Acquisition and loss of carriage of antimicrobial resistant Enterobacterales among vulnerable children in Kenya: clinical and genomic determinants

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ABSTRACT

Antimicrobial resistance (AMR) is a critical global health challenge, with sub-Saharan Africa experiencing the highest burden of AMR-related mortality. In hospitals, extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) and carbapenem-resistant Enterobacterales (CRE) significantly threaten treatment efficacy, particularly in low-resource settings with limited infection control measures. These pathogens are often carried asymptomatically in the gut, with gastrointestinal colonisation serving as a reservoir for transmission and a precursor to invasive disease. However, data on AMR acquisition, risk factors, and dynamics in African children remain scarce.

This thesis examines AMR in hospital-acquired Gram-negative bloodstream infections (HA-GNBSI) in African children, antimicrobial use patterns, and the dynamics of ESBL-E and CRE carriage among Kenyan children across hospital and community settings. Methods include a literature review, antimicrobial prescribing analyses in nine hospitals across Africa and South Asia, and a longitudinal study of Kenyan children from hospital admission to six months post-discharge. The diversity and clonality of invasive versus carriage *Escherichia coli* strains are also analysed.

Key findings reveal that *Klebsiella pneumoniae* and *Escherichia. coli* dominates HA-GNBSI in African children and neonates, with pooled prevalence of HA-GNBSI being 63%. Antimicrobial use patterns show high reliance on WHO Watch antibiotics, while Reserve antibiotics are rarely used. In Kenyan hospitals, ESBL-E carriage increased from 36% at admission to 71% at discharge, dropping to 27% after six months. Commonest ESBL-E genes

were CTX-M-15 and sequence type ST38 found in both hospital and community settings, while CRE remained restricted to urban hospitals, dominated by the NDM-5 genotype.

This study highlights the amplification of AMR during hospitalisation and the circulation of resistance genes between hospitals and communities. Findings underscore the need for enhanced antimicrobial stewardship, infection prevention strategies, and evidence-based guidelines which are integrated across hospital and community settings to mitigate AMR in low- and middle-income countries.

TABLE OF CONTENTS

1 PREFACE

1.1 ACKNOWLEDGEMENTS

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1.3 DECLARATION OF CONTRIBUTION TO THESIS

I, Caroline Tigoi, designed and conducted all the analyses presented in this thesis, with the support of my supervisors and colleagues.

Specific assistance from others in relation to work in this thesis is outlined below.

1.4 ATTRIBUTIONS

1.4.1 CHAPTER 3 and 4 – "Antibiotic use data collection and phenotypic analysis of ESBL-E and CRE"

The CHAIN cohort study [1], led by Prof Jay Berkley, enrolled hospitalised children aged 7 days to 23 months from sites across Africa and South Asia, with microbiology performed at locations equipped with microbiology capacity. Rectal swabs were collected from these children at six time points: admission, discharge, readmission, day 45, day 90, and day 180.

Additionally, 404 healthy community reference participants were swabbed once to serve as a non-hospital-exposed control group with hospital environmental samples for infection control practices. I led the collection, processing, storage and shipping of rectal swabs, environmental samples and blood culture samples for the isolation of ESBL-E/CRE using selective culture methods. At all 9 CHAIN sites in Africa and South Asia, comprehensive daily clinical review data were collected for every admitted child, documenting clinical diagnoses and treatments administered until discharge. I designed my thesis using this rich dataset, to analyse the pattern of antibiotic use and microbiological culture data alongside daily clinical data collected during hospital admissions. This analysis aimed to explore the dynamics of acquisition and loss of ESBL-E/CRE carriage among vulnerable children focusing on the longitudinal clinical and genomic determinants at hospital admission, discharge and at home post-discharge in relation to healthcare environment and antimicrobial treatment received as detailed in chapters 2–5.

1.4.2 CHAPTER 5 – "Whole genome sequencing work"

The Sequencing was done at the Wellcome Sanger Institute, Hinxton campus at Cambridge funded through Trevor Lawley's lab, which undertook library preparation and sequencing on the Illumina HiSeq and Oxford Nanopore. All raw read data were routinely processed through the Sanger bioinformatics pipelines for all sequenced samples. I conducted all the quality testing of raw reads, de novo assemblies, pangenome analysis and other downstream analysis detailed in Chapter 5.

1.5 PUBLICATIONS

1.5.1 IN RELATION TO THIS THESIS

Caroline Tigoi, Celine Bourdon, Moses Ngari, Robert Musimyi, Molly Timbwa, Shalton Mwaringa, Narshion Ngao et al. "Antimicrobial Usage Among Acutely Ill Hospitalised Children Aged 2-23 Months in Sub-Saharan Africa and South Asia." Under review at Open Forum Infectious Diseases (OFID); the manuscript is also available as a preprint at: https://ssrn.com/abstract=4757680.

1.5.2 IN RELATION TO OTHER WORK DURING THE COURSE OF THIS THESIS

Browne, Hilary P., Najeeha Talat Iqbal, Majdi Osman, **Caroline Tigoi**, Trevor D. Lawley, Jeffrey I. Gordon, Tahmeed Ahmed, and Samuel Kariuki. "Boosting microbiome science worldwide could save millions of children's lives." *Nature* 625, no. 7994 (2024): 237-240. DOI: 10.1038/d41586-024-00017-8.

Evans O. Mudibo, Jasper Bogaert, **Caroline Tigoi**, Moses M. Ngari, Benson O. Singa, Christina L. Lancioni, Abdoulaye Hama Diallo et al. "Systemic biological mechanisms underpin poor post-discharge growth among severely wasted children with HIV." *Nature Communications* 15, no. 1 (2024): 10299. DOI: 10.1038/s41467-024-54717-w.

Celine Bourdon, Abdoulaye Hama Diallo, Abu Sadat Mohammad Sayeem Bin Shahid, Md Alfazal Khan, Ali Faisal Saleem, Benson O. Singa, Blaise Siézanga Gnoumou, **Caroline Tigoi** et al. "Childhood growth during recovery from acute illness in Africa and South Asia: a secondary analysis of the childhood acute illness and nutrition (CHAIN) prospective cohort." *EClinicalMedicine* 70 (2024). DOI: 10.1016/j.eclinm.2024.102530.

Abdoulaye Hama Diallo, Abu Sadat Mohammad Sayeem Bin Shahid, Al Fazal Khan, Ali Faisal Saleem, Benson O. Singa, Blaise Siézan Gnoumou, **Caroline Tigoi** et al. "Hospital readmission following acute illness among children 2–23 months old in sub-Saharan Africa and South Asia: a secondary analysis of CHAIN cohort." *eClinicalMedicine* 73 (2024). DOI: 10.1016/j.eclinm.2024.102676.

Jacus S. Nacis, Patrick Kamande, Alemayehu Teklu Toni, Evans Mudibo, Robert Musyimi, Siam Popluechai, Trenton Dailey-Chwalibóg, **Caroline Tigoi** et al. "Barriers and enablers to the effective implementation of omics research in low-and middle-income countries." *Nature Biotechnology* 42, no. 6 (2024): 988-991. DOI: 10.1038/s41587-024-02274-4.

Patricia B Pavlinac, James A Platts-Mills, Jie Liu, Hannah E Atlas, Jean Gratz, Darwin Operario, Elizabeth T Rogawski McQuade, Dilruba Ahmed, Tahmeed Ahmed, Benson O Singa, Sarah Somji, Samba O Sow, Milagritos D Tapia, **Caroline Tigoi,** et al. "Azithromycin for Bacterial Watery Diarrhea: A Reanalysis of the AntiBiotics for Children With Severe Diarrhea (ABCD) Trial Incorporating Molecular Diagnostics." *The Journal of Infectious Diseases*, 2024;229(4):988–998. DOI: 10.1093/infdis/jiad252.

Victoria Temwanani Mukhula, Phillines Prisca Harawa, Chisomo Phiri, Stanley Khoswe, Emmie Mbale, **Caroline Tigoi,** Judd L. Walson et al. "Evaluating blood culture collection practice in children hospitalized with acute illness at a tertiary hospital in Malawi." *Journal of tropical pediatrics* 70, no. 1 (2024): fmad043. DOI: 10.1093/tropej/fmad043

Mohsen Naghavi, Stein Emil Vollset, Kevin S. Ikuta, Lucien R. Swetschinski, Authia P. Gray, Eve E. Wool, Gisela Robles Aguilar, James A Berkley, Nicole Stoesser, **Caroline Tigoi** et al. "Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050." *The Lancet* 404, no. 10459 (2024): 1199-1226. DOI: 10.1016/S0140-6736(24)01867-1.

Abdoulaye Hama Diallo, Abu Sadat Mohammad Sayeem Bin Shahid, Ali Fazal Khan, Ali Faisal Saleem, Benson O. Singa, Blaise Siezanga Gnoumou, **Caroline Tigoi** et al. "Characterising paediatric mortality during and after acute illness in Sub-Saharan Africa and South Asia: a secondary analysis of the CHAIN cohort using a machine learning approach." *EClinicalMedicine* 57 (2023). DOI: 10.1016/j.eclinm.2023.101838.

Abdoulaye Hama Diallo, Abu Sadat Mohammad Sayeem Bin Shahid, Ali Fazal Khan, Ali Faisal Saleem, Benson O. Singa, Blaise Siezanga Gnoumou, **Caroline Tigoi** et al. "Childhood mortality during and after acute illness in Africa and south Asia: a prospective cohort study." *The Lancet Global Health* 10, no. 5 (2022): e673-e684. DOI: 10.1016/S2214-109X(22)00118-8.

Tomislav Mestrovic, Gisela Robles Aguilar, Lucien R. Swetschinski, Kevin S. Ikuta, Authia P. Gray, Nicole Davis Weaver, Chieh Han, Anna Gershberg Hayoon, Daniel T Araki, Sherief Abd-Elsalam, Richard Gyan Aboagye, **Caroline Tigoi,** et al. "The burden of bacterial antimicrobial resistance in the WHO European region in 2019: a cross-country systematic

analysis." *The Lancet Public Health* 7, no. 11 (2022): e897-e913. DOI: 10.1016/S2468-2667(22)00225-0.

1.6 ABBREVIATIONS (Listed alphabetically)

ADM	Admission
aHR	Adjusted Hazard Ratios
ALRIs	Acute Lower Respiratory Infections
AMR	Antimicrobial Resistance
ARGs	Antimicrobial resistance genes
aRR	Adjusted Risk Ratios
AWaRe	Access, Watch and Reserve
bla$_{\text{CTX-M}}$	Cefotaximase beta-lactamase (CTX-M) gene/enzyme
bla$_{\text{KPC}}$	Klebsiella pneumoniae carbapenemase (KPC) gene/enzyme
bla$_{\text{NDM}}$	New Delhi metallo-beta-lactamase (NDM) gene/enzyme
bla$_{\text{OXA}}$	Oxacillinase beta-lactamase (OXA) gene/enzyme
bla$_{\text{SHV}}$	Sulfhydryl variable beta-lactamase (SHV) gene/enzyme
bla$_{\text{TEM}}$	Temoneira beta-lactamase (TEM) gene/enzyme
CAIs	Community-Acquired Infections
CARD	Comprehensive Antimicrobial Resistance Database
CAUTIs	Catheter-Associated Urinary Tract Infections
CHAIN	Childhood Acute Illness & Nutrition
CLABSIs	Central Line-Associated Bloodstream Infections
CRE	Carbapenem-Resistant Enterobacterales
DALYs	Disability-Adjusted Life-Years

DISCH	Discharge
DNA	Deoxyribonucleic Acid
EPEC	Enteropathogenic *Escherichia coli*
ESBL-E	Extended-Spectrum Beta-Lactamases
GBD	Global Burden of Disease
GDP	Gross Domestic Product
GLASS	Global Antimicrobial Resistance and Surveillance System
HA-GNBSIs	Hospital-acquired Gram-negative bloodstream infections
HAIs	Hospital-acquired infections
HGT	Horizontal gene transfer
HIV	Human Immunodeficiency Virus
ICUs	Intensive Care Units
IPC	Infection Prevention and Control
IQR	Interquartile Range
LMICs	Low- And Middle-Income Countries
MDROs	Multidrug-Resistant Organisms
MRSA	Methicillin-Resistant Staphylococcus Aureus
MW	Moderately wasted
MGEs	Mobile Genetic Elements
NICUs	Neonatal Intensive Care Units
NDM	New Delhi metallo-β-lactamase
NW	Not wasted
ONT	Oxford Nanopore Technology

PCVs	Pneumococcal Conjugate Vaccines
PPE	Personal Protective Equipment
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
SDI	Sociodemographic Index
SIRS	Systemic Inflammatory Response Syndrome
SNV	Single Nucleotide Variant
SSIs	Surgical Site Infections
sSA	sub-Saharan Africa
ST	Sequence types
SWK	Severely wasted or kwashiorkor (oedematous malnutrition)
READM	Readmission
RNA	Ribonucleic acid
TB	Tuberculosis
UPEC	Uropathogenic *Escherichia coli*
UTIs	Urinary Tract Infections
VAP	Ventilator-Associated Pneumonia
WASH	Water Supply, Sanitation and Hygiene
WGS	Whole genome sequencing
WHO	World Health Programme

2 BACKGROUND

2.1 Introduction

Antimicrobial resistance (AMR) occurs when microorganisms such as bacteria, viruses, fungi, and parasites develop the ability to withstand the effects of medications designed to kill them or stop their growth[1]. AMR, particularly resistance to multiple drugs in Gram-negative Enterobacterales, poses a significant public health threat. Resistance to commonly used antimicrobials including penicillins, cephalosporins and carbapenems is increasing globally[2] and AMR complicates and increases costs of treatment of infection, especially among more susceptible populations, increasing the risk of adverse health outcomes[3, 4]. The emergence and spread of AMR in Enterobacterales, an order of Gram-negative bacteria that includes pathogens such as *Klebsiella pneumoniae*, *Serratia marcescens, Enterobacter spp.*, *Citrobacter*, *Salmonella spp. and Escherichia coli,* has especially affected the progress made in the treatment of hospital-acquired infections (HAIs), including some pathogens resistant (*in vitro*) to all currently available antimicrobials in typical African public hospitals[5]. Rising asymptomatic carriage prevalence of ESBL-producing Enterobacterales (ESBL-E) has been observed in children and its occurrence in community-acquired infections suggests the importance of community reservoirs of AMR[2]. These may include AMR carriage in humans and animals, and transmission in the community due to limited water, sanitation and hygiene (WASH). In hospitals, persistent contamination of surfaces in healthcare settings without effective disinfection practices, including hands, equipment, cots, sinks and tap, further drives AMR transmission[6].

Antibiotics are indispensable medicines used to treat bacterial infections[7]. They are the most prescribed drugs in hospitals[8]. Up to 60% of antibiotics are reported to be used inappropriately among children in hospitals[9]. Studies in LMICs report widespread inappropriate antibiotic use in paediatric settings and challenges due to the costly, limited alternative options, especially in public hospital settings[2, 4, 10]. The WHO has identified resistance to commonly used antibiotics including penicillins, cephalosporins, carbapenems, and fluoroquinolones as central to the AMR crisis, exacerbated by the slow pace of new antibiotic development[11]. Knowledge of prescribing patterns and effectively implementing antibiotic stewardship programs are crucial to improving standards[8] and defining empiric prescribing strategies. Neonatal, intensive care units, and overcrowded paediatric wards are hotspots for AMR transmission. Neonates and malnourished children are particularly vulnerable, facing a higher mortality risks and prolonged hospital stays, which increase their exposure to antibiotics and the hospital environment. Their metabolic instability may further elevate the risk of death from infections, especially when treated with antimicrobials that do not align with pathogen's susceptibility[12].

