap (v.1.0.0). The tree was annotated and visualised in Interactive Tree of Life (iTOL) (v-6).

6.2.3 Characterisation of plasmids harbouring *bla*$_{NDM-5}$

The distribution of plasmid replicon types identified among *E. coli* isolates harbouring the *bla*$_{NDM-5}$ gene, stratified by source, is illustrated in Figure 6.7. The most frequently detected replicons were IncFIA, IncFIB, and IncFII**,** with the highest diversity and abundance observed in isolates from water and HRS. Several replicon types, such as IncFIA and IncFIB, were shared between environmental (water, fly) and clinical (UTI, SSI) sources. Analysis of plasmid replicon types revealed that the isolates frequently carried multiple replicons. Out of 27 isolates, 10 (37%) carried a single replicon, 6 (22.2%) carried two replicons, and 8 (26.6%) carried three replicons, while 3 isolates lacked any known replicon type.

In this study, thirteen isolates harboured closed circular plasmids carrying *bla*$_{NDM-5}$ (Table 6.1). Of these, six belonged to IncFII, one to IncR, and the remaining six carried multiple replicon types. PlasmidFinder and BLAST-based comparison against publicly available reference plasmids revealed that IncFIA plasmids shared ≥ 99% nucleotide identity with ≥ 98% coverage to reference plasmid (accession number AP001918). IncFIB plasmids showed ≥ 97% identity with ≥ 88% coverage to reference plasmid (accession number AP001918), IncFIC plasmids displayed ≥ 83% identity with ≥ 99% coverage to reference sequences (accession number AP001918), IncFII plasmids were 100% identical with ≥ 95% coverage to the reference sequence (accession number AY458016), IncR plasmids were 100% identical at full coverage to the reference sequence (accession number DQ449578), and IncX1 plasmids exhibited 95% identity at 100% coverage to the reference sequence (accession number JN935898).

To visualise nucleotide-level conservation and structural variation among *bla*$_{NDM-5}$–carrying plasmids within this study, BRIG analyses were performed using representative circular plasmids assembled in this study as reference sequences for respective replicons (Figure 6.8).

Genomic context analysis of *bla*$_{NDM-5}$ revealed that plasmids belonging to IncFIA, IncFIB, and IncFIC harbored a conserved 7–8 kb region comprising *bla*$_{NDM-5}$ flanked upstream by *mph*, IS30, and IS6, and downstream by ble, IS91, and *sul1*. IncFII plasmids contained a similar conserved segment like to IncFIA, IncFIB, and IncFIC. IncR plasmids displayed the same arrangement as IncFII except for the absence of *ble* downstream of *bla*$_{NDM-5}$ (Figure 6.8). Comparative genomic analysis of *bla*$_{NDM-5}$ genetic contexts across different plasmid replicon types are shown in Figure 6.9.

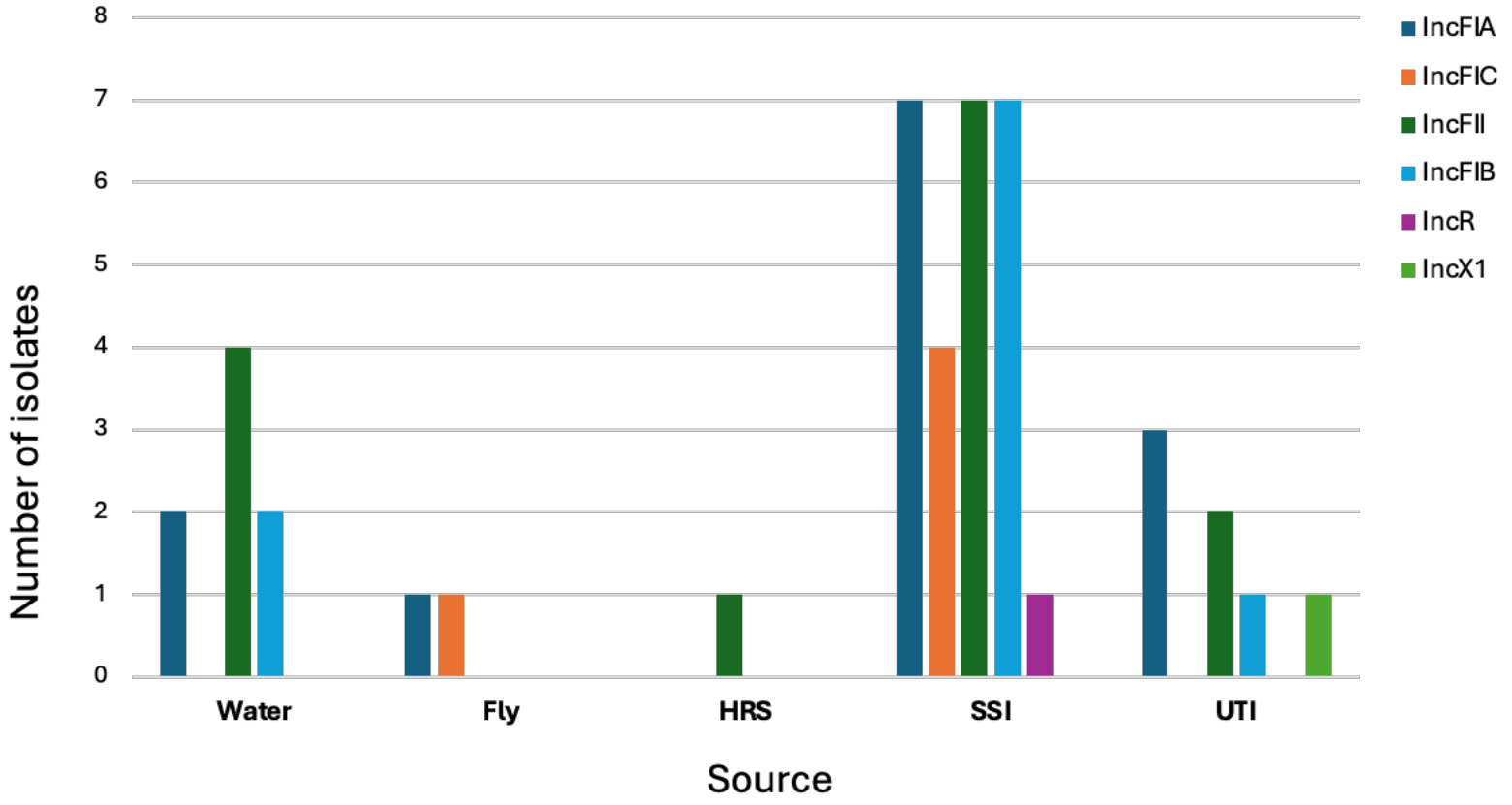


Figure 6.7 Distribution of plasmid replicon types among *E. coli* isolates carrying $bla_{\text{NDM-5}}$ from various sources.

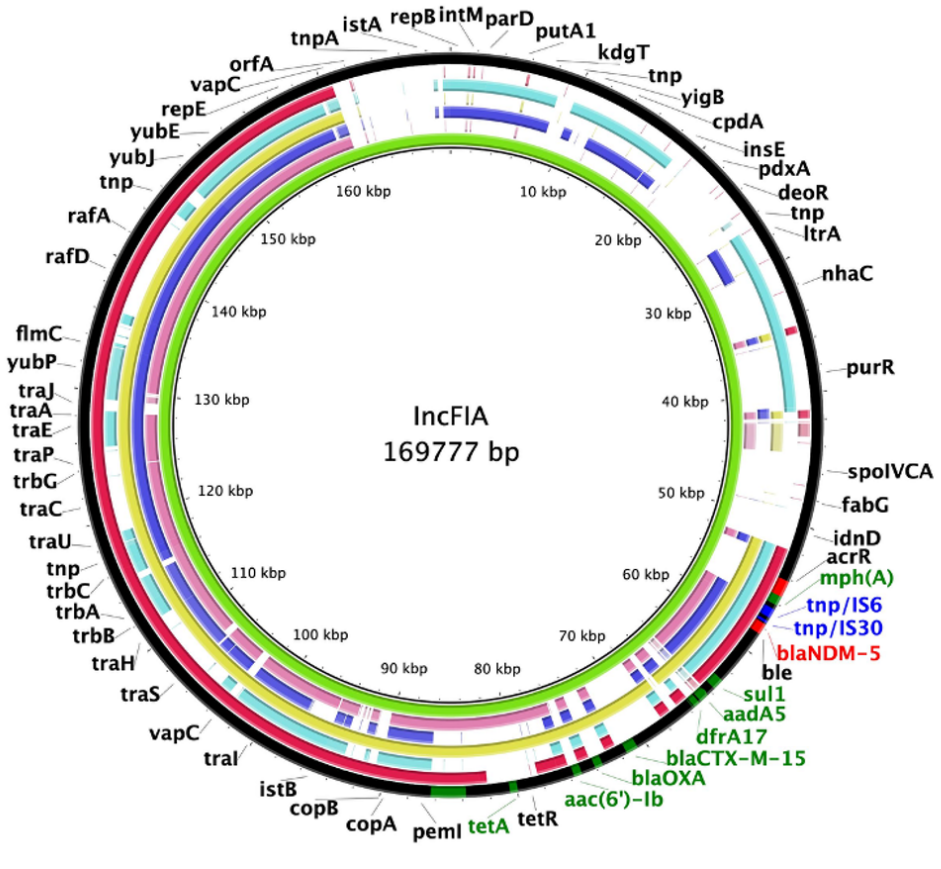


Figure 6.8.A1 Comparative analysis of plasmids belonged to IncFIA. Circular sequence alignments of reference plasmid (S504, identified in this study) with homologous contigs of IncFIA plasmids harbouring $bla_{\text{NDM-5}}$ using BLASTn and BLAST Ring Image Generator (BRIG). The concentric rings display 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 right side of the ring.

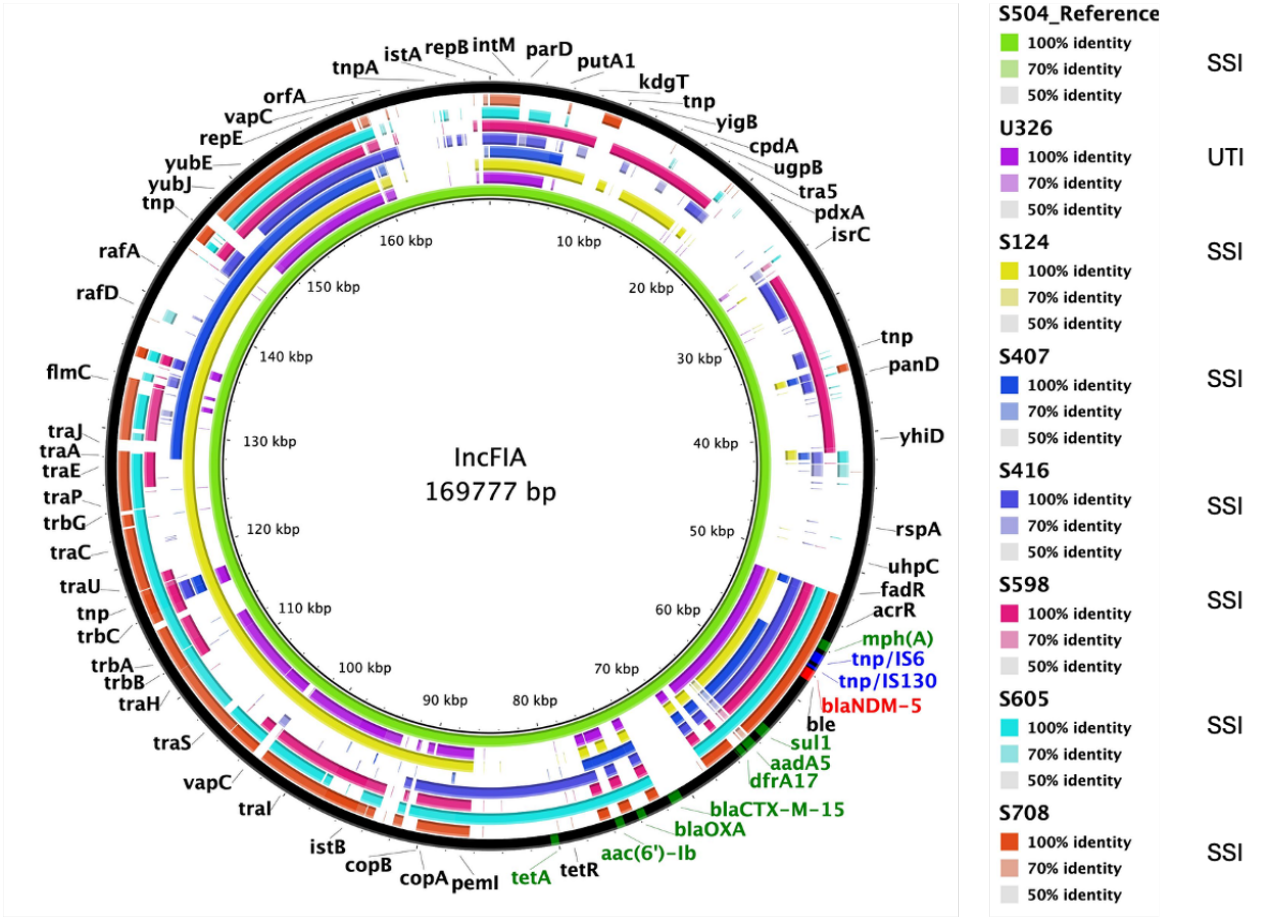


Figure 6.8.A2 Comparative analysis of plasmids belonged to IncFIA. Circular sequence alignments of reference plasmid (S504, identified in this study) with homologous contigs of IncFIA plasmids harbouring $bla_{\text{NDM-5}}$ using BLASTn and BLAST Ring Image Generator (BRIG). The concentric rings display 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 right side of the ring.

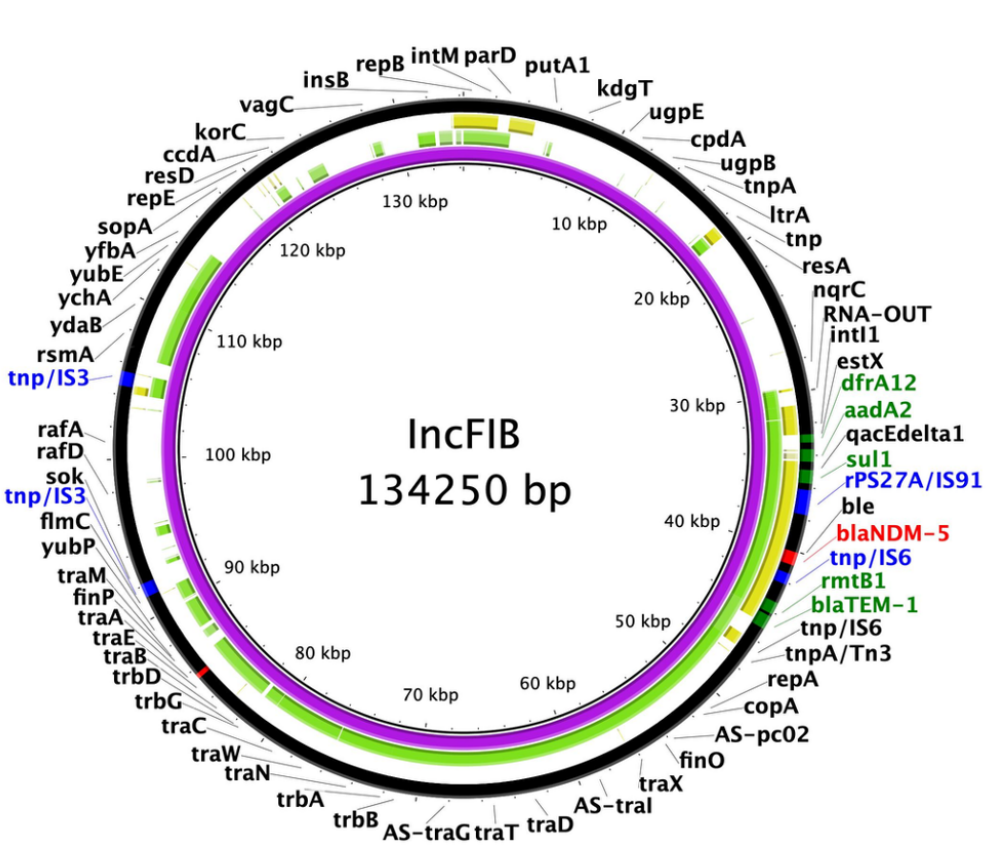


Figure 6.8.B1 Comparative analysis of plasmids belonging to IncFIB. Circular sequence alignments of reference plasmid (circular complete plasmid identified in this study was used as reference) with homologous contigs of IncFIB plasmids harbouring $bla_{\text{NDM-5}}$ using BLASTn and BLAST Ring Image 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 right side of the ring.