2.2 Burden of Bacterial Disease

2.2.1 Global Burden

Bacterial infections are highly prevalent in developing countries, posing significant challenges that affect populations differently due to variations in underlying co-morbidities, socioeconomic vulnerabilities, healthcare access, and AMR[13]. According to the Global Burden of Disease (GBD) study, in 2019, bacterial pathogens accounted for approximately 7.7 million deaths[14]. Prominent bacterial pathogens identified in the WHO 2023 report on bacterial infections and the 2024 Bacterial Priority Pathogen List include Enterobacterales, *Salmonella spp*. and *Shigella spp*., *Pseudomonas aeruginosa,*

Staphylococcus aureus and *Mycobacterium tuberculosis* amongst others[15]. These pathogens are responsible for a wide array of infections, from community-acquired pneumonia to hospital-acquired bloodstream infections, with tuberculosis (TB) alone causing an estimated 1.6 million deaths in 2021, with LMICs bearing the heaviest disease and mortality burden due to co-infections with HIV[16, 17].

The burden of bacterial diseases has significant regional disparities that impact individual health and well-being while straining healthcare systems and economies[18]. A comprehensive global study from 2000 to 2022 highlighted the impacts of bacterial pathogens across different regions[19]. While Europe has a comparatively lower overall burden of bacterial disease, there are increasing concerns regarding healthcare-associated infections[20]. In Asia, particularly South and Southeast Asia, high prevalence rates of bacterial diseases persist, with tuberculosis, pneumonia, and waterborne diseases (such as diarrhoea) being particularly problematic, especially among children under five years of age[21].

Bacterial pneumonia and meningitis in childhood remain significant public health challenges globally, especially in low- and middle-income countries (LMICs). Lower respiratory tract infections, often caused by *Streptococcus pneumoniae* and *Haemophilus influenzae*, remain a leading cause of mortality, particularly in young children and the elderly. However, the introduction of conjugate pneumococcal vaccines (PCVs) and *H. influenzae* type b (Hib) vaccines have led to substantial declines in invasive infections caused by *these bacteria*, not only reducing disease incidence but also altering nasopharyngeal carriage, thereby enhancing herd immunity. Despite these successes, life-threatening invasive infections caused by bacteria such as *K. pneumoniae*, *S. pneumoniae*,

H. influenzae, and *Neisseria meningitidis* remain major concerns. These pathogens often colonise mucosal surfaces, such as the gut, nasopharynx, or oropharynx, in an asymptomatic state before progressing to severe illness. This silent carriage complicates prevention and control efforts, as asymptomatic individuals may unknowingly contribute to the transmission. Moreover, factors such as urbanisation and population density, particularly in Asia and sub-Saharan Africa, exacerbate the burden of bacterial diseases due to overcrowded living conditions, poor sanitation, and inadequate access to clean water, facilitating the spread of infections through close human contact and contaminated environments[22].

Bacterial infections, significantly contribute to the global disease burden. For instance, bacterial skin diseases like pyoderma and cellulitis have an age-standardised incidence of 169.72 million cases and account for 0.41 million disability-adjusted life-years (DALYs) globally[23]. The burden is notably higher in low-middle socio-demographic index (SDI) areas compared to high or middle SDI areas, indicating a correlation between socioeconomic status and disease prevalence[24]. Bacterial diarrheal diseases, driven by *Shigella* spp., *Vibrio cholerae*, and toxin-producing *Escherichia coli*, continue to cause significant morbidity, particularly in areas with poor sanitation and limited access to clean water[25]. In addition, urinary tract infections, frequently due to *Escherichia coli*, contribute to a high burden of disease, with increasing antimicrobial resistance complicating treatment[26]. Enteric fever caused by *Salmonella enterica* serovars Typhi and Paratyphi, remain major contributors to disability and death, with an estimated 14.3 million cases and 135.9 thousand deaths globally in 2017. Despite a decline in incidence and mortality rates since 1990, these diseases still account for 9.8 million DALYs, with higher fatality rates among children, older adults, and those in lower-income countries[13].

2.2.2 Burden of bacterial disease in Africa

Africa bears a high burden of bacterial infections and associated mortality due to several factors, including malnutrition, chronic illnesses, inadequate healthcare infrastructure, diagnostic challenges, poor living conditions and poverty[19]. The HIV epidemic further increases susceptibility to opportunistic bacterial infections and increases the severity of disease[27]. Neonatal infections, such as sepsis and meningitis caused by *K. pneumoniae*, *Streptococcus agalactiae*, and *S. aureus*, and *E. coli*, are significant contributors to child mortality across the continent, accounting for approximately 17% of all neonatal deaths, with neonatal meningitis incidence estimated at 0–40 per 100,000 per year[27-30].

Enteric diseases, particularly typhoid fever and diarrheal infections caused by *Salmonella* spp. and *Shigella* spp., remain prevalent in African region due to poor sanitation and water quality. In 2017, typhoid fever alone caused an estimated 1.2 million illnesses and 29,000 deaths[31]. Non-typhoidal *Salmonella* (NTS) is among the four leading global causes of diarrhoea, with its zoonotic potential contributing to transmission between humans and animals. *Salmonella Typhi* and *Paratyphi*, the causative agents of enteric fever, significantly impact global morbidity, mortality, and economic stability[32].A study in 2020 estimated that approximately 1 billion cases of diarrhoea and 515,031 diarrhoea-related deaths occurred in the region[33].

Pneumonia remains a leading cause of childhood mortality in Africa as well, primarily due to *S. pneumoniae*, despite introduction of conjugate vaccines. Tuberculosis (TB) also poses a major health challenge in Africa accounting for 25% of global cases (2.5 million) and over 33% of TB-related deaths (424,000) in 2022[34]. Co-infection with HIV exacerbates the disease burden, particularly in sub-Saharan Africa, where both diseases are prevalent

with 25% of the estimated 450,000 global cases of rifampicin-resistant TB (RR-TB) occurring there and complicating treatment efforts and increasing mortality rates[34, 35].

Efforts to combat bacterial infections in Africa face significant systemic challenges, necessitating tailored interventions such as expanding access to vaccines, improving sanitation, and strengthening healthcare infrastructure to reduce the disease burden[36]. Healthcare-acquired infections(HAIs) are rising in African healthcare facilities due to overcrowding, lack of IPC measures, and limited antibiotic stewardship programs[37]. Strengthening IPC programs is critical; measures such as hand hygiene protocols, availability of PPE, sterilisation practices, and environmental cleaning can significantly reduce HAIs. Initiatives like the WHO GLASS provide a framework for tracking AMR and guiding policy to improve antibiotic use and infection control practices. Integrating these efforts with vaccination campaigns, improved Water Supply, Sanitation and Hygiene (WASH) systems, and better hospital management could form a comprehensive strategy to curb the rise of resistant infections and reduce HAIs across the region but require political commitment and investment.

AMR, especially among the ESKAPEE pathogens (*Enterococcus* spp*., S. aureus, Klebsiella* spp*., Acinetobacter* spp*., Pseudomonas aeruginosa, Enterobacter* spp*., and E. coli*) that play key roles in community- and hospital-acquired illnesses, has rendered many standard treatments ineffective, leading to prolonged illness, possibly higher mortality rates, and increased healthcare costs globally[38]. HAIs, including those caused by *S. aureus* and *K. pneumoniae*, place additional strain on healthcare systems, often necessitating intensive care and prolonged hospital stays[39]. Other clinically important bacterial pathogens associated with AMR, such as *Neisseria gonorrhoeae*, which causes

sexually transmitted infections, and *Helicobacter pylori*, which leads to gastrointestinal disorders, further complicate management efforts.

2.2.3 Surveillance and Infection Control in Kenya

Infection control measures have long been a critical component of healthcare systems, with the primary goal of preventing the transmission of infectious diseases among patients, healthcare workers, and the broader community. As AMR continues to rise, the need for robust infection control practices has become even more pronounced[40]. There is a lack of a strong IPC culture and surveillance systems in many African healthcare facilities. Surveillance systems allow healthcare facilities to monitor the incidence and prevalence of nosocomial infections, as well as to detect emerging threats and respond accordingly[41, 42].

Kenya uses different approaches to health surveillance, integrating national reporting systems, Health and Demographic Surveillance System (HDSS) sites, academic research, and advanced modelling efforts. The Kenya Health Information System (KHIS) using the DHIS 2 software, serves as the central national platform for collecting and analysing health data, informing policy and planning[43].

The country hosts several Health and Demographic Surveillance Study (HDSS) sites conducting longitudinal health and demographic research. A notable example is the Nairobi Urban Health and Demographic Surveillance System (NUHDSS), which monitors health dynamics in Nairobi's informal settlements, offering essential data on urban health challenges[44]. These HDSS efforts are often spearheaded by the African Population and Health Research Centre (APHRC), which frequently collaborates with the INDEPTH

Network, a global consortium dedicated to enhancing health information systems for evidence-based policy development[45].

Kenyan academic institutions contribute through targeted studies, providing valuable local insights, though limited scope and potential biases, require careful interpretation. Additionally, organisations such as the Institute for Health Metrics and Evaluation (IHME) use modelling to estimate health metrics where data is sparse, aiding strategic decision making[46]. Despite limitations due to data scarcity, Kenya is advancing toward a comprehensive health surveillance system to generate robust evidence, guide interventions, and improve population health outcomes and improve population health outcomes.[19].

2.2.4 Hospital-Acquired Infections (HAIs) vs. Community-Acquired Infections (CAIs)

Hospital-acquired infections (HAIs) sometimes referred to as healthcare-associated infections and community-acquired infections (CAIs) represent two classes of infections usually characterised by differences in onset, risk factors, aetiology and epidemiology. HAIs refer to infections developed by patients during treatment in healthcare institutions, typically manifesting after 48 hours of admission and were not present at the time of admission[47]. They are often associated with invasive procedures and devices, such as catheters and ventilators, and pose challenges when caused by multidrug-resistant organisms (MDROs) [48]. Common examples of HAIs include catheter-associated urinary tract infections central line-associated bloodstream infections, ventilator-associated pneumonia and surgical site infections caused by ESKAPEE pathogens [38, 49] [50]. Viral HAIs may also occur, including norovirus outbreaks and respiratory viral infections caused by coronaviruses and influenza viruses[51]. The transmission dynamics of HAIs

significantly depend on the hospital environment, particularly the selection pressures from antibiotic use[52].

Globally, the frequency of HAIs has been estimated as 14% of hospitalised patients included in a systematic review of 400 papers and increases at a rate of 6% annually, while in Africa, the estimated prevalence is higher at 27% compared to developed countries (9% for both Americas and West Pacific and 11.4% in Europe), with ICUs and neonatal wards reporting the highest percentages[53, 54].This inequity is highlighted by broad HAI rates, which range from 2.5% (2/80 patients) to 14.8% (61/412 patients) in African studies limited by identification and reporting challenges, which may also bias reports with cumulative incidences ranged from 5.7% (127/2223 patients) to 45.8% (304/664 patients) per 100 patients on surgical wards[55]. Among the various types of HAIs, surgical site infections are the most commonly reported in Africa, accounting for 41.6% of all HAIs, followed by bacteraemia and respiratory tract infections[53]. In contrast, global data mostly reveals that ventilator-associated pneumonia and catheter-related bloodstream infections are more prevalent[54], reflecting differences in resources. The principal pathogenic agents of HAIs in Africa are predominantly Gram-negative bacteria, such as *K. pneumoniae*, *E. coli,* and *P. aeruginosa*, which are also significant globally but may vary regionally with respect to prevalence and resistance patterns[49]. Factors contributing to the high prevalence of HAIs in Africa include inadequate IPC measures, such as inconsistent hand hygiene practices, insufficient sterilisation of medical equipment, and a lack of access to PPE[56]. While longer hospital stays and the presence of invasive devices, such as catheters, are contributing factors globally, these may be less common in many African settings[49]. Instead, structural challenges like overcrowded hospitals, limited healthcare infrastructure, and shortages of trained healthcare workers amplify the risk. Underlying factors such as

poor water, sanitation, and hygiene (WASH) infrastructure in healthcare facilities further exacerbate the situation, making effective IPC a critical priority for reducing HAIs in Africa[57]. The impact of HAIs extends beyond individual patients, as they have been linked to the spread of multidrug-resistant infections in communities[58].

Differentiating the causative organisms of HAIs often imported by patients and CAIs is crucial for implementing targeted prevention strategies to reduce the spread of multidrug-resistant bacteria in both healthcare and community settings, ultimately enhancing patient outcomes. However, the distinction can be challenging in individuals with frequent healthcare exposures, rendering the 48 or 72-hour post-admission windows typically used to distinguish HAI from CAI less meaningful. By recognising these complexities, healthcare providers can develop more effective treatment options and infection control measures tailored to specific risk factors and sources of infection.

CAIs contracted outside healthcare settings are commonly viral in origin, including illnesses such as the common cold, influenza, and gastroenteritis[49]. However, bacterial CAIs are also prevalent and encompass conditions like urinary tract infections (UTIs), pneumonia, sepsis, meningitis, and skin and soft tissue infections[59]. These infections are commonly caused by pathogens that are spread through person-to-person contact, contaminated food or water, or exposure to environmental sources[60]. Individuals at the extremes of age and those with comorbidities are particularly susceptible to CAIs. Notably, the vast majority of CAIs do not require hospitalisation and can be managed in outpatient settings.

CAIs exhibit high disparities in prevalence and impact among different countries and regions. In Africa, CAIs contribute significantly to mortality, with respiratory tract infections, diarrheal diseases, malaria and tuberculosis accounting for the majority of cases and ill-health instances[19, 61]. The incidence of acute lower respiratory infections (ALRIs), particularly pneumonia, among children under five in Sub-Saharan Africa is highly variable and age dependent. For instance, incidence rates from studies in Africa have ranged from 1.91 to 698 cases per 100,000 child-years, with the highest rates observed among infants under one year of age[62]. UNICEF reports global estimates of over 1,400 cases of pneumonia per 100,000 neonates and children annually, equivalent to one case per 71 children each year. The incidence in West and Central Africa reaches 1,620 cases per 100,000 children annually (UNICEF, 2023); however improved healthcare interventions and preventive measures have notably halved these figures between 2000 and 2021[63]. Additionally, tuberculosis is a major CAI, with prevalence rates reaching up to 300 cases per 100,000 people in some African nations, significantly exceeding the world average[61]. In developed countries, lower CAI rates are attributed to better healthcare infrastructure, vaccination programs, and public health interventions. The annual incidence of community-acquired pneumonia in high-income countries ranges from one to five per one thousand adults, far below that of most regions in Africa[64]. In sub-Saharan Africa, overcrowded living conditions, poor sanitation, and limited access to clean water and healthcare facilities drive the high prevalence of CAIs. Inadequate living conditions, including crowded homes and lack of clean water supply, are major factors contributing to the high incidence of community-acquired infections in Africa[65]. Strengthening public health interventions, including improved sanitation, universal vaccination coverage, and increased accessibility to healthcare services, is crucial to reducing the disease burden in these regions. Enhanced surveillance systems and effective infection control measures are

essential to improving health outcomes and mitigating the overall effects of community-acquired infections across Africa[19, 66].

2.2.5 Mortality Associated with Bacterial Disease

The Global Burden of Disease Study 2019 provides a comprehensive assessment of bacterial pathogens' contribution to global mortality, identifying 33 priority pathogens and highlighting five leading ones including *S. aureus*, *E. coli*, *S. pneumoniae*, *K. pneumoniae*, and *P. aeruginosa* as collectively responsible for more than half of these deaths (Figure 2.1)[14]. Most deaths occurred in low- and middle-income countries, with a significant burden in sub-Saharan Africa and South Asia[14]. In 2019, bacterial infections accounted for 13.6% of all global fatalities, with AMR directly responsible for 1.27 million deaths and contributing to an additional 4.95 million deaths globally [12]. Within this, approximately 929,000 deaths were directly attributable to AMR, while 3.57 million were associated with drug-resistant infections [12].

Among the three major bacterial infectious syndromes reported by the Global Burden of Bacterial Antimicrobial Resistance (GRAM) project, lower respiratory infections and bloodstream infections each caused more than 2 million deaths, while peritoneal and intra-abdominal infections accounted for over 1 million fatalities, collectively contributing to an estimated 6 million deaths in 2019 [14, 19, 67]. Children under five years of age, particularly neonates are highly vulnerable, with bacterial infections responsible for 1 in 8 deaths globally, especially in hospital and post-discharge settings[68]. Notably, in sub-Saharan Africa, the impact is severe due to limited microbiology infrastructure, which restricts effective pathogen identification, AMR monitoring, and evidence-based antibiotic usage, further complicating the region’s high child mortality rates[14, 19, 69].

Neonatal sepsis remains a major global health issue, with an incidence of 2,824 cases per 100,000 live births and a mortality rate of 17.6%, as reported in a review analysing over 2.7 million live births across 14 countries[70]. Studies by Laxminarayan *et al*. (2016) and Folgori *et al*. (2017) found that 25% of neonatal sepsis deaths were caused by bacterial infections, with a third of these directly attributable to AMR[3, 71]. Lower respiratory tract infections, bloodstream infections, and gastrointestinal diseases caused by common bacterial pathogens like *E. coli*, *S. aureus*, and *K. pneumoniae* contribute heavily to this mortality burden (Figure 2.2) [19]. In acute care settings such as intensive care units (ICUs), bacterial infections lead to mortality rates ranging from 26% to 80%, illustrating the urgency of addressing bacterial diseases and reducing related mortality[72, 73].

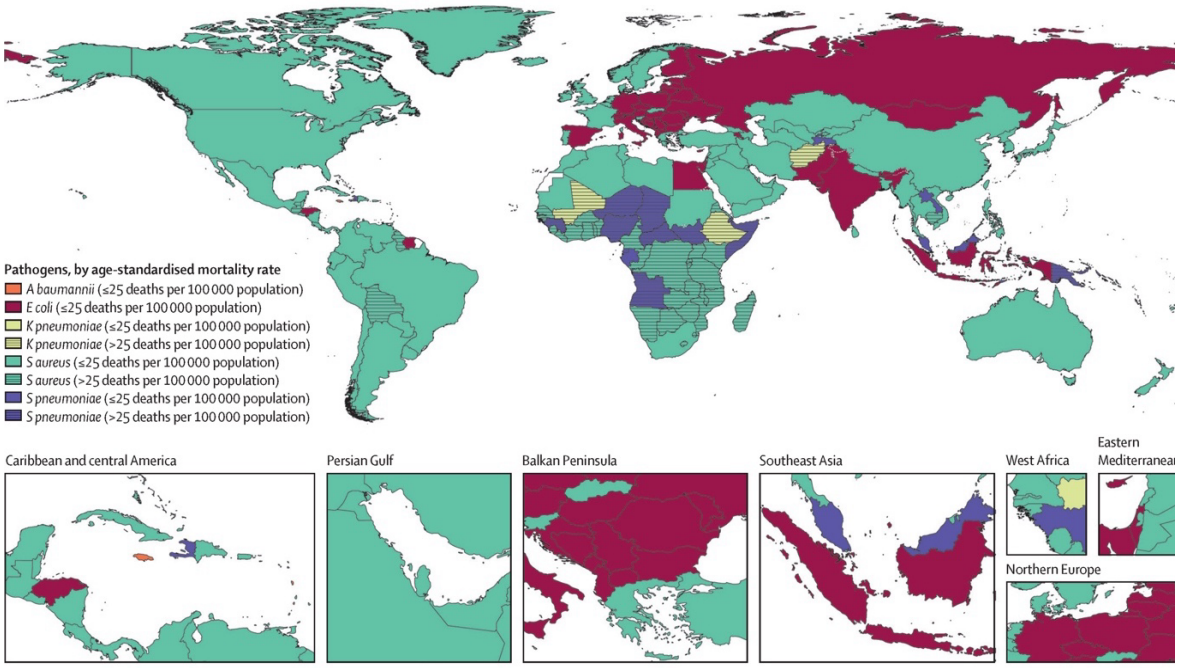


Figure 2.1: *Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019, Ikuta et al., 2022.*

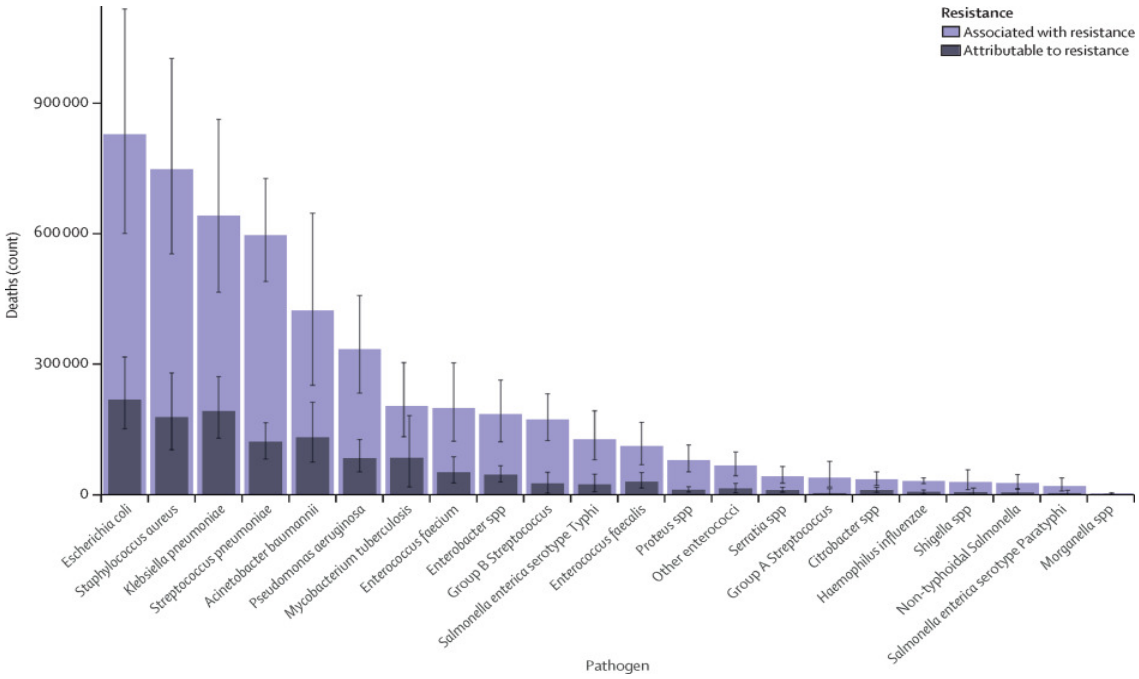


Figure 2.2. *Global deaths (counts) attributable to and associated with bacterial antimicrobial resistance by pathogen in 2019, Murray et al., 2022.*

2.3 Burden of antimicrobial resistance

2.3.1 General Concepts of Antimicrobial Resistance

AMR arises through complex biological mechanisms including genetic adaptations through mutations or via horizontal gene acquisition, drug inactivation by enzyme production or cell wall inactivation and efflux pumps expelling antimicrobial agents[1, 74]. Over time, these mechanisms enable bacteria to resist the effects of antibiotics, rendering treatments less effective. Thus, AMR leads to bacterial infections that are harder to treat.

AMR is enhanced by the misuse and overuse of antibiotics in humans, animals, and agriculture, through unnecessary prescription of antibiotics for viral infections, the incomplete use of prescribed antibiotics, and the use of antibiotics in animal husbandry contribute to the development of resistant strains[75]. This is a growing public health crisis that threatens the effectiveness of essential medical procedures, from routine surgeries to cancer treatments, which depend on effective antibiotics to prevent and treat infections[76].

2.3.2 The Global Burden of Antimicrobial Resistance

AMR has emerged as a critical global health challenge, rendering many standard treatments ineffective, leading to longer hospital stays, higher medical costs associated with treating resistant infections strain healthcare systems and increased mortality[77]. In a systematic review done in 2019 by the GBD collaboration, six pathogen–drug combinations each caused between 50 000 and 100 000 resistance-attributable deaths in 2019: MDR (excluding XDR) tuberculosis, third-generation cephalosporin-resistant *E. coli*, carbapenem-resistant *A. baumannii*, fluoroquinolone-resistant *E. coli*, carbapenem-resistant *K. pneumoniae*, and third-generation cephalosporin-resistant *K. pneumoniae* (Figure 2.3)[19]. Projections suggests that AMR could lead to 10 million deaths per year by 2050 if unaddressed[78]. AMR disproportionately affects low- and middle-income countries (LMICs), where healthcare infrastructure is limited, and the regulation of antibiotics is often inadequate[77]. Sub-Saharan Africa and South Asia experience the highest mortality rates related to AMR, largely due to the limited access to effective diagnostics, treatments, and infection control measures[79].

AMR has profound economic consequences. The World Bank estimates that by 2050, the global economy could lose up to $1 to $3.4 trillion annually due to the direct and indirect impacts of AMR[80]. As resistant infections become more difficult to treat, the costs associated with healthcare such as prolonged hospital stays, more intensive care, and the use of more expensive antibiotics continue to rise[81]. Implementing antimicrobial stewardship programs and improving access to effective diagnostics are crucial[80, 82]. A "One Health" approach [78], integrating human, animal, and environmental health, is considered essential for combating AMR[75]

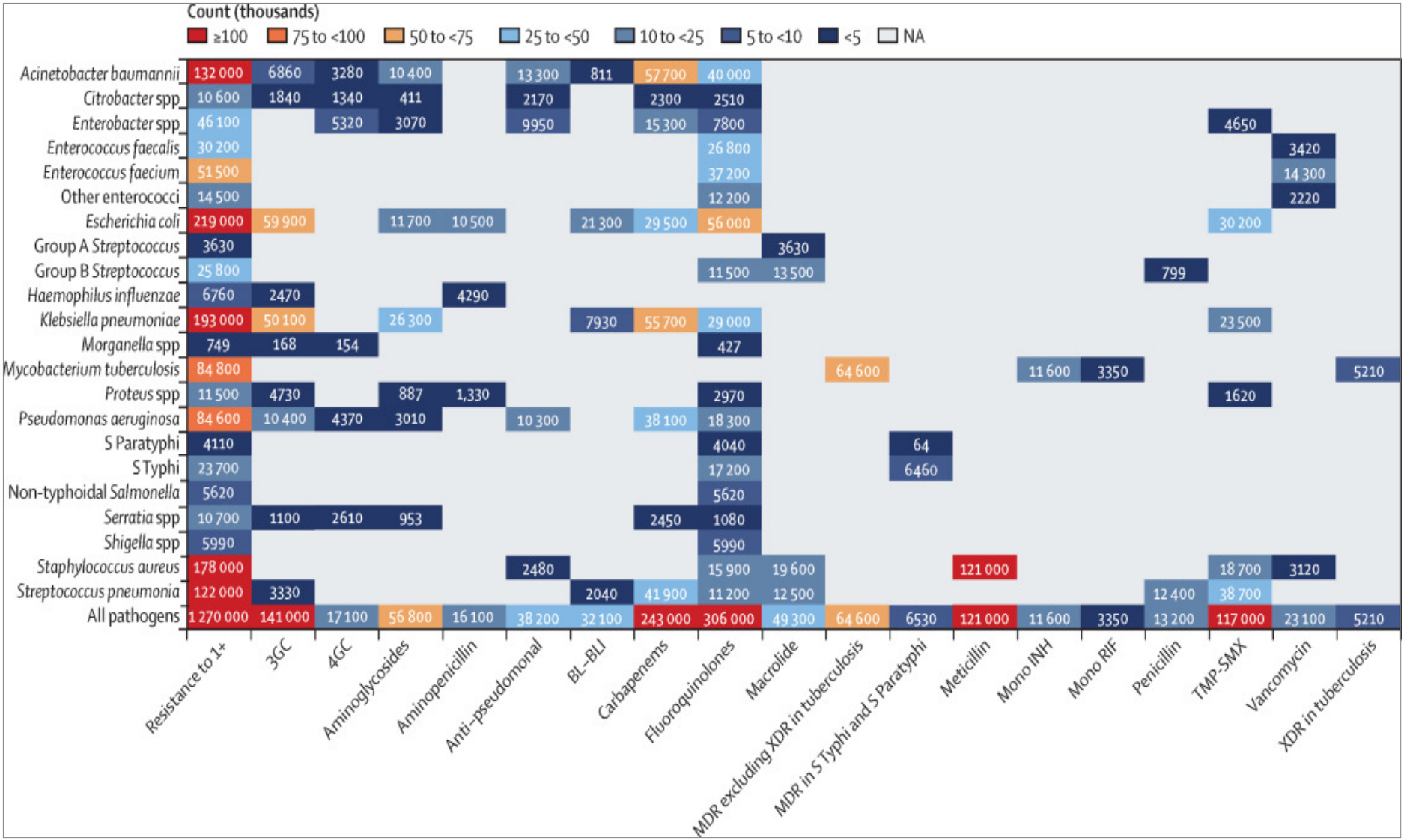


Figure 2.3: Global deaths (counts) attributable to bacterial antimicrobial resistance by pathogen–drug combination, 2019

2.3.3 AMR in Enterobacterales

One of the most concerning developments in the AMR landscape is the rise of resistance among Enterobacterales, a family of Gram-negative bacteria that includes common pathogens including *E. coli*, *K. pneumoniae*, and *Enterobacter cloacae*[83]. These pathogens are frequently implicated in serious infections, such as urinary tract infections (UTIs), bloodstream infections, and pneumonia, impacting both healthcare settings and communities[84]. This resistance can be driven by intrinsic factors, acquired traits, cross-resistance, multidrug resistance (MDR), and pan-drug resistance (PDR)[85].