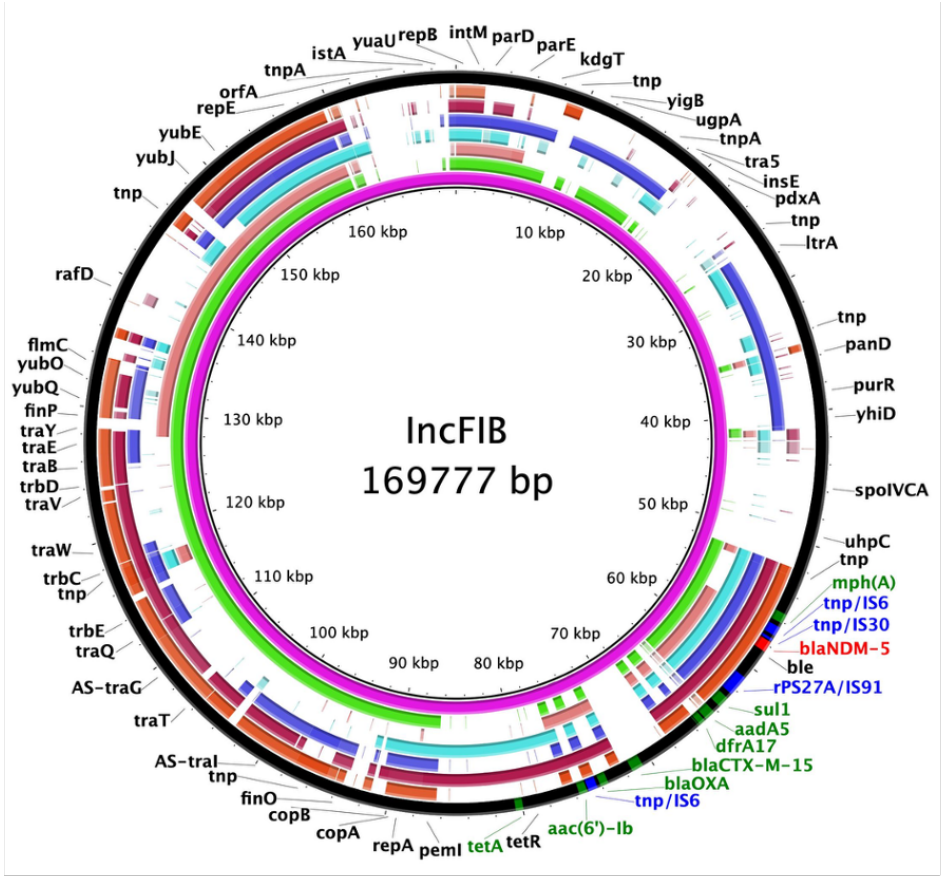


Figure 6.8.B2 Comparative analysis of plasmids belonging to IncFIB. Circular sequence alignments of reference plasmid (circular complete plasmid identified in this study was used as reference) with homologous contigs of IncFIB plasmids harbouring *bla*$_{\text{NDM-5}}$ using BLASTn and BLAST Ring Image 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 right side of the ring.

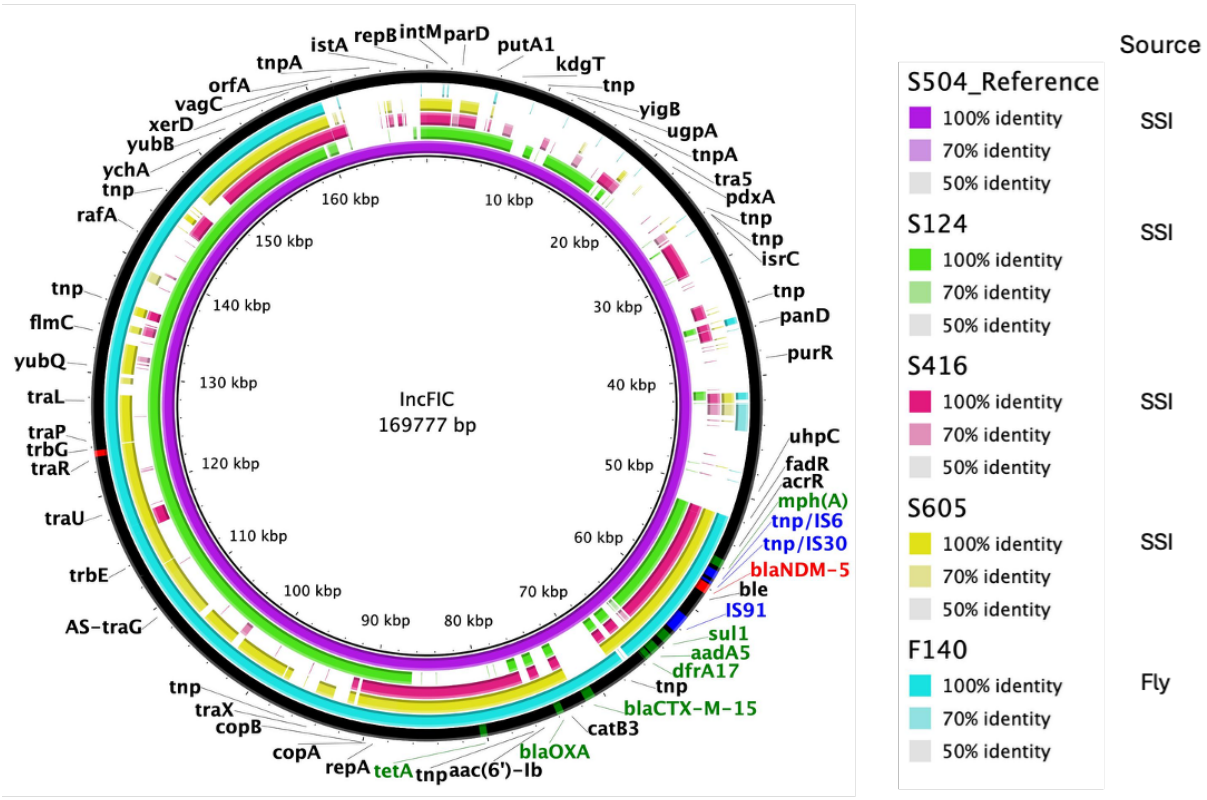


Figure 6.8.C Comparative analysis of plasmids belonging to IncFIC. Circular sequence alignments of reference plasmid (circular complete plasmid identified in this study was used as reference) with homologous contigs of IncFIC plasmids harbouring the *bla*$_{\text{NDM-5}}$ gene using BLASTn and BLAST 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 right side of the ring.

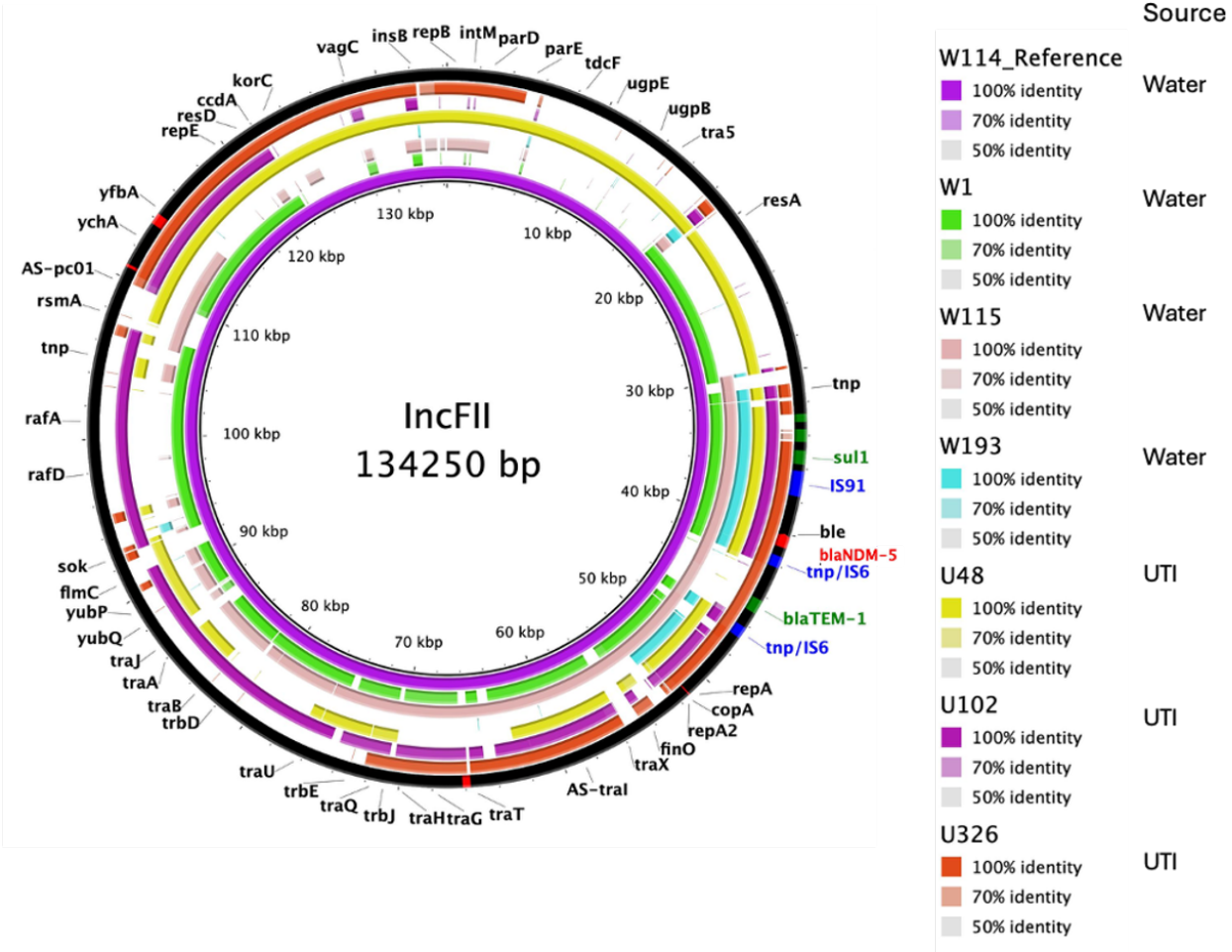
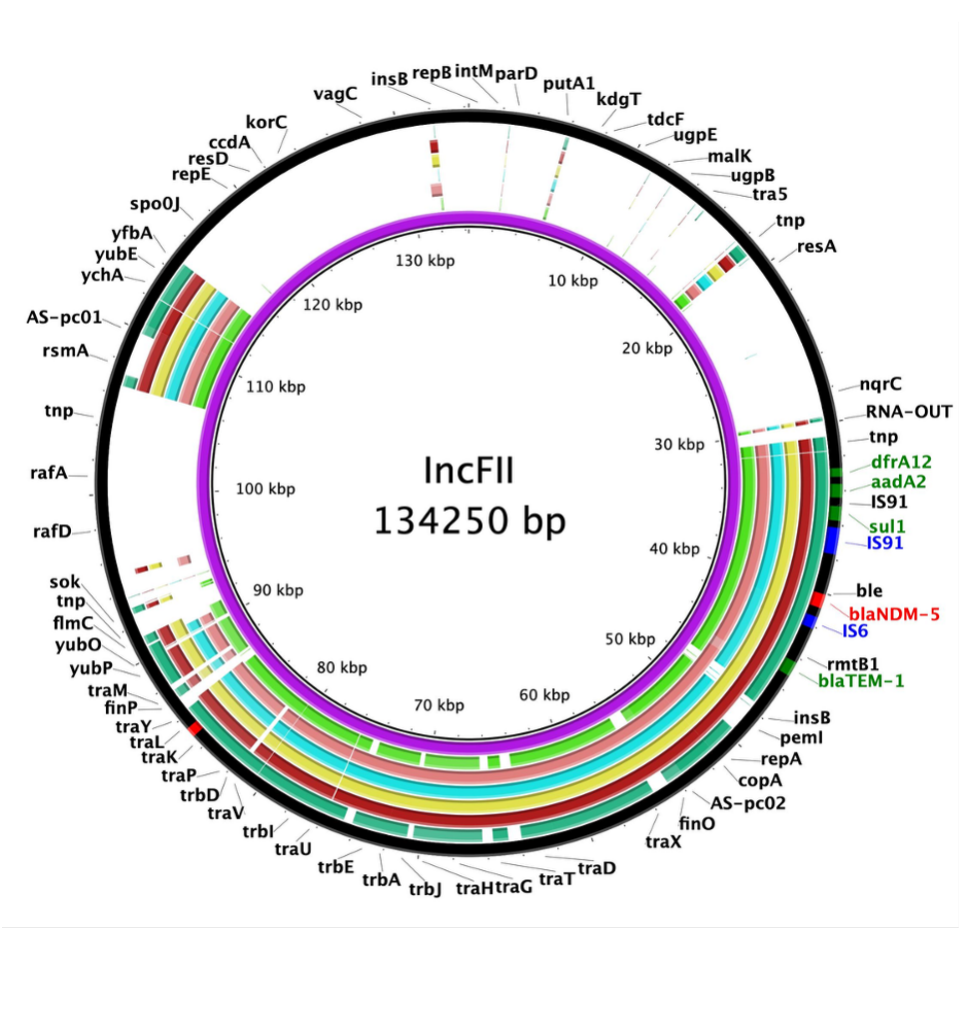


Figure 6.8.D1 Comparative analysis of plasmids belonging to IncFII. Circular sequence alignments of reference plasmid (circular complete plasmid identified in this study was used as reference) with homologous contigs of IncFII plasmids harbouring $bla_{\text{NDM-5}}$ using BLASTn and BLAST Ring Image 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 right side of the ring.



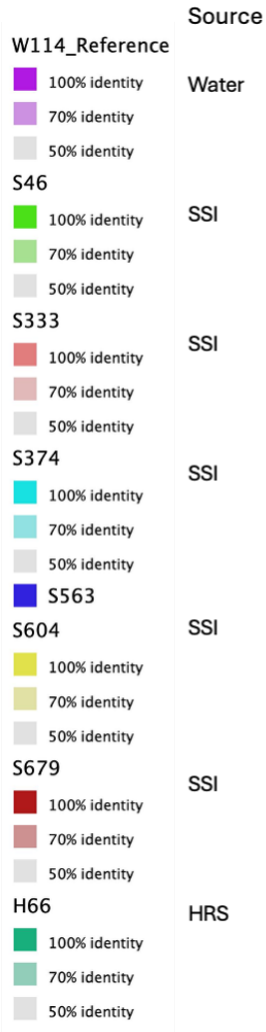


Figure 6.8.D2 Comparative analysis of plasmids belonging to IncFII. Circular sequence alignments of reference plasmid (circular complete plasmid identified in this study was used as reference) with homologous contigs of IncFII plasmids harbouring *bla*$_{\text{NDM-5}}$ using BLASTn and BLAST Ring Image 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 right side of the ring.

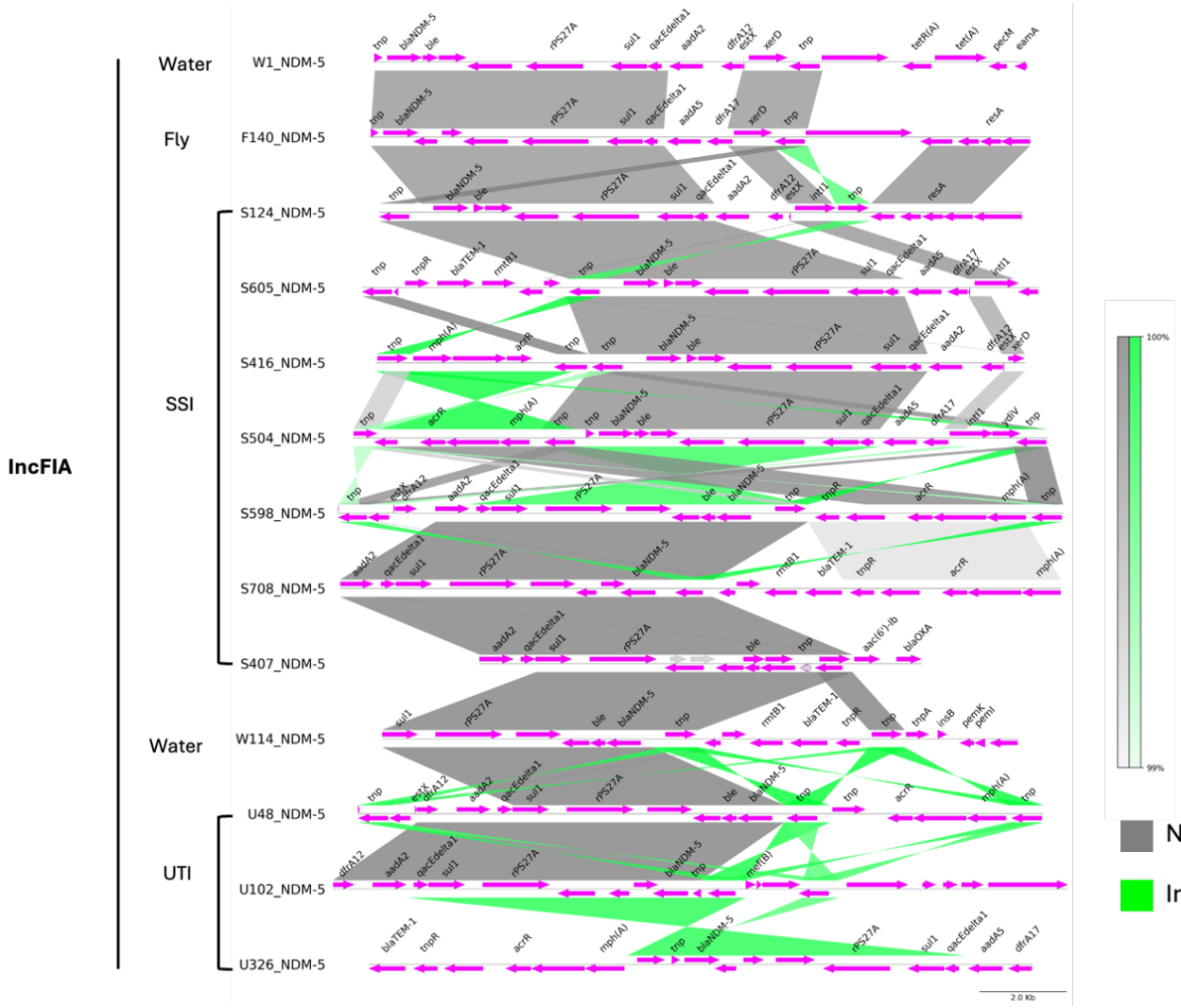


Figure 6.9.A Linear comparison of the genetic context of *bla*$_{NDM-5}$ on IncFIA plasmids. Arrows represent the position and transcriptional direction of the open reading frames. Grey shading indicates collinear homologous regions, while green shading denotes inverted regions. The genomic comparison was performed by pyGenomeViz v1.6.1. Overlapping arrows in isolate S407 indicate strand-opposed and partial CDS predicted by BAKTA within a mobile *bla*$_{NDM-5}$ region.