The WHO has classified carbapenem-resistant Enterobacterales as a critical AMR threat, emphasising the urgent need for coordinated global surveillance and interventions[86]. For example, in the United States, 4.4% of hospitalised UTI patients are infected with carbapenem-resistant pathogens, significantly increasing hospital stays and readmission rates[87]. In Moldova, *K. pneumoniae* accounts for 44.2% of carbapenem-resistant cases[88], while in Africa, *E. coli* and *K. pneumoniae* frequently harbour resistance genes such as $bla_{CTX\text{-}M}$ and bla_{NDM}[89]. A systematic review revealed ESBL production in 12.1% of bloodstream isolates across 31 African countries, highlighting the considerable burden of resistant infections[90].

The economic impact of MDR infections is substantial, with costs exceeding $202,000 per case for *K. pneumoniae*-related hospitalisations due to extended care requirements[91]. Treatment options remain limited, with older drugs like colistin serving as last-resort therapies despite safety concerns. Newer agents, such as ceftazidime-avibactam and ceftolozane-tazobactam, show promise but face limitations in efficacy and accessibility in

low- and middle-income countries (LMICs)[71, 91, 92]. These challenges underscore the need for further research and development of effective treatments to combat AMR globally.

2.3.4 Extended-Spectrum Beta-Lactamase and Carbapenem-Resistant Enterobacterales, and Other Mechanisms

Beta-lactamase-mediated resistance in Enterobacterales is a critical global health emergency, particularly due to extended-spectrum β-lactamases (ESBLs) and carbapenemases, which limit treatment options and increase healthcare costs[93, 94]. These pathogens resist penicillins, cephalosporins, and carbapenems by hydrolysing the beta-lactam ring, rendering these antibiotics ineffective[86]. Key resistance mechanisms include Extended-spectrum beta-lactamases (ESBLs), such as CTX-M, SHV, and TEM, which degrade penicillins and cephalosporins, AmpC beta-lactamases, often chromosomally encoded in species like *Enterobacter spp*., which confer cephalosporin resistance but are not inhibited by beta-lactamase inhibitors and lastly carbapenemases, including KPC, NDM, VIM, IMP, and OXA-48-like enzymes, which degrade even last-resort carbapenems, making infections extremely difficult to treat [10]. The spread of resistance genes is driven by antimicrobial use and facilitated by mobile genetic elements such as plasmids, transposons, and integrons, enabling horizontal gene transfer between bacterial strains[95].

In addition to beta-lactamase production, Enterobacterales employ additional resistance mechanisms that further limit antibiotic effectiveness like porin loss particularly in *E. coli* and *K. pneumoniae* (e.g., OmpF, OmpC) to reduce antibiotic influx, limiting drug penetration or the overexpression of efflux pumps like AcrAB-TolC, actively expels antibiotics like fluoroquinolones and beta-lactams, reducing their intracellular concentrations [87]. Additionally, target site modifications, such as mutations in DNA

gyrase (*gyrA*) and topoisomerase IV (parC) confer resistance to fluoroquinolones, a widely used antibiotic class [10].

The Ambler classification system, which categorizes beta-lactamases into four molecular classes (A, B, C, and D) based on their amino acid sequences and functional properties, is instrumental in understanding and addressing AMR[96]. Extended-spectrum beta-lactamases (ESBLs) degrade a wide range of beta-lactams, including third-generation cephalosporins, while carbapenemases, such as serine carbapenemases (Ambler class A), metallo-beta-lactamases (Ambler class B) and some oxacillinases (OXA; Ambler class D), inactivate carbapenems[97] (Figure 2.4).

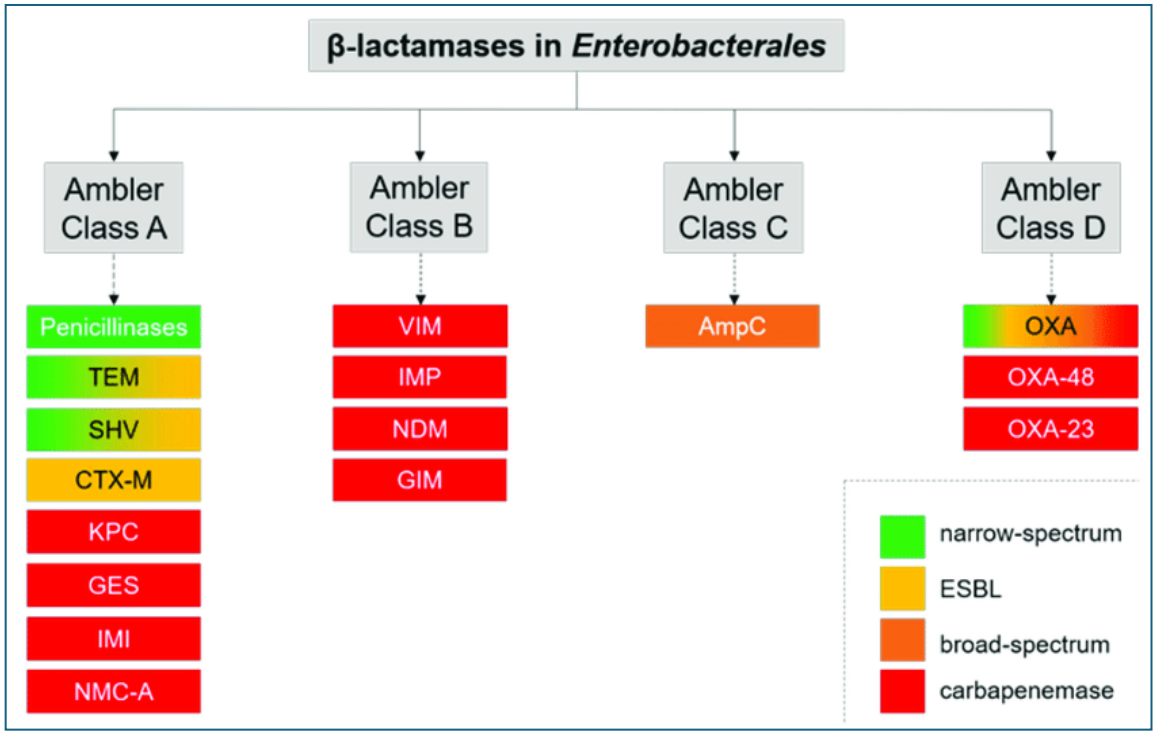


Figure 2.4: *Ambler's classification with examples of main β-lactamases in Enterobacterales[96].*

This classification underpins the mechanisms of resistance to key antibiotics like cephalosporins and carbapenems, which are vital in clinical care as detailed in **Table 2.1** below[94, 98].

Ambler Class	Enzymes	Resistance Mechanisms and Characteristics	Pathogens and Epidemiological Trends
A	TEM, SHV, CTX-M, KPC	Hydrolyse penicillins and cephalosporins; extended-spectrum β-lactamases (ESBLs, e.g., CTX-M-15) confer resistance to third-generation cephalosporins; KPC (*K. pneumoniae* carbapenemase) targets carbapenems	ESBL-producing *E. coli* and *K. pneumoniae* are widespread globally, especially CTX-M-15 in Europe; KPC predominates in North America, Southern Europe, and South America
B	NDM, VIM, IMP	Metallo-beta-lactamases (MBLs); require zinc for activity; degrade carbapenems and broad-spectrum cephalosporins	NDM-1 is prevalent in South Asia and disseminated globally via plasmids; other MBLs like VIM and IMP are prominent in Europe and Asia
C	AmpC	Resistance to penicillins, first- and second-generation cephalosporins; reduced susceptibility to β-lactamase inhibitors	Found in *E. cloacae* and *Citrobacter freundii*, with increasing prevalence in healthcare settings
D	OXA-48	Oxacillinases; carbapenemase activity but less efficient at hydrolysing carbapenems compared to KPC and NDM; complex to detect	OXA-48 is highly prevalent in the Middle East and North Africa and is spreading across Europe and Asia

Table 2.1: *Ambler's classification with examples of main β-lactamases in Enterobacterales (https://www.msdmanuals.com/professional/infectious-diseases/bacteria-and-antibacterial-medications/overview-of-beta-lactams#Beta-Lactamases_v66360398)[96].*

Historically, ESBLs were first identified in the 1980s in Europe, with the early TEM and SHV enzymes among the first to be detected[94, 98]. However, by the 2000s, CTX-M variants, particularly CTX-M-15, had become the dominant ESBL type globally. CTX-M-15, initially traced to Europe, spread rapidly due to its plasmid-based mobility[99], which facilitates efficient transmission across healthcare settings and communities[99]. Another notable CTX-M variant, CTX-M-14, is especially prevalent in East Asia and commonly associated with community-acquired infections[100].

The emergence of carbapenem-resistant Enterobacterales (CRE) is closely linked to carbapenemase production by class A (KPC), B (NDM), and D (OXA-48 group) enzymes. KPC is prevalent in North America, Southern Europe, and South America; NDM, including NDM-1, is highly distributed in South Asia, and OXA-48 dominates in the Middle East and North Africa[101, 102]. NDM variants, which confer broad-spectrum resistance, are

disseminated globally via mobile genetic elements, while OXA-48 enzymes, though less effective at hydrolysing carbapenems, evade detection and sometimes acquire additional mutations that complicate treatment[99].

AmpC β-lactamases (class C) further complicate treatment options by conferring resistance to earlier-generation cephalosporins and reducing the efficacy of β-lactamase inhibitor combinations[97]. A key challenge with AmpC enzymes is their inducibility, where exposure to certain beta-lactams, including third-generation cephalosporins, triggers increased enzyme production, leading to treatment failure[103]. The concurrent rise of resistance to aminoglycosides and fluoroquinolones leads to multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens, particularly in resource-limited regions like Africa. Nigerian hospitals report alarming rates of MDR Enterobacterales, underscoring the critical need for robust infection control measures and antimicrobial stewardship[101].

The persistence of AMR and the global spread of TEM, SHV, CTX-M, KPC, NDM, and OXA-48 highlight the urgency of a coordinated response[100]. Effective strategies include enhanced surveillance, infection control, and prudent antibiotic use to mitigate the AMR crisis[94].

2.3.5 Impact of AMR in Children

Children are particularly vulnerable to the impact of AMR because infections caused by resistant bacteria are more difficult to treat in paediatric populations due to the limited availability of age-appropriate formulations of antibiotics and the higher risks associated with severe infections in children [104]. Moreover, children, especially neonates and infants, have immature immune systems, making them more susceptible to infections[105].

In LMICs, children are disproportionately affected by AMR because of malnutrition, inadequate healthcare access, and higher exposure to pathogens including zoonotic from use in animal agriculture[106]. Prevention through immunisation, clean and regularly available water and sanitation, nutrition and education are key in mitigating the impact of AMR in children is important[107].

2.3.6 Mortality Associated with Antimicrobial Resistance (AMR)

AMR is an escalating global health crisis, with the World Health Organization (WHO) estimating that multidrug-resistant organisms (MDROs) account for 30% of healthcare-associated deaths worldwide [41]. Sub-Saharan Africa bears the highest burden, with an AMR-attributable death rate of 27.3 per 100,000 people, compared to Australasia, which has the lowest rate at 6.5 deaths per 100,000 (Figure 2.5). Lower respiratory infections are the most common AMR-associated syndrome, causing over 1.5 million deaths in 2019[14]. Estimates of AMR-related mortality vary slightly across sources due to differences in data collection methods, modelling approaches, and classification criteria. The Global Burden of Disease (GBD) study estimates that AMR was directly responsible for 1.27 million deaths, contributing to an additional 4.95 million deaths associated with drug-resistant infections in 2019[13, 14]. The GRAM project reports a slightly lower figure, with 929,000 deaths directly attributable to AMR and 3.57 million deaths associated with drug-resistant infections[13, 14]. Despite these discrepancies, both assessments highlight the significant and growing impact of AMR on global health, underscoring the urgent need for coordinated interventions to mitigate its burden.

The extent to which AMR directly causes death is unclear and the burden in children, particularly in low- and middle-income countries (LMICs), remains substantial. Global Burden of Disease (GBD) estimates show 193,000 deaths in children under 5 attributable

to AMR in 2021, down from 488,000 deaths in 1990[108]. AMR is also estimated to cause 2.67% of deaths directly in children in LMICs[109]. However, analyses using WHO recommended parallel cohort approaches comparing outcomes of both sensitive and resistant infection to no infection have not found excess mortality[110].

The U.S. Centers for Disease Control and Prevention's (CDC) *Antibiotic Resistance Threats* report emphasizes the considerable and often underestimated burden of AMR. According to recent CDC data, the United States alone experiences over 2.8 million antibiotic-resistant infections annually, resulting in more than 35,000 deaths [24]. Globally, millions of people are affected each year, with significant morbidity and mortality. The economic impact of AMR is profound, with billions of dollars in costs related to treatment, medication, hospitalisation, lost productivity, and healthcare system strain. Notably, MRSA alone is responsible for over 100,000 attributable deaths annually [25].

Mortality due to AMR is still very uncertain since the large global modelling efforts have been based on assumptions whilst more robust modelling mostly shows no impact on mortality. This situation however underscores the need for early intervention, improved treatment protocols, and effective AMR management strategies to reduce global mortality and enhance health outcomes.

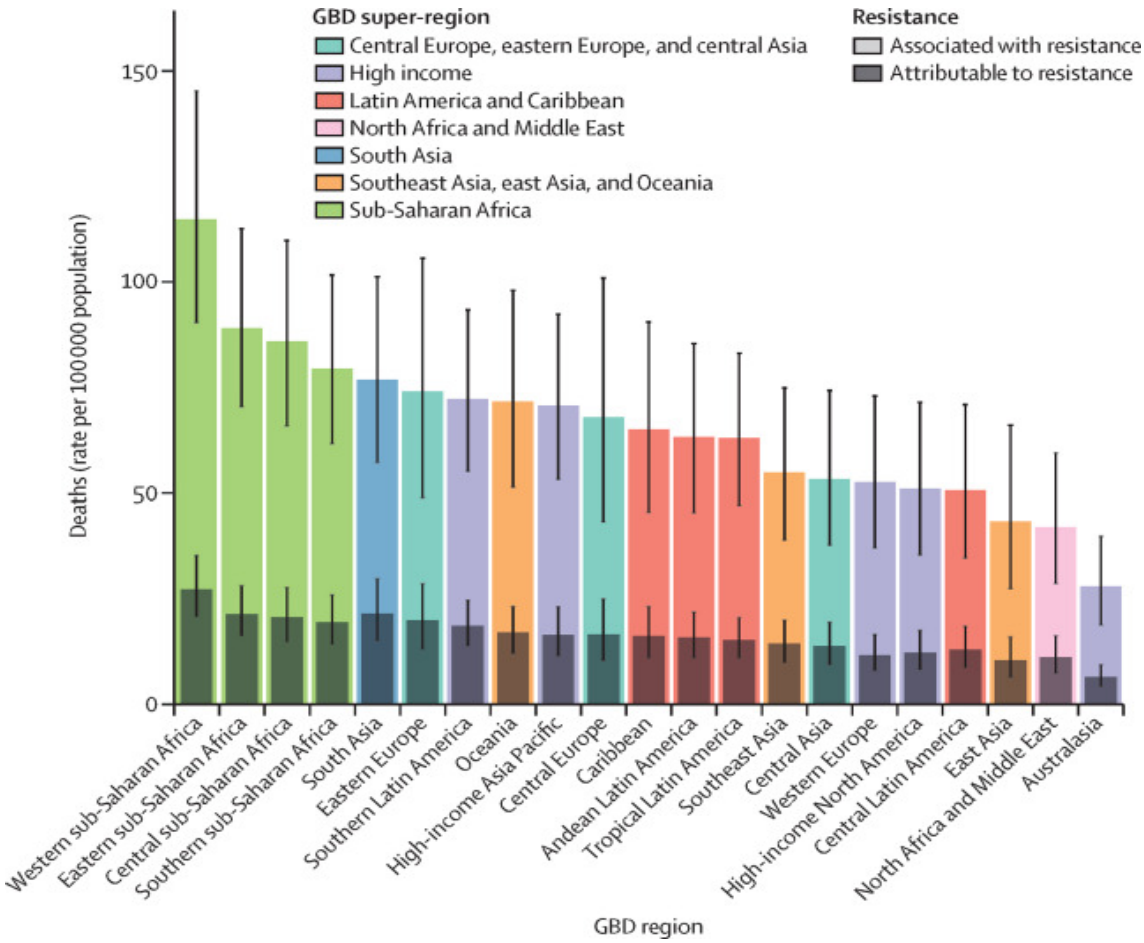


Figure 2.5: *All-age rate of deaths attributable to and associated with bacterial antimicrobial resistance by GBD region, 2019, Murray et al., 2022.*

2.4 Environmental Contamination & AMR

2.4.1 Community Environment and Antimicrobial Resistance (AMR)

AMR in community settings is largely influenced by environmental factors such as the unregulated use of antibiotics and pollution of natural resources. The widespread misuse and overuse of antibiotics in human and veterinary medicine have led to the accumulation of antimicrobial residues in water, soil, and air, creating reservoirs of antimicrobial resistance genes (ARGs) that facilitate the spread of resistance among bacterial populations[3]. Horizontal gene transfer accelerates this process, allowing resistant bacteria to thrive in environments exposed to pollutants such as heavy metals and industrial chemicals, which exert selective pressure favouring resistant strains [111].

Enterobacterales, a major group of AMR-associated bacteria, are particularly adept at persisting and spreading across diverse ecological niches, including humans, animals, wastewater and natural environments[112]. This broad adaptability contributes to their persistence in diverse settings, compounding the AMR crisis. Notably, critical resistance determinants, such as *bla*$_{CTX-M}$ family genes (responsible for extended-spectrum beta-lactamase [ESBL] production) and *mcr-1* (which confers resistance to colistin, a last-resort antibiotic), have been traced to environmental and agricultural reservoirs. The *mcr-1* gene, for instance, emerged from livestock-associated bacteria before disseminating into human pathogens, underscoring the interconnectedness of human, animal, and environmental health in the AMR crisis [113]. A "One Health" strategy, integrating human, animal, and environmental interventions, is essential to mitigating the spread of resistance and preserving the effectiveness of existing treatments.

2.4.2 The Hospital Environment and AMR

Environmental contamination with multidrug-resistant organisms (MDROs) exacerbates the risk of untreatable infections in both community and hospital settings, with hospitals serving as key reservoirs for AMR. Contaminated surfaces, medical devices, and healthcare personnel facilitate the transmission of resistant pathogens, including ESBL-E, CPE, and MRSA[114, 115].

In sub-Saharan Africa, limited infection control resources contribute to elevated rates of HAIs, with prevalence reaching up to 15.5% in some regions due to overcrowded settings, suboptimal sanitation, and insufficient infection control protocols[116]. For instance, studies in Nigerian and Kenyan hospitals have identified frequent contamination of frequently touched intensive care unit surfaces with ESBL-producing *K. pneumoniae* and *E. coli*, as well as CPE strains carrying *bla*$_{NDM-1}$, *bla*$_{CTX-M-15}$, and *bla*$_{OXA-48}$ genes[117], all

of which present a high risk for transmission to vulnerable patients through direct contact with healthcare personnel or medical equipment[118, 119]. Additionally, genomic surveillance in Ghanaian hospitals has revealed the presence of multiple *bla*$_{NDM}$-carrying species alongside ESBLs, including strains with *bla*$_{VIM-5}$, and *bla*$_{DIM-1}$, illustrating the significant diversity of carbapenemases acting as reservoirs within hospital environments[120]. Such findings underscore the need for continuous genomic surveillance and environmental monitoring to identify and contain the spread of these dangerous pathogens in healthcare facilities[120].

Addressing environmental contamination with MDROs is critical for mitigating AMR spread and protecting public health, as hospitals can act as reservoirs for resistant bacteria that cause untreatable infections. Effective intervention requires rigorous hygiene protocols, comprehensive antimicrobial stewardship programs, and targeted sterilisation of critical equipment, while ensuring thorough disinfection of hospital surfaces in resource-limited settings where AMR control measures are most challenging. To reduce AMR transmission, coordinated efforts among healthcare providers, environmental agencies, and policymakers are necessary to promote responsible antibiotic use and implement effective infection control strategies at both local and global scales[114, 121].

2.4.3 Environmental Spread of AMR

The environment plays a crucial role in the proliferation of AMR, as antibiotics, antibiotic-resistant bacteria, and resistance genes are frequently released into the surrounding ecosystem through various channels, including hospital waste, agricultural runoff, and inadequately treated sewage[122-125]. These contaminants can persist in soil, water, and air, enabling the spread of resistance genes among bacterial populations[123, 124].

The extensive use of antibiotics in livestock production, both for treatment and as growth promoters, is recognized as a significant contributor to environmental AMR[126]. However, the relationship between AMR in humans and antimicrobial use (AMU) in farmed animals remains unclear and understudied. A 2023 review underscores this gap, noting that "AMR in humans related to AMU in farmed animals has been understudied and remains unknown" [127]. Current literature often adopts cautious language, using phrases like “...are connected” or “…are correlated” to describe the potential link between AMU in agriculture and human AMR. While it is widely acknowledged that antibiotic use in food production promotes reservoirs of AMR bacteria and genes, the extent to which these reservoirs contribute to human health outcomes remains largely undefined. For instance, a 2024 review highlights that AMR bacteria can be transmitted to humans through zoonotic pathways, either directly via infection or indirectly through colonization of the human gut, where horizontal gene transfer from AMR bacteria to gut commensals may occur [123, 128]. Similarly, the CDC (2024) emphasizes the interconnected nature of AMR across humans, animals, and the environment, framing it as a One Health issue, underscoring the complex interplay between these sectors. [106].

The American Academy of Paediatrics(2024) provides insights into antibiotic use in agriculture but does not quantify the burden of AMR in children attributable to animal use[106]. It does, however, acknowledge that agricultural practices select for resistant bacteria, which can reach humans through contaminated food, direct animal contact, or environmental pathways, such as water sources. Moreover, the release of antibiotics into water bodies from pharmaceutical manufacturing processes, especially in countries with less stringent regulations, further exacerbates the environmental spread of resistance[122, 123].

The burden of human AMR linked to antibiotic use in agriculture remains unclear, with studies highlighting a correlation but lacking precise quantification. A 2023 study emphasises that AMR in humans resulting from antimicrobial use in farmed animals is understudied, while a 2024 review notes that although antibiotic use in food production promotes AMR reservoirs, its direct impact on human health is not well-defined[106].

Given the current gaps in understanding, further research is essential to accurate tent to which antibiotic use in agriculture contributes to AMR in human populations. This knowledge is crucial for developing effective policies and interventions to mitigate the spread of resistance across different sectors. The one health approach recognizes the interconnectedness of these domains and the necessity for a coordinated, multidisciplinary effort to mitigate the spread of AMR[124].

2.5 Previous studies on phenotypic and genotypic dynamics of AMR

2.5.1 Previous studies on phenotypic dynamics of AMR

The prevalence of ESBL-E and CRE varies markedly by region and healthcare setting. It is important to acknowledge potential biases in the published literature, as reported prevalence often depends on the specific contexts and samples studied. For example, reports may originate from general practice in community settings or from patients referred for failed treatments or complications. Chronic infections, particularly those persistent despite treatment, may differ significantly in bacterial species and AMR profiles compared to acute infections, such as urinary tract infections. In LMICs, much of the literature comes from university or private hospitals, intensive care units (ICUs), or a limited number of well-funded research units, which may not represent the broader situation in rural or

underserved areas. Multi-centre research efforts are essential to address these biases and provide a more comprehensive understanding of AMR across diverse settings. For instance, the NeoObs multicentre study highlights third-generation cephalosporin resistance in *K. pneumoniae* at an average of approximately 75% (96/128) and 32.6% (43/132) to meropenem in neonatal sepsis cases across a wide range of global settings, offering valuable insights into the prevalence and dynamics of AMR in secondary and tertiary level hospitals in LMICs, however it may not represent non-tertiary centres without microbiology laboratory capacity in these settings[129]. This underscores the importance of expanding surveillance and research to low-resource centres to capture the full scope of AMR challenges.

The English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) Report 2023–2024 highlighted an increase in resistance to third-generation cephalosporins in bacteraemia between 2019 and 2023, with resistance in *E. coli* rising from 14.5% to 15.8% and in *K. pneumoniae* from 16.3% to 18.8%[130]. Similarly, a study conducted in Finland from 2008 to 2019 on ESBL-producing *E. coli* found a rising prevalence of ESBL in bloodstream infections, increasing from 1.6% (28/1,806) to 6.4% (207/3,218) in females and from 2.4% (23/966) to 8.6% (190/2,197) in males. In urine cultures, the proportion of ESBL-producing *E. coli* isolates grew from 2.2% (239/10,806) to 7.2% (1,098/15,297) in males and from 1.0% (1,045/108,390) to 3.1% (3,717/120,671) in females[131]. In study done in India looking at resistance in bacterial pathogens from blood cultures at secondary care centres, carbapenem resistance increased among *E. coli* (from 7.8% ($n = 282$) in 2011 to 11.5% ($n = 426$) in 2014; $p = 0.332$) and among *K. pneumoniae* (from 41.5% ($n = 183$) to 56.6% ($n = 318$); $p < 0.001$); however the increase was statistically significant only for *K. pneumoniae*[132]. In a faecal carriage study done

in children in Bangladesh, prevalence of ESBL-producing *E. coli* and *K. pneumoniae* was 17.1% (46/269; 95% CI: 12.9%–22.7%). A total of 47 isolates were ESBL-positive, of which, 83.0% were *E. coli* and 17.0% were *K. pneumoniae*[133]. In Cusco, Peru, ESBL-producing uropathogenic *E. coli* (UPEC) isolates from urinary tract infections in a local hospital exhibited high resistance rates with half of the ESBL producers being resistant to aminoglycosides and nitrofurantoin. All isolates were multidrug-resistant (MDR) and 16.2% (16/99) classified as extensively drug-resistant (XDR). Cefoxitin and fosfomycin resistance were 29.3% (29) and 14.1% (14), respectively[134].

Phenotypic studies in Asian and African countries, including India, China, Kenya, and South Africa, have reported alarming increases in ESBL and carbapenem resistance, often linked to the misuse and overuse of antibiotics. The phenotypic dynamics of AMR, particularly regarding ESBL-Es among neonates in Kilifi, Kenya, mirror broader trends across Africa. High prevalence rates of ESBL-producing *E. coli* and *K. pneumoniae* are observed, with significant resistance to third-generation cephalosporins and other critical antibiotics. For instance, in Kenyan hospitals, 82.6% (114) of *E. coli* and 92.9% (118) of *K. pneumoniae* isolates were identified as ESBL-producers, showing substantial resistance to ceftriaxone and amoxicillin/clavulanic acid[135]. In Kiambu County, Kenya, 40% (114) of *E. coli* isolates from cattle and their environments were multidrug-resistant (MDR), with 14% (40 isolates) resistant to three different antimicrobial classes and 25% (71 isolates) resistant to between four and seven classes, indicating a potential reservoir for resistance genes that could transfer to human pathogens[136]. Similarly, research in northern Tanzania found that 29.7% (98 isolates) of bacterial isolates from clinical samples were ESBL producers, predominantly *E. coli* and *K. pneumoniae*, which exhibited high resistance to ceftazidime, cefpodoxime, and cefotaxime[137].

These phenotypic studies highlight the pervasive and evolving nature of AMR across geographic regions and underscore the necessity of understanding the functional domains in bacterial resistance. The role of environmental reservoirs, such as water bodies and agricultural settings, is critical in AMR dynamics. In sub-Saharan Africa, hospitals are increasingly reporting MDR organisms due to crowded healthcare settings and inadequate sanitation. These environments serve as reservoirs for resistant pathogens and drive AMR transmission, compounding the challenge of controlling HAIs. The widespread and growing presence of AMR underscores the urgent need for robust surveillance systems, prudent antibiotic use, and coordinated efforts between healthcare and environmental sectors to limit the expansion of resistance[138, 139].

2.6 Previous Studies on Genotypic AMR

A global one health systematic review reported an overall pooled prevalence of ESBL-producing *E. coli* genes in humans to be 35.9% (26.0–47.1), 32.3% (22.8–43.5), 31.7% (23.0–42.0), and 2.6% (8.3–42.6), for the bla_{CTX-M}, bla_{TEM}, bla_{SHV} and bla_{OXA}, respectively[140]. Additionally, the bla_{NDM-1} gene, associated with carbapenem resistance, has been detected in various regions, including Asia[141]. WHO African Region highlights the commonest ESBL AMR genes in Gram-negative bacteria to be $_{blaCTX-M}$ (522/866, 60.3%), bla_{TEM} (203/398, 51%), and bla_{SHV} (604/866, 70%) for *K. pneumoniae* and [142]. Furthermore, metallo-β-lactamase-associated genes reported in Africa include bla_{OXA} (336/686, 49%) *E. coli*, and *gyrA* mutations (487/1,948, 25%) in *Salmonella* Typhi linked to carbapenem and quinolone resistance respectively.

The genotypic dynamics of AMR focus on the genetic adaptations that enable bacteria to withstand antimicrobial agents. Bacterial resistance arises from genetic mutations, gene acquisitions, and alterations in gene expression, often as a response to selective pressures from antibiotic exposure[143]. These genetic changes introduce remarkable genome flexibility, facilitating the spread of resistance across bacterial populations[143]. Advanced molecular techniques, such as nucleic acid amplification and whole-genome sequencing (WGS), have become essential for identifying resistance genes and understanding their transmission across bacterial species and environments. Horizontal gene transfer (HGT) via mobile genetic elements (MGEs), including plasmids and transposons, plays a crucial role in the dissemination of antimicrobial resistance genes (ARGs), allowing bacteria to acquire and share resistance traits efficiently[144].