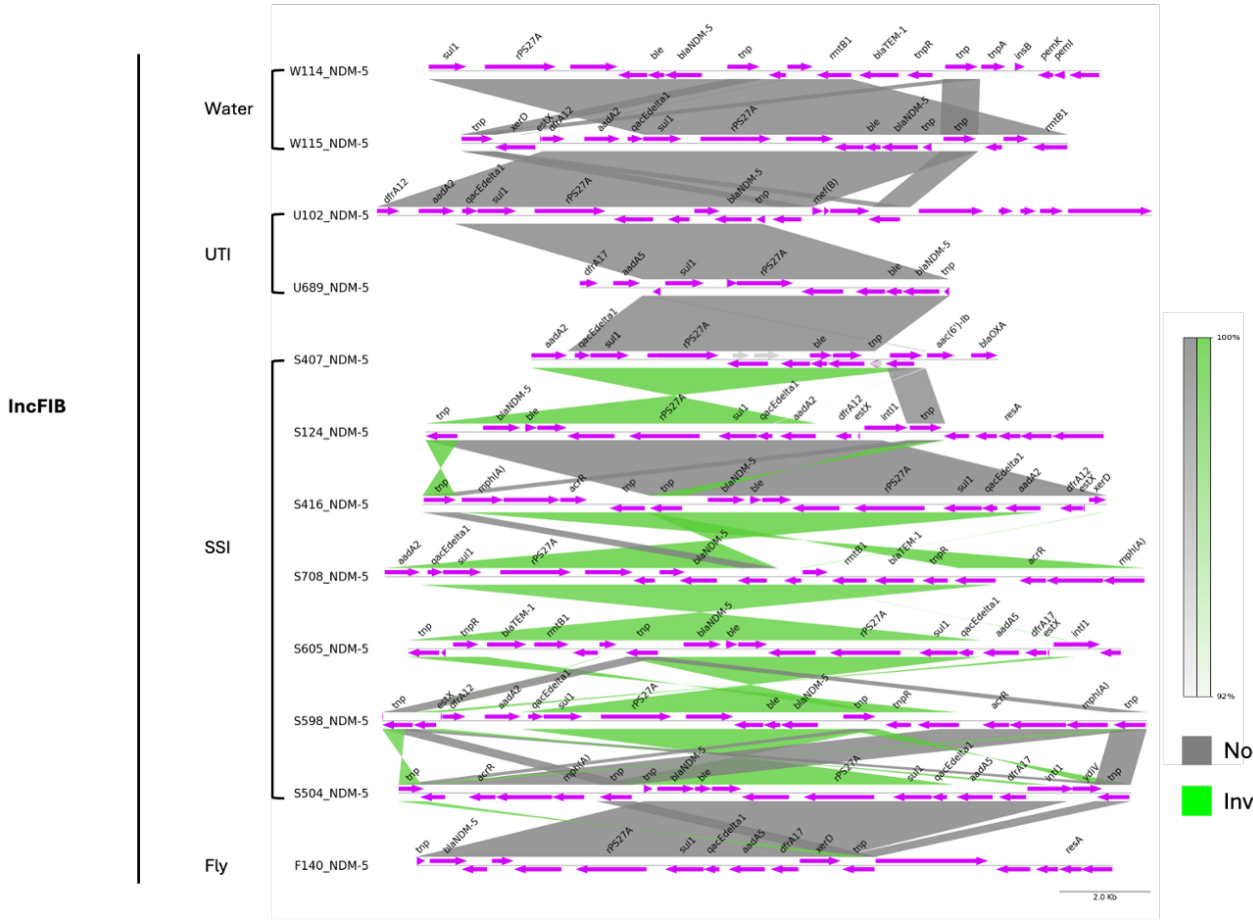


Figure 6.9.B Linear comparison of the genetic context of *bla*$_{NDM-5}$ on IncFIB plasmids. Arrows represent the position and transcriptional direction of the open reading frames. Grey shading indicates collinear homologous regions, while green shading denotes inverted regions. The genomic comparison was performed by pyGenomeViz v1.6.1. Overlapping arrows in isolate S407 indicate strand-opposed and partial CDS predicted by BAKTA within a mobile *bla*$_{NDM-5}$ region.

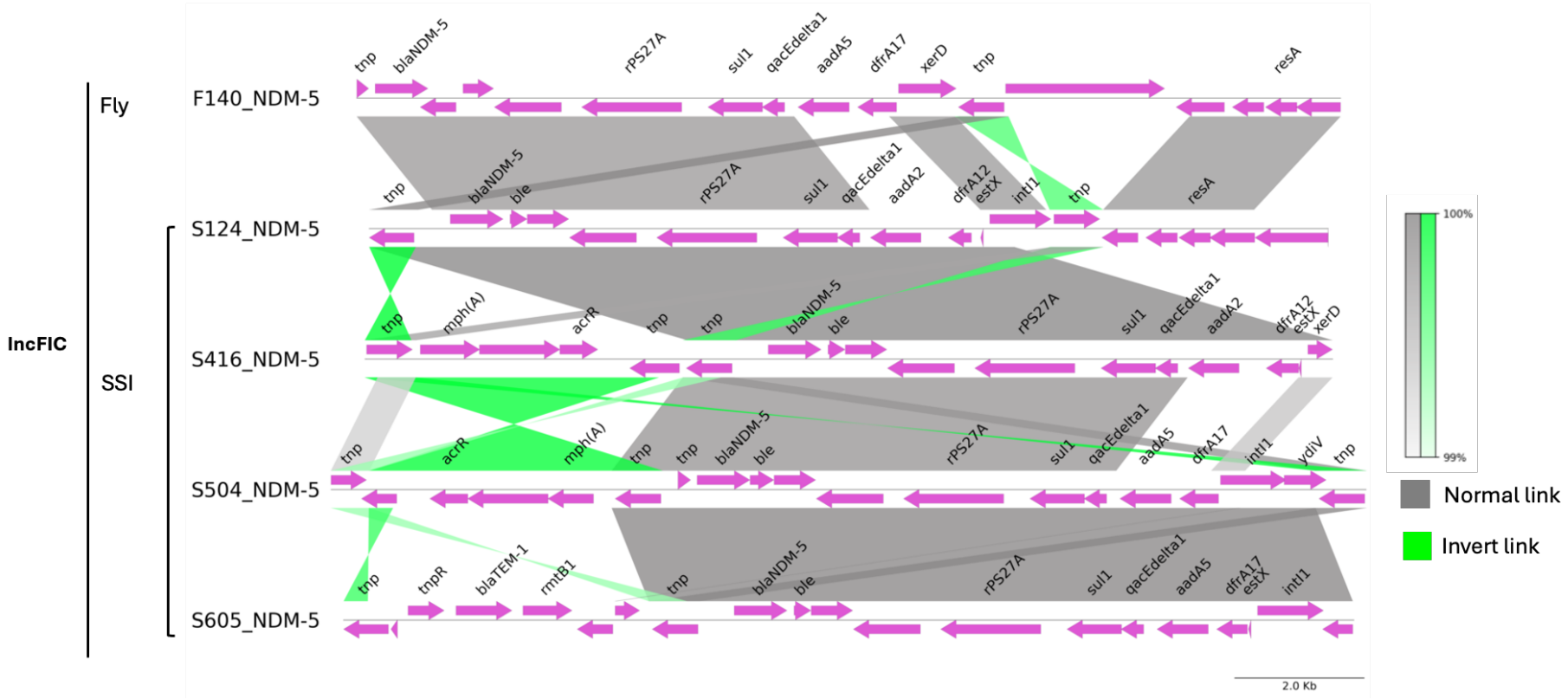


Figure 6.9.C Linear comparison of the genetic context of *bla*$_{NDM-5}$ genes on IncFIC plasmids. Arrows represent the position and transcriptional direction of the open reading frames. Grey shading indicates collinear homologous regions, while green shading denotes inverted regions. The genomic comparison was performed by pyGenomeViz v1.6.1.

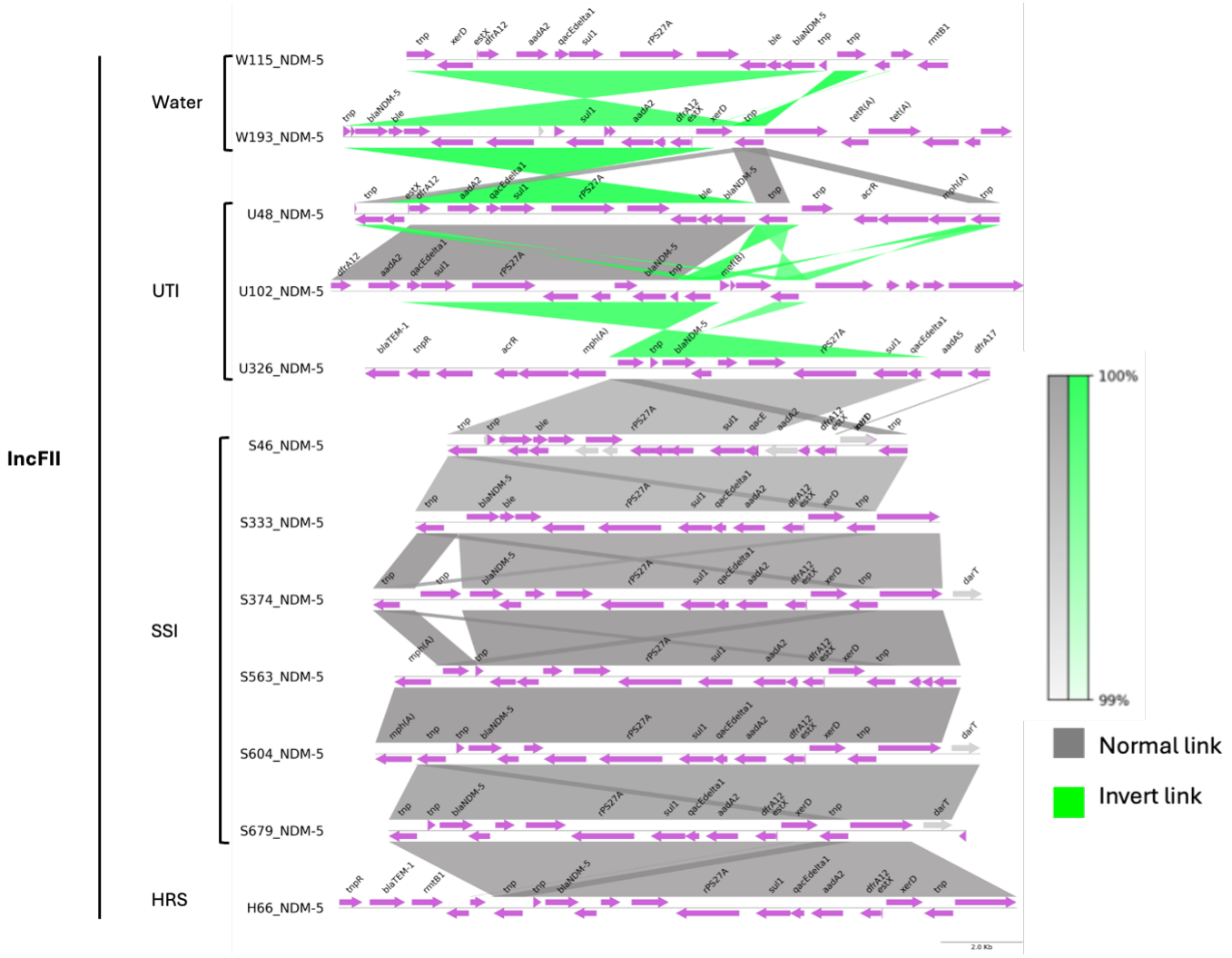


Figure 6.9.D Linear comparison of the genetic context of *bla*$_{\text{NDM-5}}$ on IncFII plasmids. Arrows represent the position and transcriptional direction of the open reading frames. Grey shading indicates collinear homologous regions, while green shading denotes inverted regions. The genomic comparison was performed by pyGenomeViz v1.6.1. Overlapping arrows in isolate S46 indicate strand-opposed and partial CDS predicted by BAKTA within a mobile *bla*$_{\text{NDM-5}}$ region.

6.2.4 Clonal distribution, resistance profile and plasmid background of *tet*(X4)

A total of eight tigecycline-resistant *E. coli* isolates carrying the *tet*(X4) gene were recovered from different sources: water (n = 1), flies (n = 4), and CMS (n = 3). They were identified by PromethION (Oxford Nanopore Technology). All isolates belonged to phylogroup A, with diverse STs, including ST6050, ST16755, ST206, ST10, and two unknown STs. Plasmid replicon typing revealed the presence of IncX1 in two fly-derived isolates, while isolates from water and CMS carried IncFIA(HI1), IncHI1B(R27), and IncHI1A replicons (Table 6.3). Three isolates, including two from flies and one from CMS, showed no known plasmid replicons.

Table 6.3 Genotypic characteristics and plasmid replicon types of *E. coli* isolates carrying *tet*(X4)

Isolate ID	Source	Phylogroup	MLST	MIC value (µg/mL) for Tigecycline	Replicon type carrying *tet*(X4) gene	Plasmid circularised (Yes or no)
W176EC	Water	A	ST6050	2	IncFIA(HI1), IncHI1B(R27)	Yes
F16EC	Fly	A	ST16755	1	IncX1	No
F22EC	Fly	A	ST16755	1	IncX1	Yes
F165EC	Fly	A	ST206	2	No replicon	No
F170EC	Fly	A	ST206	2	No replicon	Yes
M14EC	CMS	A	Unknown ST	4	IncFIA(HI1), IncHI1B(R27), IncHI1A	No
M20EC	CMS	A	Unknown ST	2	No replicon	No
M30EC	CMS	A	ST10	4	IncFIA(HI1), IncHI1B(R27), IncHI1A	No

The prevalence of *tet*(X4) among different sources is shown in Figure 6.10. A Fisher's exact test was performed to analyse the resistance profiles to antibiotics for *tet*(X4)-positive *E. coli* (tetX4PEC) and *tet*(X4) -negative *E. coli* (tetX4NEC) (Table 6.4). Resistance to most antibiotics was not significantly different between tetX4PEC and tetX4NEC, while tetX4PEC showed significantly higher resistance to CIP (100%, $p = 0.002$), LEV (100%, $p = 0.001$), and tigecycline (100%, $p < 0.001$). No significant differences were observed for carbapenems and aminoglycosides. Resistance to tigecycline was exclusive to tetX4PEC isolates, consistent with the presence of the *tet*(X4) gene. Figure 6.11 illustrates the distribution of tigecycline MIC values among tetX4PEC and tetX4NEC. MIC values for tetX4PEC ranged from 1 to 4 μg/mL, whereas tetX4NEC isolates exhibited MIC values ranging from 1 to 2 μg/mL.

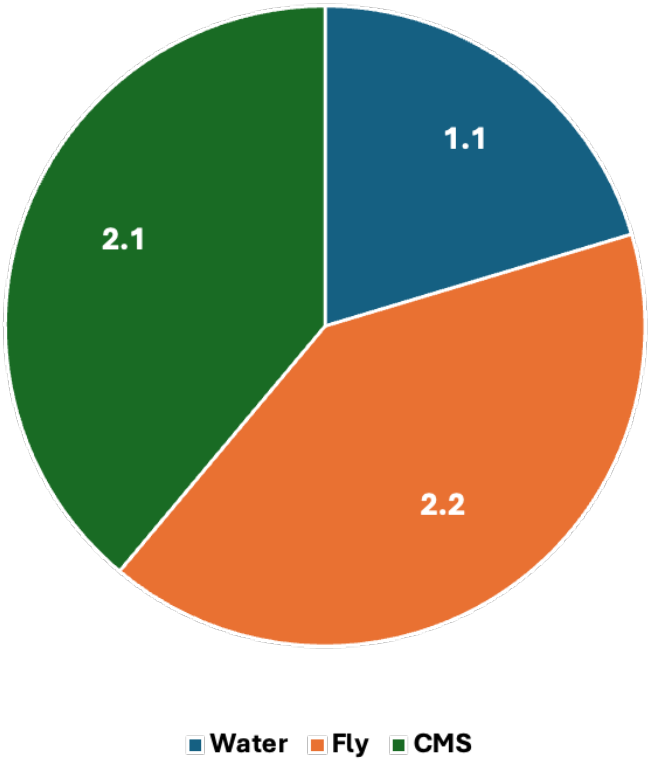


Figure 6.10 Prevalence of *tet*(X4) among the sources. The values indicate the percentage of *E. coli* isolates carrying *tet*(X4) among different sources.

Table 6.4 Resistance profile to antibiotics for tetX4PEC and tetX4NEC

Antibiotics	Resistant to respective antibiotics, n (%)		
	tetX4PEC	tetX4NEC	*p* value
AMP	6 (75)	828 (50.9)	0.289
AMC	6 (75)	689 (42.4)	0.079
TZP	2 (25)	176 (10.8)	0.214
AMK	0 (0)	54 (3.3)	1
GEN	2 (25)	210 (12.9)	0.279
AZT	0 (0)	262 (16.1)	0.369
CTX	0 (0)	267 (16.4)	0.367
CAZ	1 (12.5)	302 (18.6)	1
CAZ-AVI	0 (0)	40 (2.5)	1
CEF	0 (0)	232 (14.3)	0.61
CIP	8 (100)	729 (44.8)	0.002
LEV	8 (100)	689 (42.9)	0.001
COL	0 (0)	10 (0.6)	1
FOS	1 (12.5)	24 (1.5)	0.116
IPM	0 (0)	36 (2.2)	1
MER	0 (0)	37 (2.3)	1
TIG	8 (100)	30 (1.8)	<0.001

n, number of isolates. Values in the parentheses indicate the percentage of resistance. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; AMK, amikacin; AZT, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; CAZ-AVI, ceftazidime-avibactam; CEF, cefepime; CIP, ciprofloxacin; COL, colistin; FOS, fosfomycin; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MER, meropenem; TZP, piperacillin-tazobactam; TIG, tigecycline.

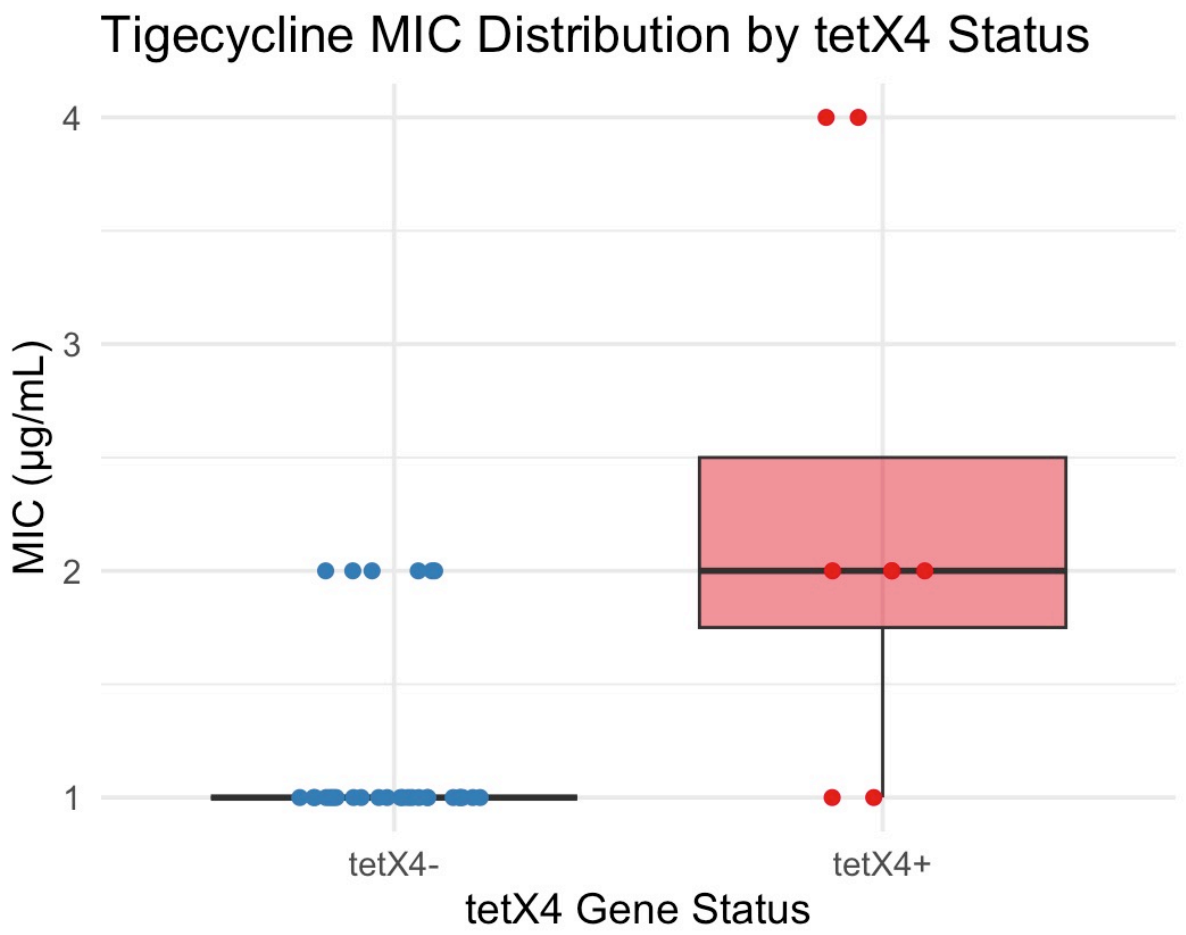


Figure 6.11 Distribution of tigecycline MIC values among *E. coli* isolates stratified by *tet*(X4) status. Boxplots show median MIC values and interquartile ranges, with individual isolates displayed as jittered points. MIC distributions are presented descriptively to visualise variation between tetX4PEC (tetX4+) and tetX4NEC (tetX4-) isolates. MIC values are expressed in µg/mL. The figure was generated using R (v4.5.1).

The phylogenetic analysis of eight *E. coli* isolates carrying the *tet*(X4) gene (Figure 6.12) showed genetic diversity, as evidenced by the distribution of isolates across multiple lineages, though they all are from the same phylogroup A. Isolates carrying *tet*(X4) were from

environmental sources (water, flies) and CMS. These isolates were sampled from five different poultry farms and environments across three quarterly time points.

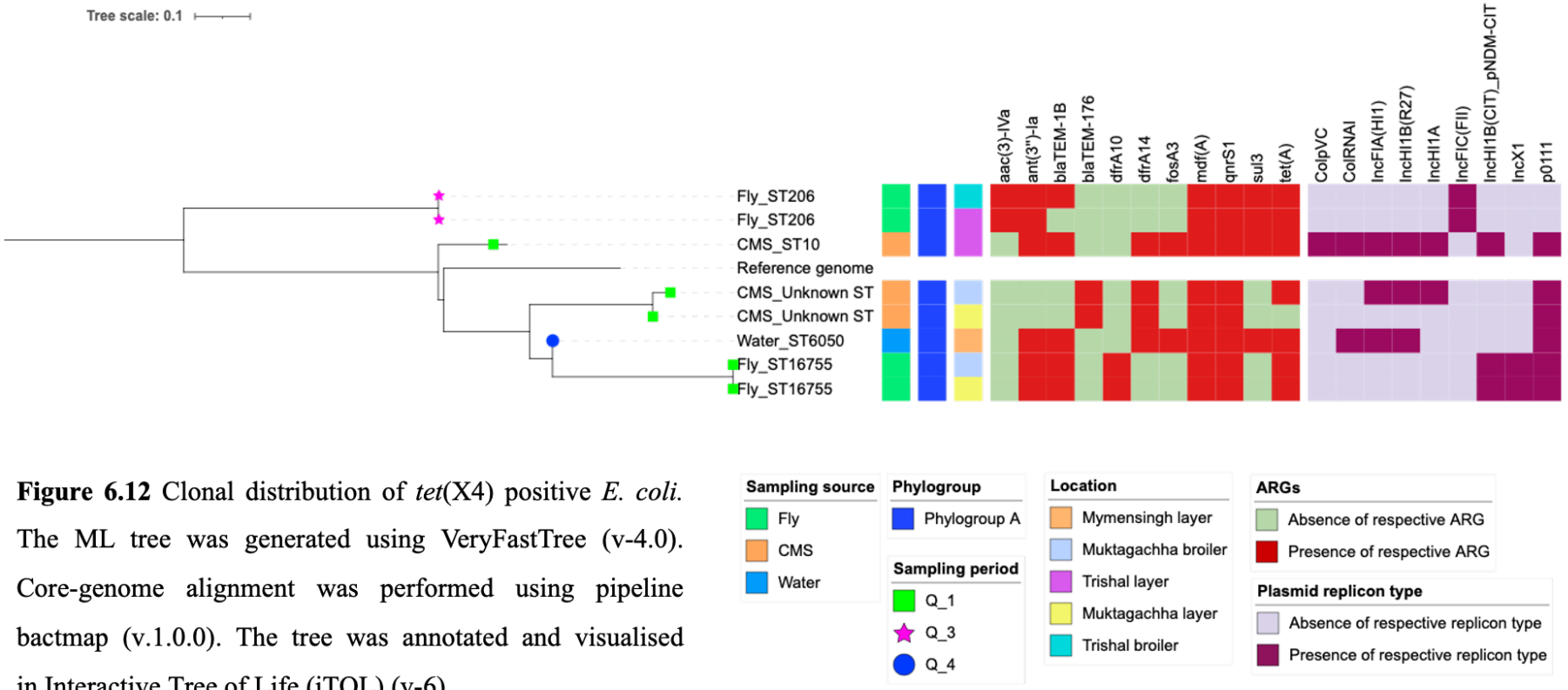


Figure 6.12 Clonal distribution of *tet*(X4) positive *E. coli*. The ML tree was generated using VeryFastTree (v-4.0). Core-genome alignment was performed using pipeline bactmap (v.1.0.0). The tree was annotated and visualised in Interactive Tree of Life (iTOL) (v-6).

6.2.5 Characterisation of plasmids harbouring *tet*(X4)

The distribution of plasmid replicon types identified among *E. coli* isolates harbouring the *tet*(X4) gene, stratified by source, is illustrated in Figure 6.13. Analysis of plasmid replicon types revealed multi-replicon carriage among the isolates. Out of 8 isolates, 2 (25%) carried a single replicon, 3 (37.5%) carried two replicons, and 3 isolates lacked any known replicon type.

In this study, three isolates harboured closed circular plasmids carrying *tet*(X4). One belonged to IncX1, one belonged to a multi-replicon type, and another one did not identify to have a known plasmid. PlasmidFinder and BLAST-based comparison against publicly available reference plasmids revealed that IncFIA plasmids shared 100% identity with 100% coverage to reference plasmids (accession number AF250878), IncHI1 plasmids showed ≥ 93% identity with ≥ 100% coverage to reference plasmid (accession number AF250878), IncX1 plasmids displayed ≥ 98% identity with 100% coverage to reference plasmid (accession number EU370913). To visualise nucleotide-level conservation and structural variation among *tet*(X4)–carrying plasmids within this study, BRIG analyses were performed using representative circular plasmids assembled in this study as reference sequences (Figure 6.14). For plasmid replicon types represented by a single circular plasmid, IncX1, nucleotide-level comparison was performed by analysing the internal structure and genetic context of the plasmid rather than by pairwise comparison with multiple plasmids of the same replicon type.

Analysis of the genomic environment surrounding *tet*(X4) showed that IncFIA and IncHI1 plasmids shared a conserved region containing *tet*(X4) and esT and IS91 upstream, and *fosA* and IS6 downstream. IncX1 plasmids possessed a different region where *tet*(X4) gene was bracketed by esT and *tet*(A) (Figure 6.14).

Comparative genomic analysis of *tet*(X4) genetic contexts across different plasmid replicon types is shown in Figure 6.15.

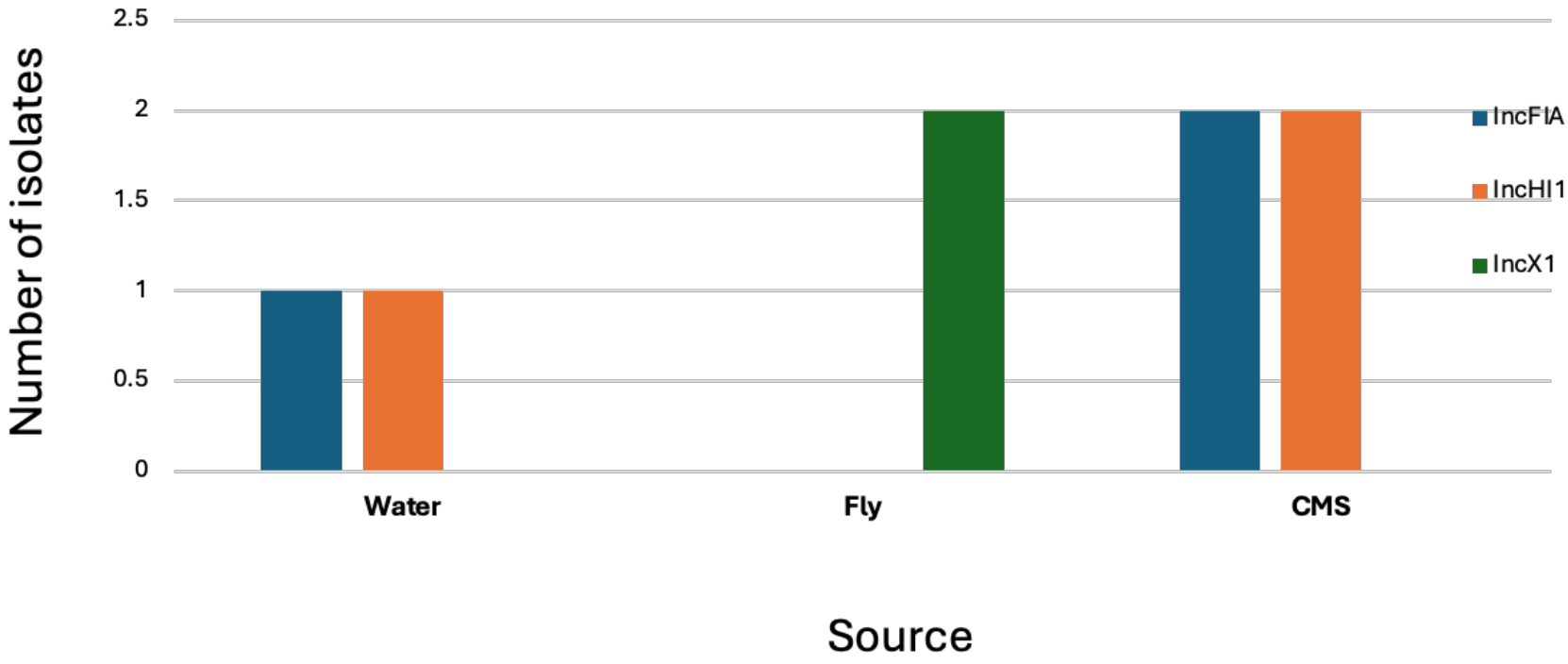


Figure 6.13 Distribution of plasmid replicon types among *E. coli* isolates carrying *tet*(X4) from various sources.

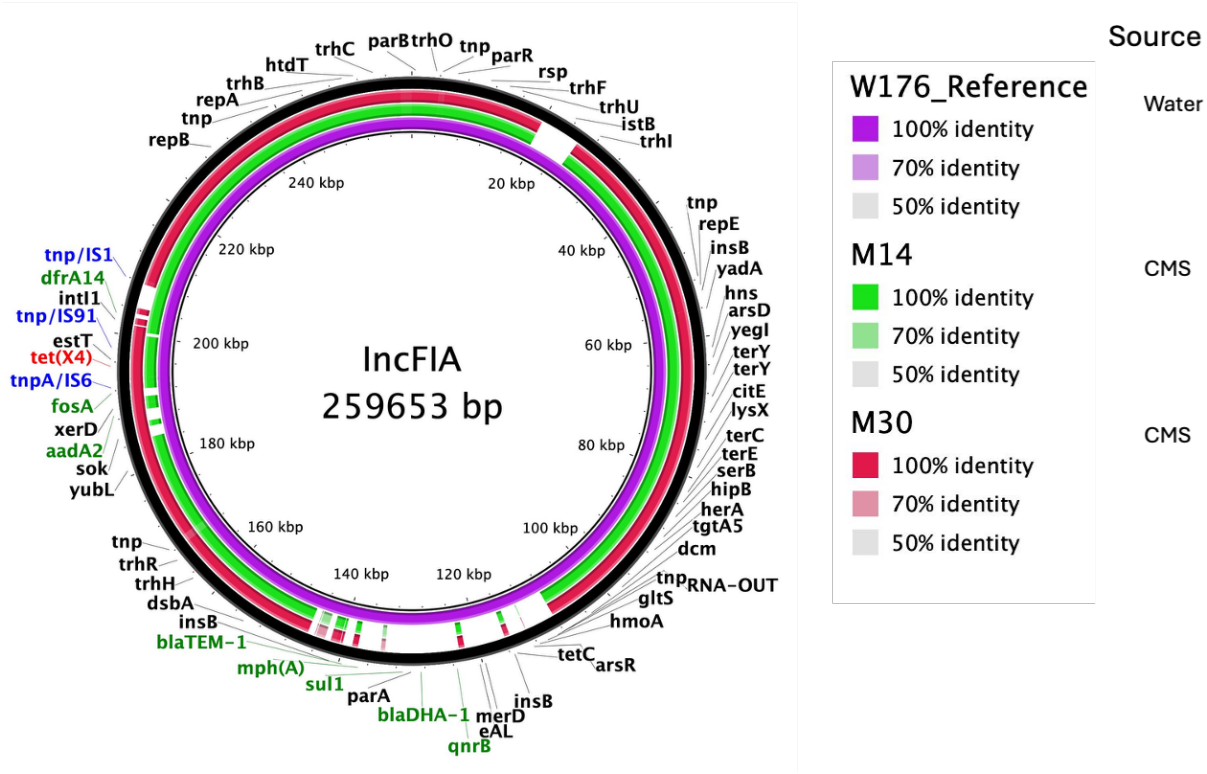


Figure 6.14.A Comparative analysis of plasmids belonging to IncFIA. Circular sequence alignments of reference plasmid (circular complete plasmid identified in this study was used as reference) with homologous contigs of IncFIA plasmids harbouring the *tet*(X4) gene using BLASTn and BLAST Ring Image Generator (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 right side of the ring.