Ecological and socioeconomic factors, such as public health standards, sanitation, and antibiotic policies, also impact AMR emergence and distribution. A global study indicated that the abundance of ARGs in sewage was more strongly linked to socioeconomic and health indicators than to direct antimicrobial usage, emphasizing the complex interplay of environmental and social factors in AMR dynamics[145]. Environmental factors also play a role in the dissemination of AMR. In a rural water body in Puducherry, India, genomic investigations identified ESBL-producing Enterobacterales with resistance genes such as $bla_{\text{VEB-6}}$, $bla_{\text{SHV-12}}$, and $bla_{\text{NDM-1}}$, indicating the environmental spread of resistance determinants[146]. Specific genetic changes in bacterial populations, such as allelic variations in the OmpU porin of *Vibrio cholerae*, demonstrate how particular genetic modifications contribute to convergent resistance phenotypes across regions[147].

Genomic studies across Africa have identified a broad array of resistance genes contributing to resistance against major antibiotic classes, including β-lactams, fluoroquinolones, aminoglycosides, sulfonamides, trimethoprim, tetracyclines, colistin, phenicols, and fosfomycin, particularly in species like *Enterobacter spp.*, *E. coli*, *Serratia marcescens*, *Salmonella enterica*, *P. aeruginosa*, and *A. baumannii*[148]. The most frequently identified resistance genes include ESBL genes (bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M-group-1}}$) and carbapenemase genes (bla_{NDM}, bla_{VIM}, bla_{OXA-48}), particularly within *K. pneumoniae*. These resistance genes have been detected across diverse ecosystems, highlighting the role of clonal expansion and horizontal gene transfer in transmitting resistance between species, regions, and environments[148].

In the context of urinary tract infections (UTIs) in Kenya, 24.2% of uropathogenic *E. coli* (UPEC) isolates were ESBL producers, mainly harbouring $bla_{\text{CTX-M}}$ and bla_{TEM} genes and falling into phylogenetic groups B2 and A[149]. While these genotypes underpin ESBL-E resistance, genomics reveals additional insights over and above phenotypic data. For example, small sub-populations of resistant bacteria, such as specific lineages within UPEC or *P. aeruginosa*, have been shown to rapidly expand under selection pressure, demonstrating their capacity for niche dominance and persistence even in adverse conditions. Additionally, in *P. aeruginosa* isolates, multidrug resistance (MDR) was linked not only to presence of AMR enzymes like VIM-6 and NDM-1 but also virulence factors such as *phzB1* and *exoU*[150]. Genomic evidence highlights the adaptability of resistance mechanisms, with globally distributed high-risk clones (e.g., ST244 and ST357) and novel MDR clones (e.g., ST3674) contributing to persistent infections and complicating treatment strategies[151]. In hospital settings, carbapenemase-producing Enterobacterales (CPE) with NDM, OXA-48-like, and VIM enzymes, further expand the resistance

spectrum. Genomic studies reveal that these clones often persist in low-abundance reservoirs, re-emerging under antibiotic pressure and contributing to outbreaks. Such findings emphasize the critical role of genomics in understanding the dynamics of resistant bacterial populations, enabling more targeted interventions to curb the spread of resistance[117].

In Nigeria, the finding of AMR genes, such as *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{CTX-M-15}}$, and *bla*$_{\text{NDM-1}}$, highlights the global dissemination of these resistance mechanisms among Enterobacterales[152]. Studies across African regions reveal unique genetic profiles, with AMR genes transmitted through clonal and horizontal gene transfer pathways, affecting both clinical and environmental bacterial populations. In Malawi, genotypic analyses identified extended-spectrum β-lactamase (ESBL) genes such as *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM,}}$ and *bla*$_{\text{SHV}}$ in clinical isolates, with some strains also harbouring carbapenemase genes like *bla*$_{\text{NDM}}$ and *bla*$_{\text{OXA-48}}$. Additionally, genotypic surveillance revealed that 24.8% of healthy children carried ESBL-producing strains, underscoring the community-level spread of these resistance determinants[153].

Overall, these findings highlight the importance of genetic surveillance in capturing the complexity of AMR and, in the future, guiding intervention strategies. While resistance genes identified in African bacterial isolates, such as *bla*$_{\text{CTX-M,}}$ *bla*$_{\text{TEM}}$, *bla*$_{\text{NDM,}}$ and *bla*$_{\text{OXA-48}}$-like variants, are commonly seen globally, their prevalence and dissemination in local contexts are influenced by unique selective pressures and ecological factors, including antibiotic use patterns and healthcare infrastructure. This underscores the critical need for coordinated genomic surveillance, infection control initiatives, and global collaboration to address AMR effectively and sustainably.

2.7 Approaches to tackling Antimicrobial Resistance (AMR)

2.7.1 Antimicrobial Stewardship Programs (ASPs)

Antimicrobial stewardship programs aim to optimize the use of antibiotics to reduce unnecessary prescribing, which in turn helps slow the development of resistance. ASPs guide healthcare providers in selecting the right antibiotic, dose, and treatment duration for each infection, focusing on evidence-based practices[154]. Studies have shown that ASPs in hospitals reduce overall antibiotic use, decrease AMR rates, and improve patient outcomes by reducing adverse effects and hospital stays and associated costs[151]. The World Health Organization (WHO) emphasizes the importance of ASPs as part of the Global Action Plan on AMR. WHO's AWaRe classification system (Access, Watch, Reserve) further supports ASPs by categorizing antibiotics based on their appropriate use[155]. This system encourages the prioritization of Access antibiotics for common infections, restricts Watch antibiotics to combat resistance, and reserves the most potent Reserve antibiotics for life-threatening infections as a last resort[156]. ASPs are increasingly essential in outpatient settings, where inappropriate prescriptions, particularly for viral infections, significantly contribute to resistance. Integrating ASPs with the AWaRe framework can enhance their effectiveness by promoting rational antibiotic use across all healthcare sectors.[157]. Additionally, ASPs in hospitals demonstrate the potential to reduce multi-drug-resistant (MDR) infections by up to 51%, particularly by addressing resistant pathogens such as *Clostridioides difficile* and various multidrug-resistant bacteria.

Despite their effectiveness, ASPs are often hindered by a lack of rapid and reliable diagnostics, especially in low-resource settings. Limited access to microbiological testing means that many infections are treated empirically, increasing the risk of unnecessary

antibiotic use. The absence of routine susceptibility testing further complicates targeted therapy, leading to overreliance on broad-spectrum antibiotics. Strengthening diagnostic capacity through improved laboratory infrastructure, affordable rapid tests, and enhanced surveillance systems is essential to maximizing the impact of ASPs and ensuring antibiotics are used appropriately[158]. Addressing these gaps is particularly critical in LMICs, where ASPs not only help curb resistance but also provide a structured approach to educating healthcare providers and the public on responsible antibiotic use, supporting long-term global health sustainability.

2.7.2 Infection Prevention and Control (IPC)

IPC measures are critical in healthcare to prevent the spread of antimicrobial-resistant bacteria and reduce HAIs [159]. Key IPC strategies include hand hygiene, use of PPE, and sterilization of medical equipment, which effectively curb the transmission of resistant pathogens like MRSA and CRE. Studies show that hand hygiene alone can reduce HAI rates by up to 50%, highlighting the importance of compliance across healthcare settings[160].

Comprehensive IPC programs integrating vaccination discussed in detail below with hygiene and disinfection practices form a robust defence against AMR, especially when implemented consistently in healthcare settings[161].

2.7.3 Vaccination

Vaccination is a powerful yet underutilized tool in combating AMR by preventing bacterial infections and reducing the need for antibiotics, which in turn limits the development and spread of resistance. For example, the pneumococcal vaccine has significantly reduced infections caused by *S. pneumoniae*, a common cause of pneumonia, meningitis, and sepsis [161]. This reduction in infections leads to decreased antibiotic use, thereby limiting the

emergence of resistant strains. Moreover, vaccines targeting pathogens like *S. pneumoniae* and *H. influenzae* reduce respiratory infections—key drivers of antibiotic prescriptions[78].

Emerging vaccines against *Enterobacterales* are increasingly recognized as critical in addressing AMR. Notably, vaccines targeting extraintestinal pathogenic *E. coli* (ExPEC) are currently in Phase III clinical trials, while organizations such as the Bill & Melinda Gates Foundation are prioritizing the development of vaccines against *Klebsiella* species [133]. However, there remains a significant knowledge gap in the genomic epidemiology of *Enterobacterales* in regions like Kenya, where these vaccines could have substantial impact. My DPhil research addresses this gap by evaluating whether these vaccines would effectively cover locally prevalent strains.

Additionally, vaccines play a crucial role in IPC by preventing viral infections that often lead to secondary bacterial infections treated with antibiotics. For instance, the influenza vaccine reduces cases of flu-related bacterial pneumonia, thereby lowering antibiotic consumption[162]. Therefore, widespread vaccination against both bacterial and viral pathogens is essential in the global strategy to mitigate AMR through reduced antibiotic use across populations.

2.7.4 Molecular pathogen detection

Rapid diagnostics are essential for early and accurate detection of resistant infections, enabling healthcare providers to make informed antibiotic choices and implement appropriate infection control measures[163]. Technologies such as polymerase chain reaction (PCR), mass spectrometry, and whole genome sequencing (WGS) hold significant promise in providing results faster than traditional culture-based methods, allowing for

more targeted treatment[164]. However, despite these advances, there remain substantial challenges in implementing next-generation sequencing (NGS) as a routine diagnostic tool, particularly in resource-limited settings[165].

For instance, Okeke et al. (2020) discuss the complexities and barriers to integrating NGS technologies into routine clinical diagnostics[166]. The study highlights challenges such as high costs, the need for specialized equipment, limited bioinformatics expertise, and the lack of standardized protocols for interpreting genomic data. These factors can limit the utility of NGS in settings where resources are constrained, thus impeding its widespread adoption.

While NGS has the potential to revolutionize diagnostics by providing comprehensive insights into pathogen resistance profiles and transmission dynamics, its implementation requires robust infrastructure, training, and sustained investment[167]. Portable Oxford Nanopore MinION devices provide a practical solution, enabling affordable, real-time sequencing for point-of-care diagnostics in resource-limited settings, bridging gaps until broader capacity is developed[168]. Until these challenges are addressed, implementing rapid diagnostics has been shown to reduce the misuse of broad-spectrum antibiotics, improving patient outcomes and slowing the spread of AMR[169].

2.7.5 Genomic Surveillance

Genomic surveillance is an advanced method that leverages whole genome sequencing (WGS) to define the genetic relatedness of bacteria within a population and track the spread of AMR genes. By analysing the genetic composition of pathogens, genomic surveillance can pinpoint specific resistance genes, monitor their transmission patterns, and detect emerging threats before they become widespread. This method has proven invaluable in

identifying outbreaks of multidrug-resistant organisms and elucidating the genetic mechanisms driving resistance[170].

However, traditional short-read sequencing platforms like Illumina have limitations, particularly when tracking AMR genes in pathogens such as ESBL-Es and carbapenemase-producing Enterobacterales (CPEs). Short-read data often fails to capture the full genetic context of resistance genes, which is critical for understanding how these genes are transmitted. This limitation is addressed by using long-read sequencing technologies, such as Oxford Nanopore, which provide the ability to resolve complex genomic structures and mobile genetic elements (MGEs) in which AMR genes are often embedded. By enabling the reconstruction of both vertical (clonal lineage) and horizontal (MGE-mediated) transmission events, long-read data significantly enhances our understanding of how resistance spreads within and between populations.

Genomic surveillance networks, such as the Global Antimicrobial Resistance Surveillance System (GLASS) coordinated by the World Health Organization (WHO), support global data sharing and tracking of resistance patterns. Insights gained from these efforts inform targeted interventions, guide infection control practices, and shape public health policies to curb the spread of AMR [170]. For instance, WGS has been pivotal in tracking carbapenem-resistant *K. pneumoniae* outbreaks and mapping the distribution of the New Delhi metallo-beta-lactamase (NDM) gene, a key driver of multidrug resistance[171].

2.7.6 Development of Novel Antibiotics and Alternative Therapies

The development of new antibiotics and alternative therapies is crucial for outpacing resistant pathogens[172]. Public-private partnerships and initiatives like the Global Antibiotic Research and Development Partnership (GARDP) work to revive antibiotic

development, particularly against resistant Gram-negative bacteria. Additionally, alternative treatments, such as bacteriophage therapy and antimicrobial peptides, offer potential solutions for infections that are difficult to treat with conventional antibiotics[169].

2.7.7 The One Health Approach

Addressing AMR requires a multifaceted approach, including adopting the One Health framework to manage the interconnected factors of resistance in humans, animals, and the environment. Effective antimicrobial stewardship, guided by global standards and strengthened by regulatory enforcement, is essential to reduce the spread of resistant bacteria and associated morbidity and mortality [40, 46].

The One Health approach is vital in combating AMR by addressing the interconnected factors driving resistance. Notably, the use of antibiotics in agriculture and animal husbandry significantly contributes to the problem, as resistant bacteria can transfer from animals to humans through food chains, water sources, or direct contact[112]. The World Health Organization (WHO) has emphasized that bacterial infections pose a critical threat, thereby advocating for vaccination programs and a One Health approach to tackle AMR[173]. The complexity of AMR, especially within community settings, necessitates integrated efforts across human, animal, and environmental sectors to prevent the cross-ecological spread of resistant pathogens [114].

Key international organizations, including the WHO, Food and Agriculture Organization (FAO), and the World Organisation for Animal Health (OIE), call for responsible antibiotic usage in livestock, improved waste management, and robust surveillance of resistance patterns across ecosystems to mitigate the global AMR challenge [174]

2.7.8 Public Awareness and Education Campaigns

Public awareness campaigns are essential for educating communities about AMR and the importance of responsible antibiotic use. Programs led by organizations like WHO and CDC focus on informing the public about completing prescribed antibiotics and discouraging self-medication[155]. Educational initiatives for healthcare providers emphasize proper prescribing practices, infection control, and the importance of diagnostics. Increased awareness leads to more responsible antibiotic use, ultimately reducing AMR[175].

2.7.9 Tackling AMR summary

It requires a comprehensive strategy that includes antimicrobial stewardship, infection control, rapid diagnostics, genomic surveillance, vaccination, and alternative therapies. Vaccination, in particular, plays a crucial role in reducing the need for antibiotics by preventing bacterial infections and associated complications[163]. Genomic surveillance provides critical insights into resistance patterns, enabling proactive interventions to prevent AMR outbreaks[171]. Strategies to decrease HAIs involve research-based infection prevention measures and antibiotic stewardship; however, these efforts have been affected by the resurgence of COVID-19, leading to the reappearance of HAIs[176]. Unique approaches must be considered for prevention and control, as CAIs are influenced by broader community-related factors, while HAIs arise from healthcare practices and environments. However, it is also important to note that these factors also represent a continuum, and the separation between hospital and community settings is incomplete, particularly for individuals who have recurrent healthcare exposures. Together, these strategies offer a robust approach to controlling AMR, but sustained global collaboration,

investment in research, and strong policy enforcement are essential to ensure success in the fight against AMR.