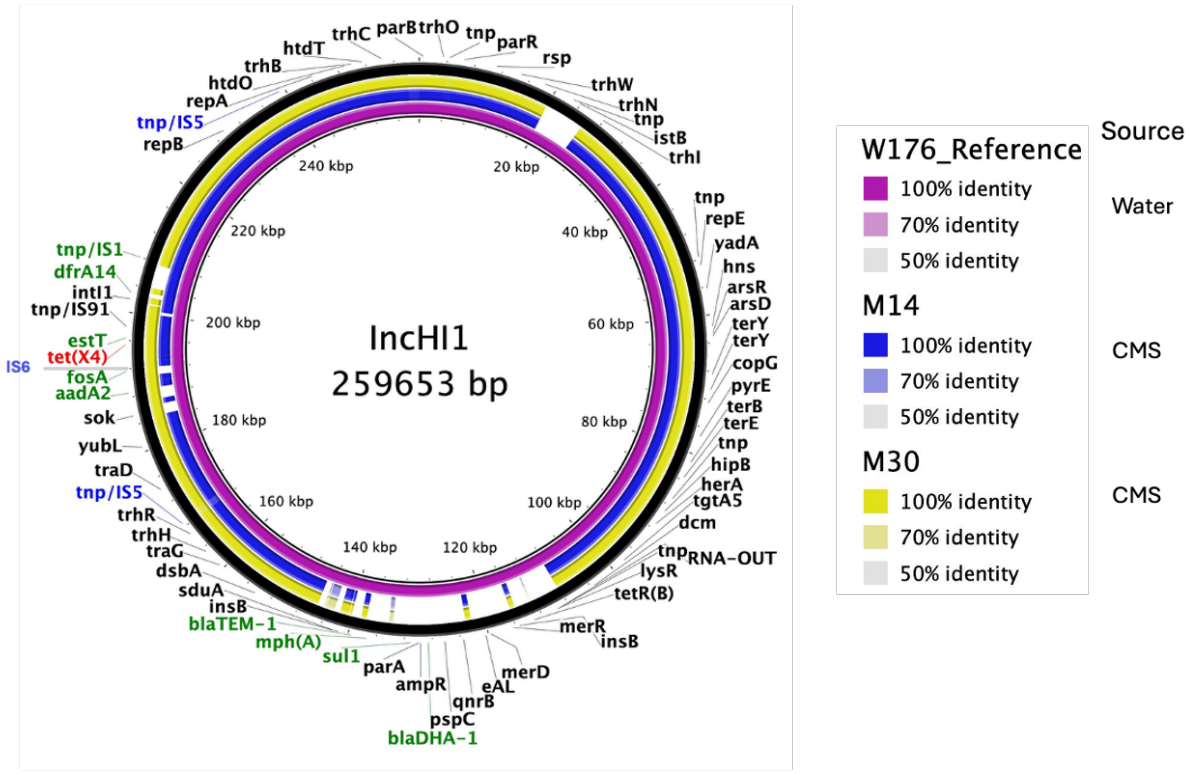


Figure 6.14.B Comparative analysis of plasmids belonging to IncHI1. Circular sequence alignments of reference plasmid (circular complete plasmid identified in this study was used as reference) with homologous contigs of IncHI1 plasmids harbouring the tet(X4) gene using BLASTn and BLAST Ring Image Generator (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 right side of the ring.

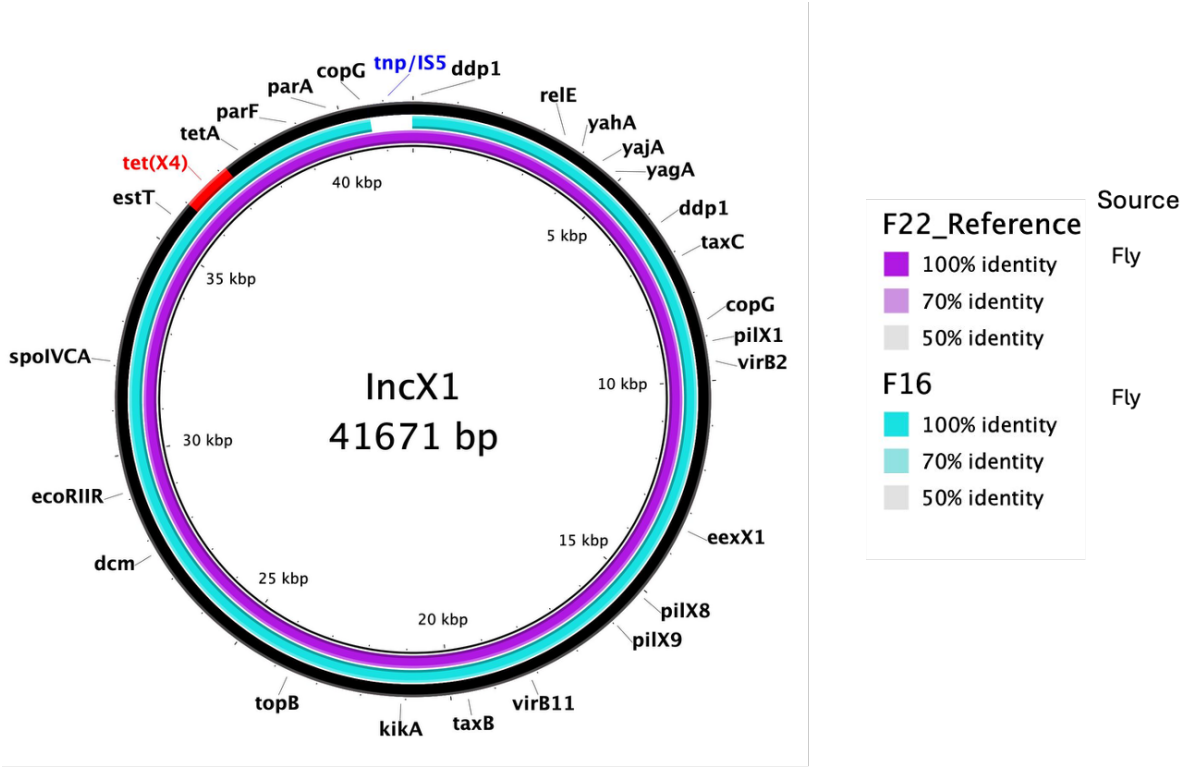


Figure 6.14.C Comparative analysis of plasmids belonging to IncX1. Circular sequence alignments of reference plasmid (circular complete plasmid identified in this study was used as reference) with homologous contigs of IncX1 plasmids harbouring the *tet*(X4) gene using BLASTn and BLAST Ring Image Generator (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 right side of the ring.

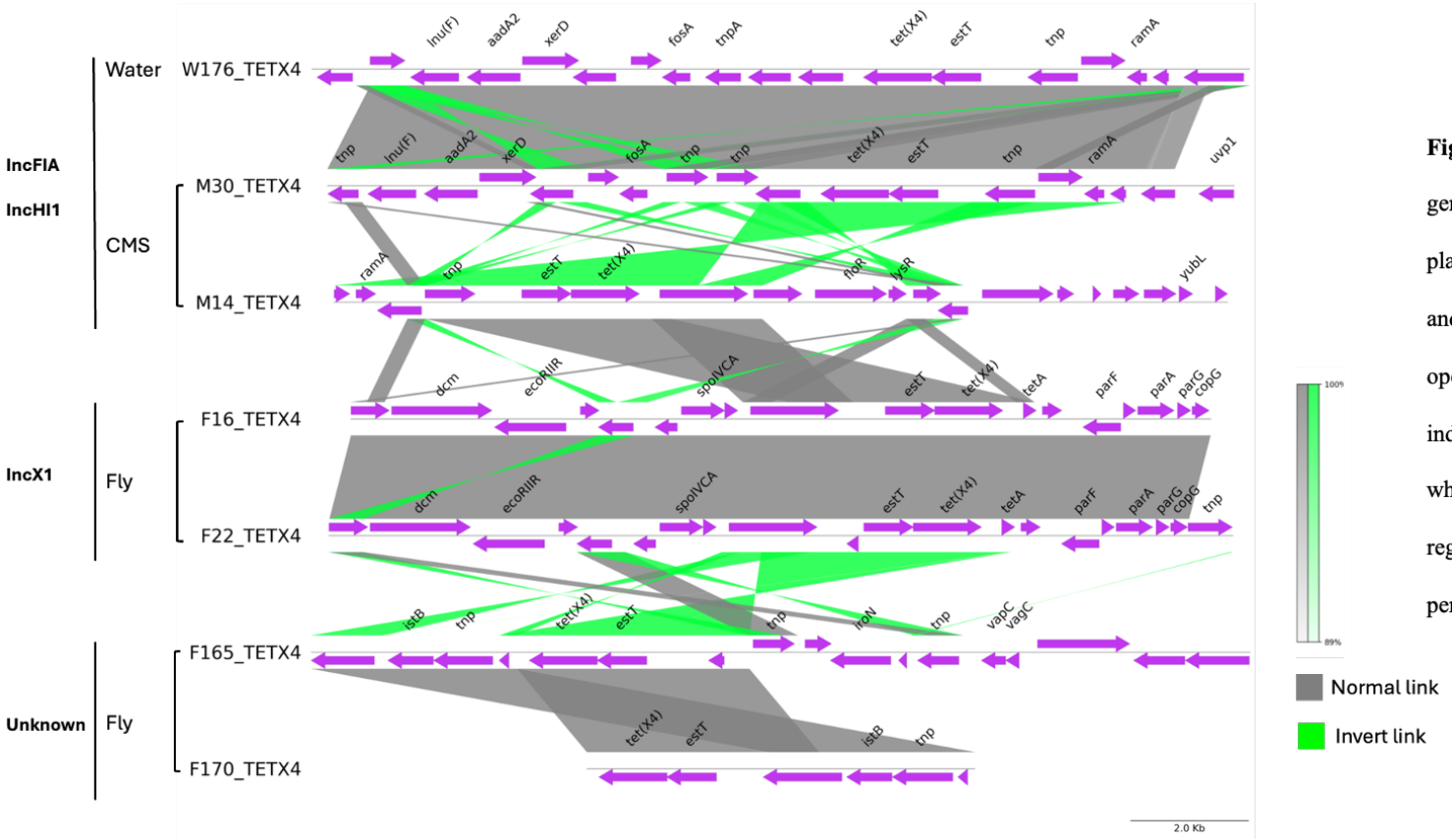


Figure 6.15 Linear comparison of the genetic context of *tet*(X4) on different plasmids. Arrows represent the position and transcriptional direction of the open reading frames. Grey shading indicates collinear homologous regions, while green shading denotes inverted regions. The genomic comparison was performed by pyGenomeViz v1.6.1.

6.3 Discussion

In the present study, *bla*$_{\text{NDM-5}}$–positive *E. coli* belonged to diverse sequence types, including ST167, ST38, ST410, ST648, ST361, ST2083, ST405, ST46, ST448, ST2851, and others, spanning multiple phylogroups and isolated from both clinical and environmental sources such as water and flies. ST167 was the most frequent, occurring in both human and non-human sources, followed by ST38 and ST410. Several of these STs, notably ST167, ST410, ST648, ST361, and ST405, are recognized high-risk international clones associated with the global spread of *bla*$_{\text{NDM-5}}$ (Huang *et al*., 2024; Linkevicius *et al*., 2023). Similar STs have been documented in Bangladesh clinical settings, Hossain *et al*. (2020) reported ST361 and ST410 among uropathogenic *E. coli* (UPEC) isolates carrying *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{NDM-7}}$, while Flatgard *et al.* (2024) detected ST167 in wastewater. Globally, an EU/EEA-wide survey (2012–2022) identified ST167, ST405, ST410, ST361, and ST648 as the predominant *bla*$_{\text{NDM-5}}$–associated sequence types (Linkevicius *et al*., 2023), and large-scale genomic analyses indicate a transition in dominance from traditionally virulent lineages such as ST131 and ST38 towards more resistant clones like ST410 and ST167 (Huang *et al*., 2024). ST410, ST648, and ST361 strains containing *bla*$_{\text{NDM-5}}$ were recovered from companion animals in Thailand (Chanchaithong *et al*., 2025). The emergence of these high-risk clones across environmental and clinical contexts underscores their epidemiological versatility and the importance of continued surveillance in Bangladesh.

The gene encoding *bla*$_{\text{NDM-5}}$ is mainly located on plasmids with diverse replicon types, which are often linked to determinants of multiple antibiotic resistance. The most frequent plasmid Inc types related to *bla*$_{\text{NDM-5}}$ were IncFII, followed by IncFIA and IncFIB. The predominance of IncF-type plasmids is consistent with multiple global and regional reports identifying IncFII, IncFIA, and IncFIB as the major backbones for *bla*$_{\text{NDM-5}}$ dissemination in high-risk *E. coli* clones (Chanchaithong *et al*., 2025; Flatgard *et al*., 2024; Linkevicius *et al*., 2023). Studies

from China, Europe, and Southeast Asia have similarly documented multi-replicon IncF plasmids with some variants co-harbouring other resistance genes such as *fosA3*, *bla*$_{\text{CTX-M-15}}$, and *mcr-1* (Chen *et al*., 2024; Liu *et al*., 2023; Zhao *et al*., 2022). Notably, this study findings of *bla*$_{\text{NDM-5}}$ in environmental water and flies parallel the detection of IncFIA and IncFII plasmids in wastewater, solid waste, and market environments in Bangladesh (Flatgard *et al*., 2024) and retail food sources in other countries (Zhao *et al*., 2022; Liu *et al*., 2023; Zhao *et al*., 2022).

In this study, plasmids carrying *bla*$_{\text{NDM-5}}$ in *E. coli* were predominantly of IncFIA, IncFIB, IncFIC, IncFII, and IncR types, harbouring a conserved 7–8 kb resistance module with *bla*$_{\text{NDM-5}}$ flanked upstream by *mph***,** IS30, and IS6 and downstream by *ble*, IS91, and *sul1*. This arrangement was consistent across multiple plasmid backbones, with IncFII plasmids containing the same elements, and IncR plasmids lacking the *ble* gene. Such architecture contrasts with most global reports, where IS26 is the dominant flanking element surrounding *bla*$_{\text{NDM-5}}$ in IncX3, IncF, and hybrid plasmids (Chanchaithong *et al*., 2025; Turton *et al*., 2022; Zhao *et al*., 2022). For instance, European and Southeast Asian studies frequently describe an IS26–*bla*NDM-5–ble–trpF–tat–ISCR1–sul1–qacEΔ1–aadA2–dfrA12 cassette conserved within high-risk STs such as ST167, ST410, and ST648, while Chinese and Thai isolates often display IS26-mediated transposition within IncX3 or IncFII plasmids. The absence of IS26 in my plasmids and its replacement with IS6, IS30, and IS91 may reflect insertion of varied mobile elements around *bla*$_{\text{NDM-5,}}$ with putatively different transposition events of *bla*$_{\text{NDM-5}}$ found in *E. coli* in this study than the classical dissemination mechanism. Although IS6/IS26 is the best-known driver of resistance gene mobilisation in Enterobacterales other insertion sequences such as IS30 and IS91 have also been detected in multi-resistance plasmids, sometimes linked with *bla*$_{\text{NDM}}$, suggesting they may provide functional redundancy in plasmid adaptation (Varani *et al*., 2021). This finding expands the

known diversity of genetic environments around *bla*$_{\text{NDM-5}}$ and suggests that Bangladesh and neighbouring regions may harbour distinct mobilisation pathways, underscoring the importance of genomic surveillance across environmental and clinical compartments.

The *bla*$_{\text{NDM-5}}$ gene is frequently associated with MDR plasmids, often found alongside other resistance determinants and contributing to wider antimicrobial evasion. For instance, IncF plasmids harbouring *bla*$_{\text{NDM-5}}$ have been implicated in the rapid global spread of carbapenem resistance across human and animal populations, which frequently co-harbour *bla*$_{\text{NDM-5}}$ with ESBLs, aminoglycoside resistance, and sulfonamide resistance genes, resulting in highly resistant MDR plasmids in clinical strains (Zou *et al*., 2020). An additional study of a novel IncFIB-type plasmid from a Chinese clinical isolate demonstrated that *bla*$_{\text{NDM-5}}$ exists within a modular resistance region that also includes *bla*$_{\text{TEM-1B}}$, *sul1*, *dfrA12*, *aadA2*, and *rmtB*, collectively contributing to wide-ranging resistance across antibiotic classes (Han *et al*., 2024). These findings reflect the situation in this study isolates, where *bla*$_{\text{NDM-5}}$ harbouring plasmids also carry multiple ARGs such as *mph*, *tet*(A), *tet*(B), *sul1*, *qnrS1*, *rmtb*, *bla*$_{\text{CTX-M-15}}$ and others reinforcing the role of these plasmids as MDR vectors and aligning with global patterns of resistance gene clustering around *bla*$_{\text{NDM-5}}$.

In this study, eight *E. coli* isolates across diverse sequence types (ST6050, ST16755, ST206, ST10, and two untyped) harboured *tet*(X4) from non-clinical sources: water, flies, and CMS. All isolates belonged to phylogroup A. Plasmid replicon analysis showed IncFIA and IncHI1 predominating in water and CMS isolates, while IncX1 occurred in flies; three isolates lacked recognized replicon types, suggesting the possible existence of yet-uncharacterized plasmid lineages. These findings are consistent with recent research identifying IncHI1 (n = 67), IncX1 (n = 3), and IncFIA(HI1) hybrids (n = 2) as the principal *tet*(X4)-bearing plasmids in *E. coli* from animal-associated environments (Zhang *et al*., 2023). The genetic context varied by backbone: IncFIA/IncHI1 plasmids carried an *esT–IS91* upstream and *fosA–*

IS6 downstream arrangement, whereas IncX1 plasmids showed an *esT–tet(A)* configuration. Both configurations are distinct from the canonical ISCR2–*tet*(X4)–abh–ISCR2 cassette commonly reported in China and globally (Fan *et al*., 2024; He *et al*., 2019). The absence of ISCR2 and the presence of IS91/IS6 in this study plasmid contexts suggest alternate mobilisation mechanisms of *tet*(X4) in Bangladeshi strains.

All *tet*(X4)-positive isolates in this study exhibited a MDR phenotype, co-harbouring additional resistance determinants such as *floR*, *dfrA14*, and *fosA*, mirroring global reports where *tet*(X4) is frequently linked with florfenicol, aminoglycoside, and β-lactam resistance genes Similar MDR genetic linkages have been described in plasmids from China, Vietnam, and Pakistan, where *tet*(X4) is often part of complex resistance regions with multiple insertion sequences, facilitating co-selection under non-tigecycline antibiotic use (Mohsin *et al*., 2021; Sun *et al*., 2019).

South Asian studies report *tet*(X4) in poultry, food, and environmental niches (Mohsin *et al*., 2021); however, Bangladesh data have so far documented only a single *tet*(X4) positive isolate from chicken caeca (Davies *et al*., 2024). As per my knowledge, there is no report in Bangladesh on the presence of *tet*(X4) in water and flies. By identifying *tet*(X4) in water and fly isolates, this findings extend the known Bangladeshi reservoir beyond poultry retail and highlight emergent environmental and vector pathways for the dissemination of tigecycline resistance.

The concurrent detection of $bla_{\text{NDM-5}}$ and *tet*(X4) in *E. coli* from both environmental and non-human sources underscores an alarming expansion of the resistance beyond clinical boundaries. These findings reveal that both resistance genes occur in multi-replicon plasmid backgrounds and co-exist with diverse ARGs, enabling co-selection under multiple antimicrobial pressures. The detection of $bla_{\text{NDM-5}}$ within plasmids containing insertion

sequences such as IS6, IS30, and IS91, alongside the discovery of *tet*(X4) in unique IS91/IS6 contexts, rather than the classical ISCR2 module, suggests an alternative transmission mechanism in Bangladesh's bacterial populations.

While this study provides high-resolution insights into the plasmid-mediated dissemination of bla_{NDM-5} and *tet*(X4), several considerations related to plasmid analysis should be acknowledged. Plasmids carrying multiple replicon types were treated as single circular molecules for comparative analyses rather than being partitioned by replicon. This approach reflects the biological reality of hybrid plasmids and preserves backbone continuity. In isolates lacking detectable replicons, the precise genomic location of bla_{NDM-5} could not be determined and may reflect either chromosomal integration or carriage on divergent plasmids. Nucleotide-level comparisons and BRIG visualisations used representative circular plasmids assembled in this study as internal reference sequences. Although this strategy enables accurate, high-resolution comparison among closely related plasmids, it limits direct inference regarding similarity to globally circulating plasmid lineages. Plasmid replicon identification relied on existing reference databases, and plasmids lacking detectable replicons may represent divergent or previously uncharacterised plasmid backbones.

Globally, the convergence of carbapenem and tigecycline resistance within shared ecological niches represents a worst-case scenario for the continued efficacy of last-resort antibiotics. The findings of this study highlight Bangladesh as an important setting for the emergence and environmental dissemination of high-priority resistance genes, including bla_{NDM-5} and *tet*(X4), which have previously been reported predominantly in clinical or poultry-associated contexts (Shafiq *et al*., 2022; Lu *et al*., 2022). The detection of these resistance determinants across environmental sources underscores the critical need for integrated One Health genomic surveillance to monitor plasmid backbones, genetic contexts, and transmission pathways.

Chapter 7

General discussion and future perspectives

7.1 Unravelling the web of AMR: A One Health perspective

This study combines stands of evidence, ranging from field-based sampling to phenotypic resistance profiling, epidemiological assessment, clonal dissemination analysis, and plasmid characterisation to provide a comprehensive view of AMR within a One Health framework in a confined area in Bangladesh. These findings highlight how human, animal, and environmental reservoirs are not only interconnected but also mutually reinforcing the persistence and spread of AMR and ARGs.

This study also demonstrates the value of adopting a One Health framework for AMR surveillance strategy, aligning with global calls for integrated approaches to tackle this public health challenge. While earlier work in South and Southeast Asia has typically focused on isolated sectors or limited sampling designs (Zhou *et al*., 2022b; Nobel *et al*., 2021; Rousham *et*., 2018; Liu *et al*., 2016), this research illustrates the advantages of simultaneously considering human, animal, and environmental reservoirs. By situating the Bangladeshi context within this wider discourse, present study highlights shared challenges, such as unregulated antimicrobial use, inadequate waste management and a threat to food security and livelihoods due to the emergence of AMR in poultry farming (World Bank, 2025; Zhao *et al*., 2024; Musoke *et al*., 2021). Together, these insights contribute to the global evidence base that effective AMR mitigation requires transdisciplinary surveillance and policies tailored to local realities, an approach embraced by the International Centre of Antimicrobial Resistance Studies (ICARS) (https://icars-global.org).

Across sectors, *E. coli* prevalence and resistance patterns reflect the convergence of microbial, clinical, and socio-economic drivers. Chicken cloacal swabs and domestic animal rectal swabs

showed the highest *E. coli* prevalence rates of 97% and 95%, respectively consistent with other Bangladeshi and international studies (Mudenda *et al*., 2023; Mandal *et al*., 2022). In contrast, the prevalence of *E. coli* in human rectal swab was comparatively lower (80.9%) than expected globally (Martinson & Walk, 2020). Clinical isolates, particularly from SSIs, displayed extensive MDR (>95%), dominated by resistance to third-generation cephalosporins and fluoroquinolones, in line with global AMR surveillance data (WHO, 2022; Tacconelli *et al*., 2018). Prior antibiotic exposure consistently emerged as the strongest predictor of AMR across surgical, urinary, and human rectal swab isolates, echoing global concerns that antimicrobial use is the primary driver of MDR (High-Level Meeting on Antimicrobial Resistance, 2024; Tiseo *et al*., 2020; Woerther *et al.,* 2013).

Genomic analysis further demonstrates the convergence of AMR and virulence across diverse *E. coli* lineages. High-risk clones such as ST131, ST648, ST410, and ST38 were prominent among clinical isolates but also detected in environmental reservoirs, highlighting their potential for cross-host transmission and persistence beyond healthcare settings. The widespread detection of $bla_{\text{CTX-M-15}}$, particularly in ST131, mirrors findings from both Bangladesh and global studies where $bla_{\text{CTX-M-15}}$ remains the dominant ESBL determinant (Abdelrahim *et al*., 2021; Mazumder *et al*., 2020). The emergence of $bla_{\text{NDM-5}}$ in both clinical and environmental isolates underscore its growing epidemiological significance, consistent with regional reports of its circulation in South Asia (Nisa *et al*., 2024; Khan *et al*., 2018). While colistin resistance via *mcr-1.1* was observed at lower frequencies than earlier Bangladeshi poultry studies (Ahmed *et al*., 2020; Islam *et al*., 2020), its persistence in poultry sources continue to pose a risk for foodborne transmission. The detection of *tet*(X4) in water, flies, and chicken meat is particularly concerning, given its plasmid-mediated ability to inactivate tigecycline, and aligns with emerging reports from China and Southeast Asia (Davies *et al*., 2024; Yu *et al*., 2021). Clinical *E. coli* isolates, particularly ST131 and ST73 in

phylogroup B2, harboured multiple ExPEC-associated virulence traits, while poultry and environmental isolates carried a more limited but consistent set of colonisation and iron-acquisition genes, such as *fimH*, *iutA*, and *iroN*, reflecting patterns seen in avian *E. coli* from Bangladesh and India (Kumari, 2024; Saha *et al*., 2020a).

Plasmid analysis revealed the role of mobile genetic elements in amplifying AMR threats across clinical and environmental compartments. *bla*$_{\text{NDM-5}}$ was widely distributed among high-risk clones, such as ST167, ST410, and ST648, exhibiting similar patterns to those observed in global surveillance, where these sequence types dominate carbapenem-resistant *E. coli* (Huang *et al*., 2024; Linkevicius et al., 2023). The detection of *E. coli* positive *bla*$_{\text{NDM-5}}$ in flies and water indicates the spillover of carbapenem resistance beyond hospital settings, in line with recent studies on wastewater in Bangladesh and Southeast Asia (Chanchaithong *et al*., 2025; Flatgard *et al*., 2024). In addition to *bla*$_{\text{NDM-5}}$, the detection of *tet*(X4) in water, flies, and chicken meat points to emerging environmental and foodborne pathways for tigecycline resistance, previously reported only sporadically in South Asia (Davies *et al*., 2024; Mohsin *et al*., 2021). Both *bla*$_{\text{NDM-5}}$ and *tet*(X4) were embedded in multi-replicon plasmids harbouring additional resistance determinants, reinforcing their potential for horizontal transfer and co-selection under antibiotic pressure. Findings from this study parallel global warnings that the convergence of carbapenem and tigecycline resistance in shared ecological niches represents a worst-case scenario for last-resort antibiotics (Korczak *et al*., 2024; Zhang *et al*., 2020). The identification of such plasmid-mediated resistance in clinical, animal, and environmental reservoirs in Bangladesh highlights the urgency of integrated genomic surveillance to monitor and contain these high-risk elements before they become entrenched.

7.2 Tackling antibiotic misuse in a One Health context

While the findings from this study highlight the interconnected reservoirs of AMR, they also raise an urgent question: what underlying drivers sustain and amplify such resistance patterns? One of the most critical issues is the widespread misuse of antibiotics in both the human and animal sectors and one of the most pressing challenges in Bangladesh's AMR landscape. A recent survey found that 56.6% of community pharmacy antibiotic sales occurred without prescription, reflecting the ease of access and lack of prescription enforcement in Bangladesh (Al Masud *et al*., 2024). In rural areas, unqualified village doctors and drug sellers often serve as the first point of care, contributing to inappropriate antibiotic use and incomplete treatment courses (Bepari *et al*., 2023). On the animal side, antibiotics such as tetracyclines and fluoroquinolones are commonly used for treatment and prophylaxis (disease prevention), with a smaller but notable proportion used as growth promoters in poultry and livestock production (Chowdhury *et al*., 2022; Imam *et al*., 2020). The overlapping use of the same antimicrobial classes in human and veterinary medicine further accelerates AMR transmission across reservoirs. The interconnectedness of these practices is best illustrated through a One Health framework, where antibiotic misuse in any sector can drive AMR across humans, animals, and the environment sectors (Figure 7.1).

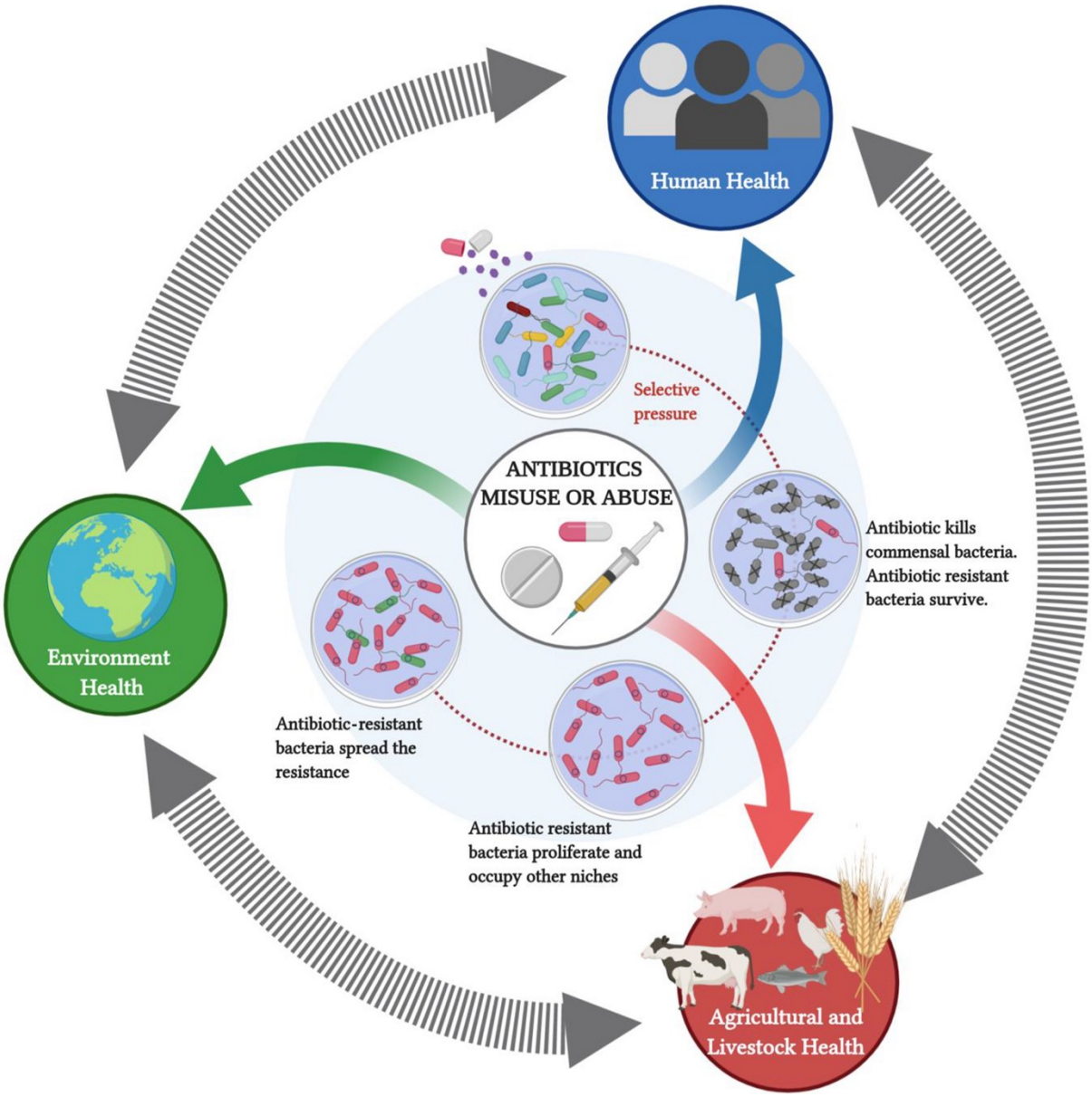


Figure 7.1 Pathways of AMR dissemination across human, animal, and environmental sectors. Conceptual schematic created by the author, synthesising information from Walsh (2018).

While this broad perspective illustrates how antibiotic misuse drives antimicrobial resistance across different reservoirs, the underlying microbiological mechanisms are equally important. Antibiotic pressure preferentially kills or suppresses susceptible bacterial populations, while resistant subpopulations survive and proliferate. Sub-therapeutic antibiotic treatment is particularly problematic because it exposes bacteria to antibiotic concentrations insufficient to

eradicate the entire population, thereby creating a selective environment that favours the survival of partially resistant or tolerant cells (Andersson & Hughes, 2014). These surviving bacteria may carry pre-existing resistance determinants or acquire additional mutations and mobile resistance elements during prolonged exposure (Perry & Wright, 2013). Incomplete or inappropriate treatment, whether in clinical care or agricultural settings, directly accelerates the selection, amplification, and persistence of multidrug-resistant organisms (Figure 7.2).