2.8 THESIS OUTLINE

The overarching aim of this thesis is to generate novel insights into determinants – including genetic determinants – that are contributing to the spread of AMR in Kenyan children. Firstly, I have determined the pattern of antibiotic use in children in Africa (including Kenya) and South Asia as part of a multinational study to quantify a major selection pressure for the emergence of AMR in Enterobacterales. I have then focused on Kenyan sites to undertake more detailed research to characterise the longitudinal dynamics of acquisition and loss of ESBL-E and CRE among young children as they progress from admission through to hospital discharge, and subsequently in the community environment post-discharge. As part of this cohort study to determine the prevalence of ESBL-E/CRE carriage and the risk factors for ESBL-E/CRE acquisition at admission, discharge, and post-discharge from hospital, I have gone on to undertake WGS of isolates to explore the genomic epidemiology of important ESBL and carbapenemase genes in this context. Understanding the burden and sources of AMR is essential to revising treatment guidelines, developing antimicrobial stewardship and infection control initiatives, and to generating systems for conducting pragmatic intervention trials in African settings.

My introduction chapter 2 has discussed the burden of bacterial diseases, burden of AMR, mortality associated with bacterial disease and AMR, the contribution of hospital environmental contamination to AMR, hospital- verses community-acquired infections and a summary of previous studies on phenotypic and genotypic dynamics of AMR including both local and international studies.

Chapter 3 is a review of existing literature and an update on healthcare-acquired Gram-negative blood stream infections caused by ESBL-E and CRE in children living in Africa. The chapter discusses metanalysis of prevalence of hospital-acquired (HA) GNBSI, prevalence of ESBL-E and CRE, aetiology of HA bloodstream infections and a metanalysis of risk factors associated with these infections.

Chapters 4 and 5 present analyses of a stratified prospective cohort study in which acutely-ill children enrolled into the CHAIN cohort study were under observation during their index hospital admission (of variable duration) and then for a 180 day follow-up period post-discharge[177]. Chapter 4 discusses antimicrobial usage among acutely ill hospitalised children aged 2-23 months at nine hospitals in sub-Saharan Africa and South Asia, presenting proportion of children given antibiotics and classes prescribed at admission, number of hospital-days spent on each class of antibiotics and the incidence of use of second and third-line antibiotics and factors are associated with receiving specific classes of antibiotics.

Chapter 5 presents data on characterisation of the phenotypic prevalence of gastrointestinal ESBL-E/CRE carriage at hospital admission, acquisition whilst in hospital, and loss during 180 days after discharge in comparison with the prevalence among healthy, non-hospitalised children within the same communities. The analyses in this chapter utilised rectal swab culture data performed at different longitudinal follow-up timepoints using selective and differential media to isolate and identify common enteric pathogens and determine their antimicrobial resistance status as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Finally, in Chapter 6, I used WGS to investigate the genomic characteristics of longitudinal ESBL-E/carbapenem Resistant (CRE) *Escherichia coli* isolates, phylogenetic clustering of lineages in relation to hospital environmental, faecal carriage isolates, blood culture invasive isolates and correlates between AMR genes, sequence types, mobile genetic elements and markers associated with bacterial virulence or pathogenicity. This chapter utilised a total of 538 stored Enterobacterales isolates from CHAIN study, selected from a library of ESBL-E and CRE positive rectal swabs, invasive isolates, and environmental swab isolates from the three Kenyan sites (Kilifi, Mbagathi and Migori) stored in -80^{o}C freezers. A random selection of longitudinal ESBL-E positive and negative *E. coli* from rectal isolates (408) were picked. These included rectal swabs that were positive at admission and discharge plus any other time point for each individual child without a missing rectal swab. All ESBL-E and CRE positive environmental and invasive isolates (total of 92 for both) and 38 CRE positive carriage isolates from all timepoints were included in the analysis. This chapter presents invasive and carriage *E. coli* diversity, sequence types, AMR and virulence genes causing paediatric invasive disease, being carried at admission to hospital, acquired or transmitted in hospital environment and in the community. A detailed map showing ESBL-E and CRE acquisition and loss pattern during admission and follow-up is presented in this chapter and the location of these AMR genes within the plasmids. In addition, an assessment has been done to determine if there is any genotypic overlap between colonising ESBL-E and CRE isolates taken at different time points from patients, from the hospital environment (in both the hospitals and community) and causing invasive disease.

REFERENCES

1. Shahid A, Arshad A, Aslam B, Ali MA, Munir S, Aslam S, Khurshid M: **Antimicrobial Resistance: Causes, Mechanisms, Origins and Characterization**. In: *Handbook of Molecular Biotechnology.* edn.: CRC Press; 2024: 382-396.
2. Moxon CA, Paulus S: **Beta-lactamases in Enterobacteriaceae infections in children**. *Journal of Infection* 2016, **72**:S41-S49.
3. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H: **Antibiotic resistance—the need for global solutions**. *The Lancet infectious diseases* 2013, **13**(12):1057-1098.
4. Gerber JS, Kronman MP, Ross RK, Hersh AL, Newland JG, Metjian TA, Zaoutis TE: **Identifying targets for antimicrobial stewardship in children's hospitals**. *Infection Control & Hospital Epidemiology* 2013, **34**(12):1252-1258.
5. Hsu AJ, Tamma PD: **Treatment of multidrug-resistant Gram-negative infections in children**. *Clinical infectious diseases* 2014, **58**(10):1439-1448.
6. Kramer A, Schwebke I, Kampf G: **How long do nosocomial pathogens persist on inanimate surfaces? A systematic review**. *BMC infectious diseases* 2006, **6**(1):130.
7. **How to investigate drug use in health facilities: selected drug use indicators** [https://www.who.int/publications/i/item/who-dap-93.1]
8. Demoz GT, Kasahun GG, Hagazy K, Woldu G, Wahdey S, Tadesse DB, Niriayo YL: **Prescribing Pattern of Antibiotics Using WHO Prescribing Indicators Among Inpatients in Ethiopia: A Need for Antibiotic Stewardship Program**. *Infection and Drug Resistance* 2020, **13**:2783.
9. Shiva F, Ghanaie R, Shirvani F, Armin S, Tabatabaei SR, Fahimzad SA, Fallah F, PourMoshtagh H, Karimi A: **Pattern of antibiotic usage in children hospitalized for common infectious diseases**. *Archives of Pediatric Infectious Diseases* 2018, **6**(1).
10. Paterson DL: **Resistance in gram-negative bacteria: Enterobacteriaceae**. *American journal of infection control* 2006, **34**(5):S20-S28.
11. Ventola CL: **The antibiotic resistance crisis: part 1: causes and threats**. *Pharmacy and therapeutics* 2015, **40**(4):277.
12. Russell AB, Sharland M, Heath PT: **Improving antibiotic prescribing in neonatal units: time to act**. *Archives of Disease in Childhood-Fetal and Neonatal Edition* 2012, **97**(2):F141-F146.
13. Fongang H, Mbaveng AT, Kuete V: **Global burden of bacterial infections and drug resistance**. In: *Advances in Botanical Research. Volume 106*, edn.: Elsevier; 2023: 1-20.
14. Ikuta KS, Swetschinski LR, Aguilar GR, Sharara F, Mestrovic T, Gray AP, Weaver ND, Wool EE, Han C, Hayoon AG: **Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019**. *The Lancet* 2022, **400**(10369):2221-2248.
15. Organization WH: **WHO Bacterial Priority Pathogens List**. *Geneva: World Health Organization* 2024:12-13.
16. Kamboj A, Lause M, Kamboj K: **The Problem of Tuberculosis: Myths, Stigma, and Mimics**. In: *Tuberculosis: Integrated Studies for a Complex Disease.* edn.: Springer; 2023: 1047-1062.

17. MD AK, Fahim S, Muheb R, Ahmadi S: **Short Overview of The Burden of Tuberculosis**. *Clinical Medicine And Health Research Journal* 2024, **4**(2):829-832.
18. Ait Ouakrim D, Cassini A, Cecchini M, Plauchoras D: **The health and economic burden of antimicrobial resistance**. *European Journal of Public Health* 2020, **30**(Supplement_5):ckaa165. 1201.
19. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, Han C, Bisignano C, Rao P, Wool E: **Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis**. *The Lancet* 2022, **399**(10325):629-655.
20. Mestrovic T, Aguilar GR, Swetschinski LR, Ikuta KS, Gray AP, Weaver ND, Han C, Wool EE, Hayoon AG, Hay SI: **The burden of bacterial antimicrobial resistance in the WHO European region in 2019: a cross-country systematic analysis**. *The Lancet Public Health* 2022, **7**(11):e897-e913.
21. Zaidi AK, Awasthi S, DeSilva HJ: **Burden of infectious diseases in South Asia**. *Bmj* 2004, **328**(7443):811-815.
22. Misra R, Virmani R, Dhakan D, Maji A: **Tackling the antibiotic resistance: the "gut" feeling**. *Drug resistance in bacteria, fungi, malaria, and cancer* 2017:325-338.
23. Trakatelli M, Richard MA, Rouillard A, Paul C, Röcken M, Stratigos A: **The burden of skin disease in Europe**. In., vol. 37: Wiley Online Library; 2023: 3-5.
24. Xue Y, Zhou J, Xu B-N, Li Y, Bao W, Cheng XL, He Y, Xu CP, Ren J, Zheng Yr: **Global burden of bacterial skin diseases: a systematic analysis combined with sociodemographic index, 1990–2019**. *Frontiers in Medicine* 2022, **9**:861115.
25. Mokomane M, Kasvosve I, Melo Ed PJ, Goldfarb D: **The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management. Therapeutic advances in infectious disease. 2018; 5 (1): 29–43**. In.
26. Li X, Fan H, Zi H, Hu H, Li B, Huang J, Luo P, Zeng X: **Global and regional burden of bacterial antimicrobial resistance in urinary tract infections in 2019**. *Journal of clinical medicine* 2022, **11**(10):2817.
27. Williams PC, Isaacs D, Berkley JA: **Antimicrobial resistance among children in sub-Saharan Africa**. *The Lancet Infectious Diseases* 2018, **18**(2):e33-e44.
28. Abate BB, Kasie AM, Reta MA, Kassaw MW: **Neonatal sepsis and its associated factors in East Africa: a systematic review and meta-analysis**. *International journal of public health* 2020, **65**:1623-1633.
29. Kim J-H, Choi J, Kim C, Pak GD, Parajulee P, Haselbeck A, Park SE, Mogasale V, Jeon HJ, Browne AJ: **Mapping the incidence rate of typhoid fever in sub-Saharan Africa**. *PLoS neglected tropical diseases* 2024, **18**(2):e0011902.
30. Barichello T, Rocha Catalão CH, Rohlwink UK, Kuip Mvd, Zaharie D, Solomons RS, van Toorn R, Tutu van Furth M, Hasbun R, Iovino F: **Bacterial meningitis in Africa**. *Frontiers in neurology* 2023, **14**:822575.
31. Ekwanzala MD, Dewar JB, Kamika I, Momba MNB: **Systematic review in South Africa reveals antibiotic resistance genes shared between clinical and environmental settings**. *Infection and drug resistance* 2018:1907-1920.
32. Popa GL, Papa MI: **Salmonella spp. infection-a continuous threat worldwide**. *Germs* 2021, **11**(1):88.
33. Thystrup C, Majowicz SE, Kitila DB, Desta BN, Fayemi OE, Ayolabi CI, Hugho E, Buys EM, Akanni GB, Machava NE: **Etiology-specific incidence and mortality of diarrheal diseases in the African region: a systematic review and meta-analysis**. *BMC Public Health* 2024, **24**(1):1864.

34. Floyd K, Glaziou P, Zumla A, Raviglione M: **The global tuberculosis epidemic and progress in care, prevention, and research: an overview in year 3 of the End TB era**. *The Lancet Respiratory Medicine* 2018, **6**(4):299-314.
35. Nyirenda MJ: **Non-communicable diseases in sub-Saharan Africa: understanding the drivers of the epidemic to inform intervention strategies**. *International health* 2016, **8**(3):157-158.
36. Akpan MR, Isemin NU, Udoh AE, Ashiru-Oredope D: **Implementation of antimicrobial stewardship programmes in African countries: a systematic literature review**. *Journal of global antimicrobial resistance* 2020, **22**:317-324.
37. Igwe U, Okolie OJ, Ismail SU, Adukwu E: **Effectiveness of infection prevention and control interventions in healthcare facilities in Africa: a systematic review**. *American Journal of Infection Control* 2024.
38. Bereanu A-S, Bereanu R, Mohor C, Vintilă BI, Codru IR, Olteanu C, Sava M: **Prevalence of Infections and Antimicrobial Resistance of ESKAPE Group Bacteria Isolated from Patients Admitted to the Intensive Care Unit of a County Emergency Hospital in Romania**. *Antibiotics* 2024, **13**(5):400.
39. Irek EO, Amupitan AA, Aboderin AO, Obadare TO: **A systematic review of healthcare-associated infections in Africa: An antimicrobial resistance perspective**. *African journal of laboratory medicine* 2018, **7**(2):1-9.
40. Nazarko L: **Standard principles of infection control: infection control series, part 2**. *British Journal of Healthcare Assistants* 2014, **8**(5):226-233.
41. Morley PS: **Surveillance for nosocomial infections in veterinary hospitals**. *Veterinary Clinics: Equine Practice* 2004, **20**(3):561-576.
42. Carrico RM, Garrett H, Balcom D, Glowicz JB: **Infection prevention and control core practices: a roadmap for nursing practice**. *Nursing2023* 2018, **48**(8):22-28.
43. Manya A, Nielsen P: **Reporting practices and data quality in health information systems in developing countries: an exploratory case study in Kenya**. *Journal of Health Informatics in Developing Countries* 2016, **10**(1).
44. Beguy D, Elung'ata P, Mberu B, Oduor C, Wamukoya M, Nganyi B, Ezeh A: **Health & demographic surveillance system profile: the Nairobi urban health and demographic surveillance system (NUHDSS)**. *International journal of epidemiology* 2015, **44**(2):462-471.
45. Ng N, Van Minh H, Juvekar S, Razzaque A, Huu Bich T, Kanungsukkasem U, Ashraf A, Masud Ahmed S, Soonthornthada K: **Using the INDEPTH HDSS to build capacity for chronic non-communicable disease risk factor surveillance in low and middle-income countries**. *Global Health Action* 2009, **2**(1):1984.
46. Mahajan M: **The IHME in the shifting landscape of global health metrics**. *Global policy* 2019, **10**:110-120.
47. Beauvais B, Dolezel D, Shanmugam R, Wood D, Pradhan R: **An Exploratory Analysis of the Association between Healthcare Associated Infections & Hospital Financial Performance**. In: *Healthcare: 2024*: MDPI; 2024: 1314.
48. ECDC: **Healthcare-associated infections acquired in intensive care units**. *Annual Epidemiological Report for 2017* 2019.
49. Bunduki GK, Masoamphambe E, Fox T, Musaya J, Musicha P, Feasey N: **Prevalence, risk factors, and antimicrobial resistance of endemic healthcare-associated infections in Africa: a systematic review and meta-analysis**. *BMC Infectious Diseases* 2024, **24**(1):158.
50. McFee RB: **Nosocomial or hospital-acquired infections: an overview**. *Disease-a-Month* 2009, **55**(7):422.
51. Monegro AF, Muppidi V, Regunath H: **Hospital-acquired infections**. 2017.