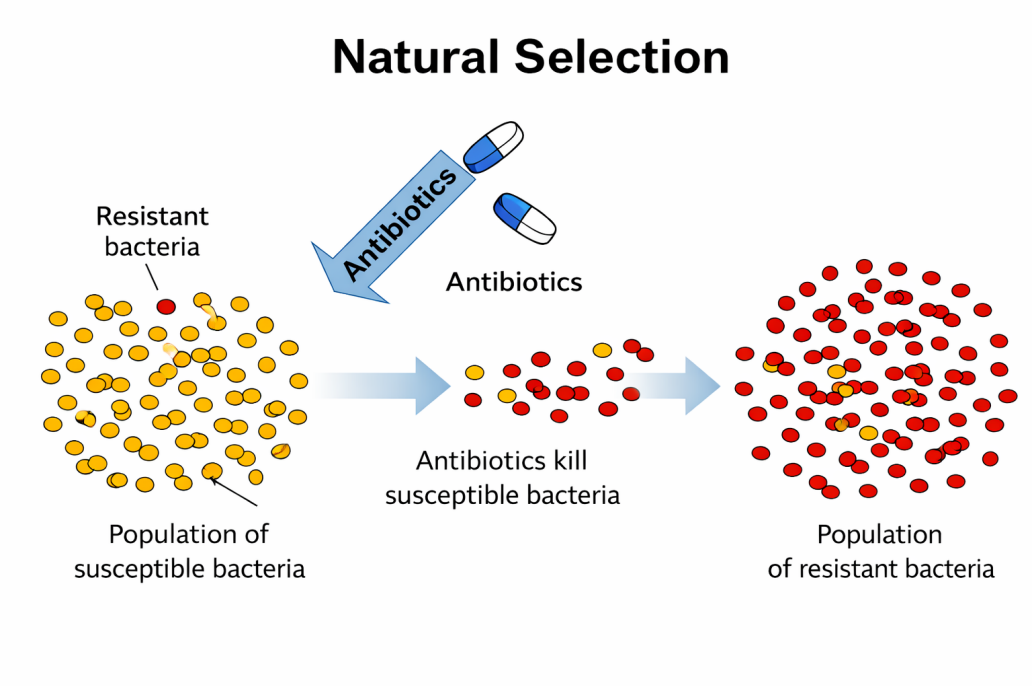


Figure 7.2 Natural selection of AMR bacteria under antibiotic pressure. Antibiotics eliminate susceptible bacteria, allowing AMR strains to dominate. (Figure was produced using Microsoft PowerPoint).

Recognising these risks, Bangladesh has introduced regulatory measures in recent years. The Fish Feed & Animal Feed Act (2010) and Animal Feed Rules (2013) prohibited antibiotic growth promoters in livestock production; in 2019, combination veterinary products containing colistin were withdrawn, and in 2022, the Directorate General of Drug Administration (DGDA) formally banned colistin use in food animals (DGDA, 2022). The Bangladeshi

National Strategy and Action Plan for AMR 2021–2026, approved in 2022, emphasizes stricter enforcement of prescription-only sales and improved pharmacovigilance, while FAO- and WHO-supported stewardship initiatives promote rational antimicrobial use in veterinary practice (WHO, 2022; FAO, 2021). According to the Bangladesh AMR Multi-Partner Trust Fund (MPTF) report, the Bangladesh AMR Response Alliance (BARA) expanded its in-service training program in 2024 to include veterinarians and human health practitioners. This training focused on AMR and antimicrobial usage (AMU) and was delivered as a three-day Continuing Professional Development (CPD) course across Dhaka, Savar, and other parts of the country. In parallel, the BARA has been training farmers on the rational use of antibiotics and alternative farming practices, aiming to reduce reliance on antimicrobials for prophylaxis and growth promotion. Yet enforcement remains inconsistent, and informal antibiotic-sales markets continue to thrive. The persistence of the over the counter (OTC) antibiotics sales, coupled with limited public awareness and weak monitoring capacity, undermines these policy advances.

Against this backdrop, findings from present study underscore the consequences of unregulated antibiotic use, with high rates of MDR among *E. coli* isolates from both humans and poultry. Although the percentage of colistin-resistant isolates was lower than that reported in some previous studies, this reduction may reflect recent regulatory measures restricting the use of colistin in food-producing animals, including bans or tighter controls on its use as a growth promoter and for prophylaxis. These results provide locally relevant evidence to strengthen antimicrobial stewardship initiatives, inform DGDA enforcement priorities, and support integrated One Health approaches that address antibiotic misuse through regulation, education, and the promotion of sustainable alternatives for farmers and communities.

7.3 Sanitation under the microscope: Implications for One Health in Bangladesh

Antibiotic misuse is only one component of the AMR landscape. Even in contexts where antibiotic use is relatively controlled, inadequate sanitation and unsafe water systems can sustain faecal–oral transmission pathways that facilitate the persistence and spread of resistant *E. coli*. Within a One Health framework, sanitation and hygiene therefore remain important contextual factors for understanding AMR dynamics in Bangladesh.

Although sanitation was not presented as details in the Results chapter, data about sanitation status were collected and variables related to drinking water source**,** toilet facilities, and access to water and soap at the toilet were included in the risk assessment analyses conducted among SSI, UTI patients and healthy human volunteers (Tables 4.9, 4.11, 4.13a and 4.13b). Across antibiotics and study populations, these sanitation-related indicators did not show statistically significant associations with antimicrobial resistance.

The study population was not intended to be nationally representative with respect to WASH access; rather, sanitation variables were included to explore potential household-level risk patterns within the sampled cohorts. Maximum participants reported access to a toilet facility and a drinking water source, although the type of facility varied. This relative homogeneity in basic WASH access may have limited the ability to detect statistically significant associations between sanitation indicators and antimicrobial resistance, particularly in settings where exposure pathways extend beyond individual households.

National-level data nonetheless provide important context for interpreting these findings. According to the WHO/UNICEF Joint Monitoring Programme, approximately 98% of the Bangladeshi population has access to at least basic drinking water services, yet only 42–43% benefit from safely managed water, with microbial and arsenic contamination remaining widespread (UNICEF & WHO, 2021). Sanitation access is more uneven: while 59% of the population uses basic sanitation facilities, only around 39–41% have safely managed sanitation, and a large proportion of toilets lack appropriate faecal sludge management (Bangladesh Bureau of Statistics, 2022; GWSC, 2025). These deficiencies create persistent environmental reservoirs of faecal bacteria that facilitate AMR circulation beyond the household level. At a population level, inadequate sanitation and unsafe water systems create multiple interconnected pathways through which resistant bacteria can circulate between the environment, humans, and animals. Key national trends in access to drinking water and sanitation services in Bangladesh are summarised in Table 7.1.

Table 7.1 National trends in water and sanitation access in Bangladesh (contextual background data).

Year	Access to improved water (%)	Safely managed water (%)	Improved sanitation (%)	Safely managed sanitation (%)	Open defecation (%)
2000	77.0	20.0	39.0	10.0	~30.0
2010	81.0	30.0	46.0	20.0	21.5
2015	87.0	35.0	61.0	28.0	3.0
2019	98.5	42.6	64.4	39.0	1.0
2022	98.0	43.0	69.0	41.0	<1.0

Sources: Data compiled from UNICEF & World Health Organisation (2021); Bangladesh Bureau of Statistics (2011,2019, 2022)

Urban centres such as Dhaka face acute challenges related to faecal sludge management, with substantial volumes of untreated waste released into surface waters each day, contributing to environmental contamination (UNICEF & WaterAid, 2025). In rural areas, reliance on shallow tubewells remains high, and many sources classified as "improved" are contaminated with faecal bacteria (Bangladesh Bureau of Statistics, 2019). Schools and marginalised communities further illustrate persistent inequities in access to functional water, sanitation, and hygiene facilities (Alam & Sheoti, 2024).

Within the present study, drinking water exposure was assessed through reported source type rather than microbiological testing. While this approach did not reveal significant associations with resistance, multiple studies in Bangladesh have documented widespread *E. coli* contamination in both source and stored drinking water, often linked to inadequate sanitation and unsafe handling practices (Mahmud *et al*., 2020; Luby *et al*., 2015). Gendered sanitation barriers further compound exposure risks, with limited access to hygiene facilities contributing to urogenital infections frequently caused by *E. coli* (Neugent *et al*., 2020; Alam *et al*., 2017; Sommer *et al*., 2016; Das *et al*., 2015).

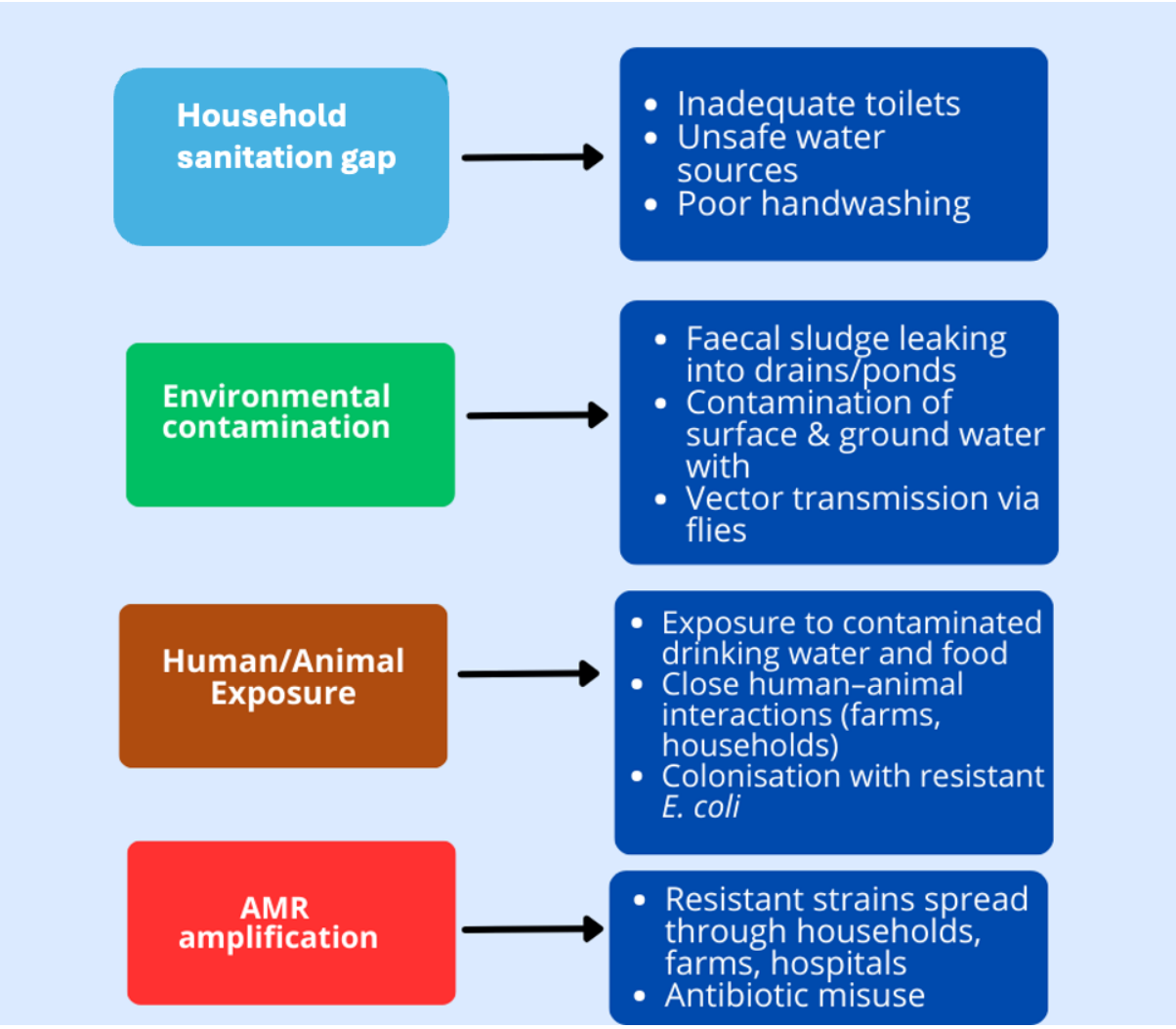


Figure 7.3 Flowchart showing the pathways linking sanitation gaps to AMR amplification in Bangladesh.

The conceptual pathways linking sanitation gaps to environmental contamination, human–animal exposure, and AMR amplification are summarised in Figure 7.3**.** While household-level sanitation indicators did not demonstrate strong associations with resistance in this study, these pathways remain highly relevant at community and population scales. Collectively, these findings underscore the need to integrate sanitation and hygiene considerations into One Health AMR surveillance frameworks alongside antimicrobial stewardship, with particular emphasis on faecal sludge management, water quality monitoring, and equitable access to WASH services in Bangladesh.

7.4 Emergence of *tet*(X4) in Bangladesh: Expanding the AMR threat

These environmental gaps not only perpetuate existing resistance but also create pathways for the emergence of novel and clinically important ARGs. The detection of *tet*(X4) in Bangladesh exemplifies this growing threat. One of the most notable findings of this study is the detection of *tet*(X4)-positive *E. coli* in environmental and non-clinical sources in Bangladesh, including water, flies, and poultry samples. *tet*(X4), first reported in China in 2019 (He *et al*., 2019), confers resistance to tigecycline, a last-resort antimicrobial often reserved for MDR infections. While *tet*(X4) has been documented in multiple countries across Asia, Europe, and Africa, its presence in Bangladesh has been limited to isolated reports from poultry (Davies *et al*., 2024). To my knowledge, this study is among the first to demonstrate its occurrence in environmental reservoirs such as water and flies, thereby expanding the potential pathways for dissemination beyond the food chain.

The environmental detection of *tet*(X4) is particularly concerning, as plasmid-mediated transmission enables rapid horizontal gene transfer between commensal and pathogenic strains, thereby increasing the risk of AMR community-level spread (Mohsin *et al*., 2021). This risk is amplified by the extensive and largely unregulated use of tetracyclines in Bangladeshi poultry and livestock production, where they are widely administered not only for therapeutic purposes but also for prophylaxis and growth promotion. Such practices create sustained selective pressure, which may facilitate the co-selection and persistence of *tet*(X4) even in the absence of tigecycline use (Chowdhury *et al*., 2022). Importantly, although tigecycline itself is not used in veterinary medicine, exposure to older tetracyclines can still select for *tet*(X4), thereby indirectly promoting tigecycline resistance in bacterial populations.

Given Bangladesh's dense population, limited wastewater treatment infrastructure, and widespread misuse of antibiotics in both humans and livestock, conditions are conducive to the

establishment of *tet*(X4) within local microbial communities. The convergence of *tet*(X4) with other resistance determinants, as observed in this study, allows for co-selection under non-tigecycline antimicrobial pressure. These findings underscore the urgent need to integrate tigecycline resistance into routine AMR surveillance in Bangladesh and highlight the critical role of environmental monitoring within a One Health framework.

7.5 Strengthening One Health AMR surveillance: From gaps to integrated action

The emergence of such MDR underscores the pressing need for robust surveillance systems. Without integrated One Health monitoring, signals like *tet*(X4) may remain undetected until they are already widespread. While this study underscores the interconnected nature of AMR across human, animal, and environmental reservoirs, translating such insights into sustained national surveillance remains challenging. Despite Bangladesh's formal commitment to One Health surveillance and participation in global initiatives like GLASS, data representation remains limited by uneven quality and poor integration across sectors. Additionally, surveillance is hindered by community-level challenges in sample and data collection (Joh *et al*., 2023a; CAPTURA/IEDCR, 2023). The National AMR Surveillance Strategy 2020–2025 (DGHS, 2020) commits to a coordinated One Health system and GLASS reporting, but human-health surveillance still relies on a small network of sentinel hospitals with uneven geographic coverage and variable data quality, while animal and environmental surveillance streams remain only partially integrated. Recent IEDCR reports indicate that case-based surveillance has been conducted at 9–11 sentinel sites since 2017, utilising a public dashboard. However, coverage and representativeness beyond participating facilities remain limited, and linkage across sectors is still in development.

International support has been crucial in building this capacity. The UK-funded Fleming Fund has played a pivotal role in strengthening laboratory systems, training personnel, and improving data flows in LMICs, including Bangladesh. Its closure in 2026 might leave critical gaps in AMR surveillance, risking the sustainability of data systems and slowing progress in integrating human, animal, and environmental surveillance (Burki, 2025).

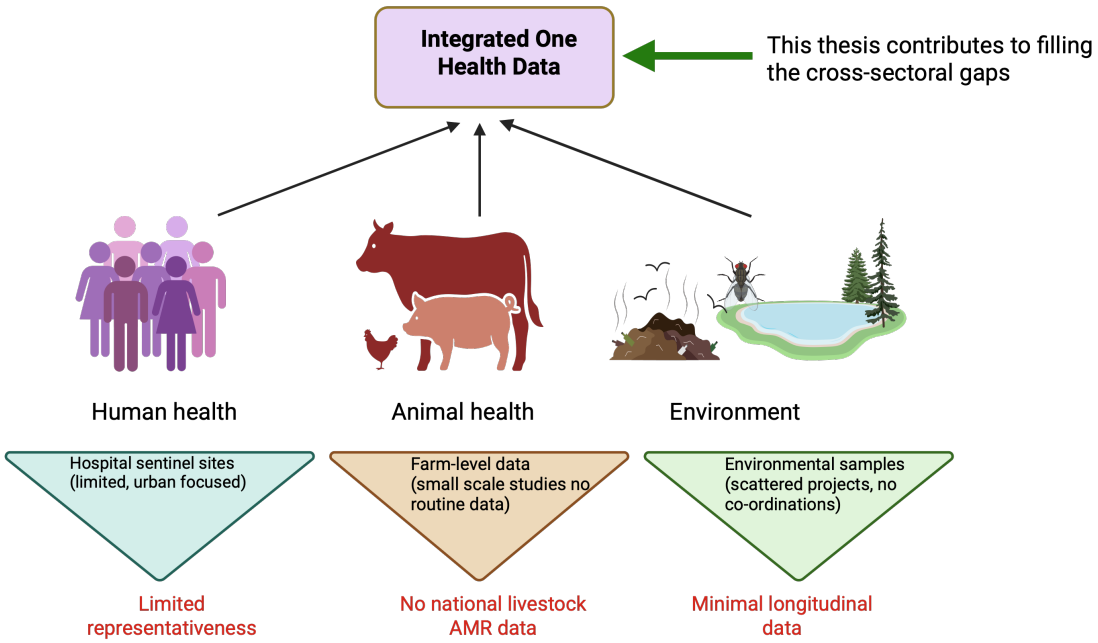


Figure 7.4 Gaps in current AMR surveillance in Bangladesh. Existing systems rely heavily on urban hospital sentinel sites, lack national livestock data, and have minimal environmental coverage, resulting in fragmented and poorly integrated One Health data. (Figure was created with BioRender.com).

Independent assessments such as the CAPTURA project have confirmed these weaknesses, showing that many laboratories still rely on paper records, have inconsistent antibiotic susceptibility testing (AST) practices, and possess incomplete metadata, which hinders

harmonised analyses and the development of national antibiograms (Holm *et al*., 2023; Joh *et al*., 2023a). Even where laboratory information systems exist, poor data entry often prevents usable exports. Bangladesh participates in GLASS, but persistent heterogeneity in inputs and constrained specimen coverage, reflect common LMIC challenges. Notwithstanding, several initiatives are working to strengthen capacity. For example, WHO is supporting targeted AMR surveillance in Cox's Bazar, FAO is promoting stewardship programmes in the livestock sector, and the BARA is leading professional training and awareness efforts. However, a fully integrated and routinely linked cross-sector dataset is still not in place and remains more of an aspiration than a reality. The National Action Plan (NAP) on AMR 2021–2026 (DGHS, 2021) has further emphasised integrated surveillance, prudent antimicrobial use, and multisectoral collaboration, but translation into practice remains uneven. Against this context, this study provides one of the more comprehensive integrated datasets to date from Bangladesh, combining human clinical and community carriage isolates with animal and environmental samples, enriched with phenotypic, genomic, and epidemiological data. These findings not only complement existing national surveillance but can also inform submissions to GLASS, contribute to FAO/WOAH stewardship efforts (Aboushady *et al*., 2023; Joh *et al*., 2023b).

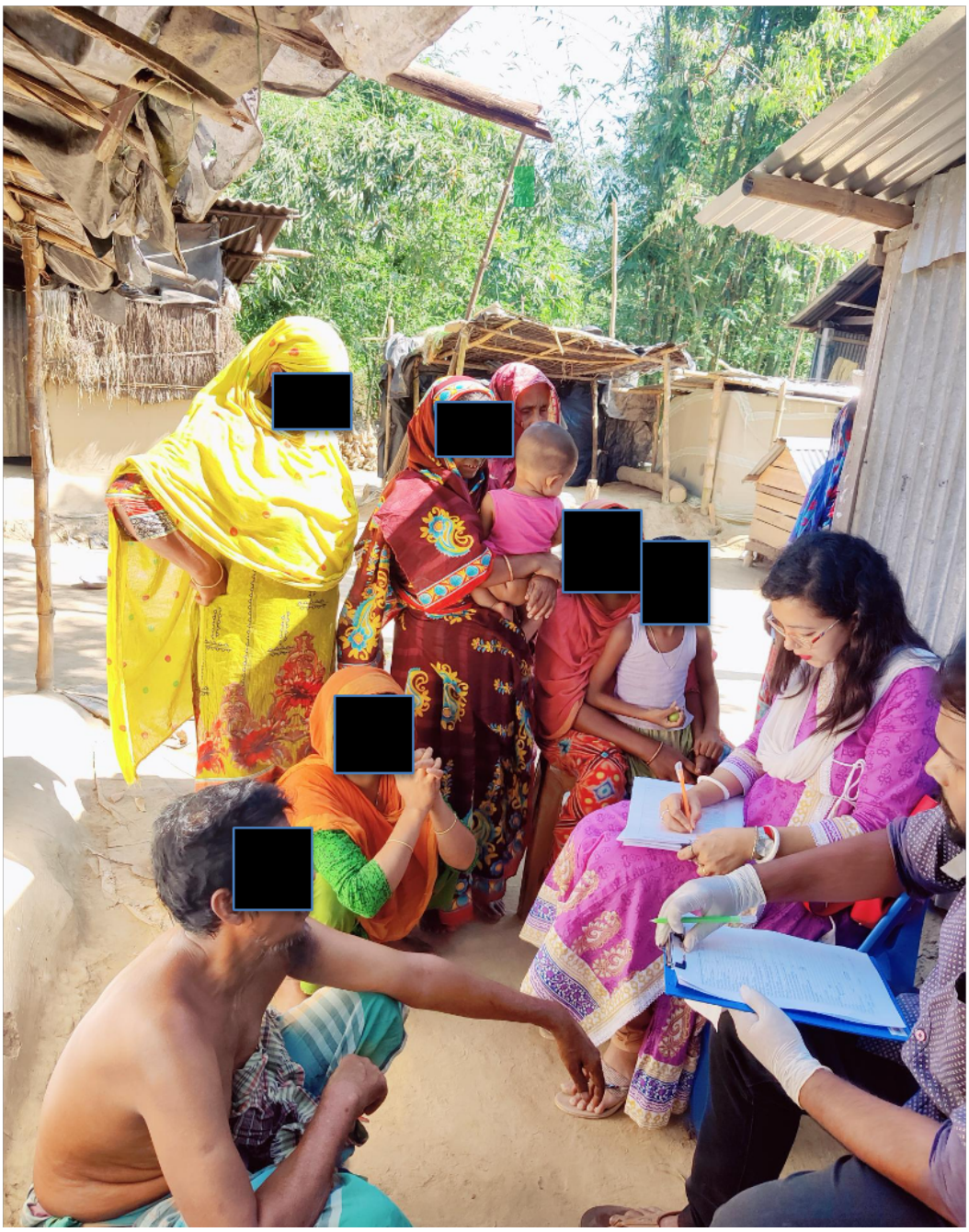
7.6 Ground realities of One Health AMR research: Challenges in sampling

However, building such systems requires acknowledging the realities on the ground. My own fieldwork revealed the social, cultural, and logistical barriers that shape the feasibility of One Health AMR surveillance in Bangladesh. Conducting large-scale One Health AMR research in Bangladesh also revealed the practical and social challenges that accompany field-based sampling. I personally travelled across the sampling sites to collect samples and epidemiological data, where awareness of AMR remains limited.

Figure 7.5 Water sample collection sites. Representative images of water sampling from diverse sources, including ponds, rivers, canals, and drains, located around farms and hospitals in the study area

Sample collection across human, animal, and environmental compartments required navigating diverse social and operational contexts. Environmental sampling involved collecting water from ponds, rivers, canals, and drainage systems surrounding farms and Mymensingh Medical College Hospital to capture heterogeneous contamination sources (Figure 7.5). In the human component, recruitment for rectal swab sampling highlighted important acceptability barriers. Many participants required detailed explanation and reassurance regarding the purpose of sampling, data use, and confidentiality. Hesitation was particularly evident among women, reflecting broader cultural norms, privacy concerns, and gendered sensitivities associated with

invasive sample collection. Despite extensive engagement and trust-building, some individuals declined participation, underscoring persistent knowledge gaps, stigma, and misconceptions related to AMR research (Figure 7.6). Importantly, participants were not provided with financial compensation, which may have further influenced willingness to participate, particularly given the time required for discussion and sample collection.



7.6a

7.6b

7.6c

Figure 7.6 Community engagement with healthy volunteers. **a.** The first image shows discussions with local residents to explain the study and encourage participation. **b.** In the second image, a few women initially showed interest. **c**. But eventually, only one agreed to provide rectal swabs and data. The third image also captures the process of recording information and obtaining consent from the volunteer. This figure illustrates the challenges

encountered during human rectal swab collection across different sampling sites throughout the study. Photograph taken by a member of the sampling team.

For the chicken meat swab collection, it was also challenging to convince both the buyer of the chicken and the seller to collect the swab samples, despite all hygienic measures being well-adapted (Figure 7.7). Collecting domestic animal rectal swabs from grazing animals around the farms was also very difficult due to handling and restraining the animals for sampling, as well as convincing the owner of the animal (Figure 7.8).

Figure 7.7a Picture of one of the wet meat markets, from where I sampled. This represents most of the wet meat market in Bangladesh, where the hygienic practice is low. Photograph by the author.

Figure 7.7b Collection of chicken meat swab samples from a wet poultry market, conducted with the consent of buyers. Photograph taken by a member of the sampling team.

7.8a

7.8b

Figure 7.8 Sampling site for domestic animal rectal swabs, showing goats and cattle reared near poultry farms in the study area. The circles show the poultry farms and a farmer's house. Photograph by the author.

Compounding these challenges, the study commenced immediately after the COVID-19 pandemic, and the initial tranche of sampling in December 2021 was particularly challenging, as communities were cautious about close contact, and these safety precautions further slowed progress. Ironically, despite these measures, the intensity of fieldwork interacting with many individuals in diverse settings eventually led to my own infection with COVID-19 at the end of the first term, which was just the day before my departure to the UK and required

rescheduling my flight, causing a slight delay in starting my lab work at Oxford. This temporary personal setback highlighted the occupational risks inherent in field-based AMR studies. Ultimately it was manageable managed to collect all the targeted samples and data despite all these difficulties and challenges. Together, these experiences shifted the narrative of this thesis from laboratory analysis to the real-world realities of conducting One Health research in Bangladesh. They demonstrate how cultural acceptance, socio-economic instability, and unforeseen global crises shape scientific ambitions. More importantly, they suggest that successful AMR surveillance in LMICs will require not only technical skills but also social engagement strategies, community trust-building, and resilient planning to withstand practical disruptions. Similar challenges have been reported in other low- and middle-income settings, where stigma, mistrust of research activities, and socio-economic vulnerabilities complicate participation in community-based surveillance (Omulo *et al*., 2021; Okeke, 2010). To close this gap, engagement strategies must be tailored and developed jointly with local leaders and health workers. These strategies should explain AMR surveillance in simple language, protect participant confidentiality, and share results with the communities involved. Embedding social science approaches into AMR fieldwork could also help bridge these divides by identifying perceptions, fears, and incentives that shape participation. Recognising and addressing these "ground realities" is critical if Bangladesh is to scale up truly representative One Health surveillance and generate reliable data that can inform local NAPs and global AMR action.

7.7 Reflections on study limitations

While this study identifies several potential interventions, it is also essential to consider its limitations. Like any complex field-based investigation, this work has constraints that should be acknowledged when interpreting the findings.

Firstly, although the study employed a One Health approach with extensive sampling across humans, animals, and environmental sources, it was geographically restricted to the Mymensingh district and therefore may not fully capture the national diversity of AMR dynamics in Bangladesh.

Secondly, rectal swabs from healthy human volunteers were self-collected, which may have resulted in suboptimal sampling and under-detection of *E. coli*. In contrast, the numbers of UTI and SSI samples exceeded the recommended sample sizes. These patient samples were collected continuously throughout the study period (December 2021 to March 2023) to allow assessment of seasonal variation. Ethical considerations required that all eligible patient samples submitted to the microbiology laboratory during this period be included, as diagnostic testing and reporting were integral to clinical care. Sampling of bird faeces was also limited and did not reach the recommended sample size due to the restricted availability of free-flying birds during the sampling period.

Thirdly, data on antibiotic use in humans and animals were primarily based on participant recall and farm observations rather than verified prescription or pharmacy records, raising the possibility of recall bias and underreporting.

Fourthly, strain transmission inferences were based on single-colony isolates, which may not fully capture within-host genetic diversity and could therefore underestimate transmission events.

Fifthly, antimicrobial susceptibility testing for colistin was performed using the agar dilution method rather than the broth microdilution method recommended by international guidelines. This approach was adopted to maintain methodological uniformity across minimum inhibitory

concentration assays for the full antibiotic panel and to ensure feasibility when processing a large number of isolates.

Sixthly, whole-genome sequencing was performed for all 1,634 *E. coli* isolates recovered in this study, providing comprehensive genomic coverage across study populations. However, long-read sequencing was conducted for a selected subset of 65 isolates carrying epidemiologically and clinically important resistance determinants, identified through short-read sequencing. This targeted strategy was implemented due to time and resource constraints and prioritised isolates of highest public health relevance.

Finally, although phylogenetic and plasmid analyses revealed overlaps among human, animal, and environmental isolates, definitive evidence of direct transmission would require long-term, longitudinal source-tracking studies incorporating higher-resolution genomic approaches.

In addition, the sudden termination of my fellowship funding at the end of the third year, due to political unrest in Bangladesh, created further challenges. It caused a few delays in subsequent analyses, as I had to seek alternative funding, and also imposed considerable personal stress. Despite these limitations, this study still provides one of the most comprehensive datasets on *E. coli* AMR in Bangladesh.

7.8 Future horizons: Positioning Bangladesh in the global fight against AMR

Despite the constraints outlined above, the dataset and experience generated through this study open substantial opportunities for both immediate follow-up work and longer-term strategic development. This study provides one of the most comprehensive One Health datasets on *E. coli* in Bangladesh to date, encompassing 1,634 isolates supported by extensive phenotypic,

genomic, and epidemiological information. Beyond addressing the primary research objectives, this collection constitutes a valuable national resource that can be further developed to strengthen AMR surveillance, research capacity, and policy engagement.

In the immediate term, several specific extensions could directly enhance and refine the current study. First, expansion of long-read sequencing to additional isolates, particularly those sharing closely related core genomes or resistance profiles, would improve resolution of plasmid diversity, resistance gene mobility, and horizontal transfer events. This would allow confirmation of whether resistance determinants observed across hosts are carried on shared plasmids or arise through independent acquisition. Second, inclusion of environmental microbiological sampling, particularly of drinking water, would address a key limitation of the present work and enable more definitive tracing of transmission pathways across the human–animal–environment interface.

Third, future sampling efforts could prioritise longitudinal follow-up of selected households, farms, and clinical sites to capture temporal dynamics of AMR acquisition and persistence. Repeated sampling would enable differentiation between transient carriage and stable colonisation, as well as more robust inference of strain transmission. Fourth, targeted resampling of wildlife, especially birds, during periods of greater availability would strengthen assessment of wildlife-associated AMR reservoirs, which were underrepresented in the current study due to field constraints.

Analytically, the existing dataset offers immediate opportunities for deeper investigation. Pan-genome and phylogenomic analyses could be extended to identify host-adapted lineages, high-risk clones, and resistance-associated accessory genes. Integrating genomic data with refined exposure variables, such as antibiotic class–specific use, farm management practices, and sanitation quality rather than access alone, could improve sensitivity for detecting risk

associations. In addition, harmonising colistin susceptibility testing with broth microdilution in future work would allow direct comparison with international datasets and further validate resistance estimates.

At the national level, the findings and genomic resources generated here can contribute directly to Bangladesh's National AMR Action Plan and ongoing BARA- and FAO-supported initiatives. The dataset can serve as a reference framework for integrating genomic surveillance into routine clinical, veterinary, and environmental monitoring, helping to bridge existing gaps between sectors. Public deposition of genome sequences in platforms such as NCBI Pathogen Detection, Pathogenwatch, and WHO GLASS would further embed Bangladesh within global genomic AMR surveillance efforts and facilitate international comparison.

In the longer term, this work provides a foundation for establishing a national genomic AMR research hub in Bangladesh. Such a hub could support advanced sequencing, bioinformatics training, and coordinated One Health research, while fostering collaboration between academic institutions, diagnostic laboratories, and policy stakeholders.

In summary, this PhD advances beyond fragmented evidence to deliver an integrated One Health perspective on AMR in Bangladesh, while also identifying clear, actionable next steps for strengthening surveillance and research. By combining field epidemiology, phenotypic profiling, and genomic analysis, the study establishes a scalable framework that can be immediately refined and expanded. Through sustained investment, targeted methodological improvements, and cross-sectoral collaboration, Bangladesh has the potential to transition from a data-scarce setting to a regional leader in One Health genomic AMR surveillance, ensuring that locally generated evidence informs both national policy and global strategies to combat AMR.

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Zuhora, F. T., Hosen, M. A., & et al. (2023). Molecular characterization of multidrug-resistant bacteria isolated from the external and internal parts of the housefly. Journal of Advanced Biotechnology and Experimental Therapeutics, 6*(3),* 597–609. https://doi.org/10.5455/jabet.2023.d156

Appendices

Appendix A: Ethical approvals

Animal Welfare and Experimentation Ethics Committee
Bangladesh Agricultural University
Mymensingh-2202, Bangladesh
E-mail: aweec@bau.edu.bd
Fax: +880-91-61510

Animal Welfare and Ethical Committee Estt. 2017 BAU, Mymensingh-2202

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:

BA168DF4624B463...

Dr Karen Melham
Sponsorship and Ethics Lead
for
Research Ethics Manager, OxTREC

Appendix B

Case Record Form (CRF) and consent form for collecting data from human participants

Case Record Form

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.

Consent form (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 আঞ্জমান 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:	অংশগ্রহণকারীর নাম:
সাক্ষাৎকার গ্রহণকারীর স্বাক্ষর:	অংশগ্রহণকারীর স্বাক্ষর:
তারিখঃ	তারিখঃ