52. Sartelli M: **Healthcare-associated infections in the surgical setting: How to prevent and treat them**. *Advances in Human Biology* 2022, **12**(2):127-137.
53. Abubakar U: **Point-prevalence survey of hospital acquired infections in three acute care hospitals in Northern Nigeria**. *ANTIMICROBIAL RESISTANCE AND INFECTION CONTROL* 2020, **9**(1):-.
54. Raoofi S, Pashazadeh Kan F, Rafiei S, Hosseinipalangi Z, Noorani Mejareh Z, Khani S, Abdollahi B, Seyghalani Talab F, Sanaei M, Zarabi F: **Global prevalence of nosocomial infection: A systematic review and meta-analysis**. *PLoS One* 2023, **18**(1):e0274248.
55. Nejad SB, Allegranzi B, Syed SB, Ellis B, Pittet D: **Health-care-associated infection in Africa: a systematic review**. *Bulletin of the World Health Organization* 2011, **89**(10):757-765.
56. Allegranzi B, Pittet D: **Role of hand hygiene in healthcare-associated infection prevention**. *Journal of hospital infection* 2009, **73**(4):305-315.
57. Hutton G, Chase C, Kennedy-Walker RJ: **Costs of Health Care Associated Infections from Inadequate Water and Sanitation in Health Care Facilities in Eastern and Southern Africa**. *Policy Research Working Paper* 2024, **10708**.
58. Melariri H, Freercks R, van der Merwe E, Ten Ham-Baloyi W, Oyedele O, Murphy RA, Claasen C, Etusim PE, Achebe MO, Offiah S: **The burden of hospital-acquired infections (HAI) in sub-Saharan Africa: a systematic review and meta-analysis**. *Eclinicalmedicine* 2024, **71**.
59. Goossens H, Sprenger MJ: **Community acquired infections and bacterial resistance**. *Bmj* 1998, **317**(7159):654-657.
60. Muvunyi V, Mpirimbanyi C, Katabogama JB, Cyuzuzo T, Nkubana T, Mugema JB, Musoni E, Urimubabo C, Rickard J: **Community-and Hospital-Acquired Infections in Surgical patients at a Tertiary Referral Hospital in Rwanda**. *World Journal of Surgery* 2020, **44**(10):3290-3298.
61. Bagcchi S: **WHO's global tuberculosis report 2022**. *The Lancet Microbe* 2023, **4**(1):e20.
62. Sarfo JO, Amoadu M, Gyan TB, Osman A-G, Kordorwu PY, Adams AK, Asiedu I, Ansah EW, Amponsah-Manu F, Ofosu-Appiah P: **Acute lower respiratory infections among children under five in Sub-Saharan Africa: a scoping review of prevalence and risk factors**. *BMC pediatrics* 2023, **23**(1):225.
63. Al-Dalfi MHK, Al Ibraheem SAH, Al-Rubaye AKQ: **The severity of pneumonia and its association with socio-demographic factors among children under five years old in Wasit governorate hospitals, Iraq**. *Journal of Public Health in Africa* 2023, **14**(8).
64. Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, Reed C, Grijalva CG, Anderson EJ, Courtney DM: **Community-acquired pneumonia requiring hospitalization among US adults**. *New England Journal of Medicine* 2015, **373**(5):415-427.
65. Prüss-Ustün A, Wolf J, Bartram J, Clasen T, Cumming O, Freeman MC, Gordon B, Hunter PR, Medlicott K, Johnston R: **Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: an updated analysis with a focus on low-and middle-income countries**. *International journal of hygiene and environmental health* 2019, **222**(5):765-777.
66. Nguyen LH, Drew DA, Graham MS, Joshi AD, Guo C-G, Ma W, Mehta RS, Warner ET, Sikavi DR, Lo C-H: **Risk of COVID-19 among front-line health-care workers and the general community: a prospective cohort study**. *The Lancet Public Health* 2020, **5**(9):e475-e483.

67. Antimicrobial Resistance C: **Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis**. *Lancet* 2022, **399**(10325):629-655.
68. Watson RS, Carrol ED, Carter MJ, Kissoon N, Ranjit S, Schlapbach LJ: **The burden and contemporary epidemiology of sepsis in children**. *The Lancet Child & Adolescent Health* 2024, **8**(9):670-681.
69. Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, Knight GM, Robotham JV: **Estimating the burden of antimicrobial resistance: a systematic literature review**. *Antimicrobial Resistance & Infection Control* 2018, **7**(1):58.
70. Fleischmann C, Reichert F, Cassini A, Horner R, Harder T, Markwart R, Tröndle M, Savova Y, Kissoon N, Schlattmann P: **Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis**. *Archives of disease in childhood* 2021, **106**(8):745-752.
71. Folgori L, Ellis SJ, Bielicki JA, Heath PT, Sharland M, Balasegaram M: **Tackling antimicrobial resistance in neonatal sepsis**. *The Lancet Global Health* 2017, **5**(11):e1066-e1068.
72. Prayag PS, Patwardhan SA, Joshi RS, Panchakshari SP, Rane T, Prayag AP: **Enzyme Patterns and Factors Associated with Mortality among Patients with Carbapenem Resistant Acinetobacter Baumannii (CRAB) Bacteremia: Real World Evidence from a Tertiary Center in India**. *Indian Journal of Critical Care Medicine: Peer-reviewed, Official Publication of Indian Society of Critical Care Medicine* 2023, **27**(9):663.
73. Morris S, Cerceo E: **Trends, epidemiology, and management of multi-drug resistant gram-negative bacterial infections in the hospitalized setting**. *Antibiotics* 2020, **9**(4):196.
74. Naaz T, Singh V, Ray S, Sharma K, Kumar B, Pandit S: **Fundamentals of bacterial quorum sensing and antimicrobial resistance**. In: *Natural products.* edn.: CRC Press; 2024: 62-78.
75. Ahmed SK, Hussein S, Qurbani K, Ibrahim RH, Fareeq A, Mahmood KA, Mohamed MG: **Antimicrobial resistance: impacts, challenges, and future prospects**. *Journal of Medicine, Surgery, and Public Health* 2024, **2**:100081.
76. Mammen NK: **Antimicrobial resistance**.
77. Ezeonu IM, Iroha I, Esiobu ND: **Antibiotic Resistance: Global Trends, Impact and Mitigation**. In: *Medical Biotechnology, Biopharmaceutics, Forensic Science and Bioinformatics.* edn.: CRC Press; 2022: 99-113.
78. O'Neill J: **Tackling drug-resistant infections globally: final report and recommendations**. 2016.
79. Sartorius B, Gray AP, Weaver ND, Aguilar GR, Swetschinski LR, Ikuta KS, Mestrovic T, Chung E, Wool EE, Han C: **The burden of bacterial antimicrobial resistance in the WHO African region in 2019: a cross-country systematic analysis**. *The Lancet Global Health* 2024, **12**(2):e201-e216.
80. Azad MAK: **Antimicrobial resistance: Real threat for the clinician**. *Bangladesh Journal of Medicine* 2023:131-131.
81. Poudel AN, Zhu S, Cooper N, Little P, Tarrant C, Hickman M, Yao G: **The economic burden of antibiotic resistance: A systematic review and meta-analysis**. *Plos one* 2023, **18**(5):e0285170.
82. Okeke IN, de Kraker ME, Van Boeckel TP, Kumar CK, Schmitt H, Gales AC, Bertagnolio S, Sharland M, Laxminarayan R: **The scope of the antimicrobial resistance challenge**. *The Lancet* 2024, **403**(10442):2426-2438.
83. Claycamp HG, Hooberman BH: **Antimicrobial resistance risk assessment in food safety**. *Journal of food protection* 2004, **67**(9):2063-2071.

84. Collignon P, Courvalin P, Aidara-Kane A: **Clinical importance of antimicrobial drugs in human health**. *Guide to Antimicrobial Use in Animals* 2008:44-58.
85. Devi NS, Mythili R, Cherian T, Dineshkumar R, Sivaraman G, Jayakumar R, Prathaban M, Duraimurugan M, Chandrasekar V, Peijnenburg WJ: **Overview of Antimicrobial Resistance and mechanisms: the Relative Status of the Past and Current**. *The Microbe* 2024:100083.
86. Henriksen T-H, Abebe W, Amogne W, Getachew Y, Weedon-Fekjær H, Klein J, Woldeamanuel Y: **Association between antimicrobial resistance among Enterobacteriaceae and burden of environmental bacteria in hospital acquired infections: analysis of clinical studies and national reports**. *Heliyon* 2019, **5**(7).
87. Shields RK, Zhou Y, Kanakamedala H, Cai B: **Burden of illness in US hospitals due to carbapenem-resistant Gram-negative urinary tract infections in patients with or without bacteraemia**. *BMC infectious diseases* 2021, **21**(1):572.
88. Anton BM, Pantea L, Burduniuc PO, Chilianu M, Bucov V, Tapu L: **Evaluation of costs related to antimicrobial resistance of priority Gramnegative bacilli**. *One Health and Risk Management* 2024(1):43-50.
89. Osei Sekyere J, Reta MA: **Genomic and resistance epidemiology of Gram-negative bacteria in Africa: a systematic review and phylogenomic analyses from a one health perspective**. *Msystems* 2020, **5**(6):10.1128/msystems. 00897-00820.
90. Toy T, Pak GD, Duc TP, Campbell JI, El Tayeb MA, Von Kalckreuth V, Im J, Panzner U, Cruz Espinoza LM, Eibach D: **Multicountry distribution and characterization of extended-spectrum β-Lactamase–associated gram-negative bacteria from bloodstream infections in sub-saharan africa**. *Clinical Infectious Diseases* 2019, **69**(Supplement_6):S449-S458.
91. Cerceo E, Deitelzweig SB, Sherman BM, Amin AN: **Multidrug-resistant gram-negative bacterial infections in the hospital setting: overview, implications for clinical practice, and emerging treatment options**. *Microbial Drug Resistance* 2016, **22**(5):412-431.
92. Jean S-S, Harnod D, Hsueh P-R: **Global threat of carbapenem-resistant gram-negative bacteria**. *Frontiers in cellular and infection microbiology* 2022, **12**:823684.
93. Da Silva GJ, Domingues S: **Insights on the horizontal gene transfer of carbapenemase determinants in the opportunistic pathogen Acinetobacter baumannii**. *Microorganisms* 2016, **4**(3):29.
94. White A, Hughes JM: **Critical importance of a one health approach to antimicrobial resistance**. *EcoHealth* 2019, **16**:404-409.
95. Partridge SR, Kwong SM, Firth N, Jensen SO: **Mobile genetic elements associated with antimicrobial resistance**. *Clinical microbiology reviews* 2018, **31**(4):e00088-00017.
96. Noster J, Thelen P, Hamprecht A: **Detection of multidrug-resistant Enterobacterales—from ESBLs to carbapenemases**. *Antibiotics* 2021, **10**(9):1140.
97. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VH, Takebayashi Y, Spencer J: **β-Lactamases and β-Lactamase Inhibitors in the 21st Century**. *Journal of molecular biology* 2019, **431**(18):3472-3500.
98. Lascols C: **Global Molecular Characterization of Extended-Spectrum ß-lactamases and carbapenemases in Enterobacterales**. Université Paris-Saclay; 2024.

99. Zhang L, Ma X, Luo L, Hu N, Duan J, Tang Z, Zhong R, Li Y: **The prevalence and characterization of extended-spectrum β-lactamase-and carbapenemase-producing bacteria from hospital sewage, treated effluents and receiving rivers**. *International journal of environmental research and public health* 2020, **17**(4):1183.
100. Toner E, Adalja A, Gronvall GK, Cicero A, Inglesby TV: **Antimicrobial resistance is a global health emergency**. *Health security* 2015, **13**(3):153-155.
101. Yusuf I, Arzai A, Haruna M, Sharif A, Getso M: **Detection of multi drug resistant bacteria in major hospitals in Kano, North-West, Nigeria**. *Brazilian Journal of Microbiology* 2014, **45**:791-798.
102. Stone G, Wise M, Utt E: **In vitro activity of ceftazidime–avibactam and comparators against OXA-48-like Enterobacterales collected between 2016 and 2020**. *Microbiology Spectrum* 2024, **12**(3):e01473-01423.
103. Jones RN, Baquero F, Privitera G, Inoue M, Wiedemann B: **Inducible β-lactamase-mediated resistance to third-generation cephalosporins**. *Clinical Microbiology and Infection* 1997, **3**:s7-s20.
104. Yu D, Zheng Y, Shen A, Wu F, Dmitriev AV, Kilian M, Yang Y: **Antimicrobial resistance in pediatric infectious diseases: antimicrobial resistance, resistance mechanisms and antimicrobial use**. In., vol. 13: Frontiers Media SA; 2023: 1287051.
105. Basha S, Surendran N, Pichichero M: **Immune responses in neonates**. *Expert review of clinical immunology* 2014, **10**(9):1171-1184.
106. Katz SE, Banerjee R, O'Leary ST, Campbell JD, Ardura MI, Bryant KA, Caserta MT, Frenck RW, Gerber JS, John CC: **Use of Antibiotics in Animal Agriculture: Implications for Pediatrics: Technical Report**. *Pediatrics* 2024, **154**(4).
107. Romandini A, Pani A, Schenardi PA, Pattarino GAC, De Giacomo C, Scaglione F: **Antibiotic resistance in pediatric infections: global emerging threats, predicting the near future**. *Antibiotics* 2021, **10**(4):393.
108. Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, Aguilar GR, Mestrovic T, Smith G, Han C: **Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050**. *The Lancet* 2024, **404**(10459):1199-1226.
109. McDonnell A, Klemperer K: **Drug-Resistant Infections Are One of the World's Biggest Killers, Especially for Children in Poorer Countries. We Need to Act Now**. *Center for Global Development blog* 2022, **22**.
110. Aiken AM, Rehman AM, de Kraker ME, Madrid L, Kebede M, Labi A-K, Obeng-Nkrumah N, Nyamwaya B, Kagucia E, Cocker D: **Mortality associated with third-generation cephalosporin resistance in Enterobacterales bloodstream infections at eight sub-Saharan African hospitals (MBIRA): a prospective cohort study**. *The Lancet Infectious Diseases* 2023, **23**(11):1280-1290.
111. Polianciuc SI, Gurzău AE, Kiss B, Ştefan MG, Loghin F: **Antibiotics in the environment: causes and consequences**. *Medicine and pharmacy reports* 2020, **93**(3):231.
112. Manyi-Loh C, Mamphweli S, Meyer E, Okoh A: **Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications**. *Molecules* 2018, **23**(4):795.
113. Heuer H, Schmitt H, Smalla K: **Antibiotic resistance gene spread due to manure application on agricultural fields**. *Current opinion in microbiology* 2011, **14**(3):236-243.

114. Tang KWK, Millar BC, Moore JE: **Antimicrobial resistance (AMR)**. *British Journal of Biomedical Science* 2023, **80**:11387.
115. Chemaly RF, Simmons S, Dale Jr C, Ghantoji SS, Rodriguez M, Gubb J, Stachowiak J, Stibich M: **The role of the healthcare environment in the spread of multidrug-resistant organisms: update on current best practices for containment**. *Therapeutic advances in infectious disease* 2014, **2**(3-4):79-90.
116. Saleem Z, Hassali MA, Godman B, Versporten A, Hashmi FK, Saeed H, Saleem F, Salman M, Rehman IU, Khan TM: **Point prevalence surveys of antimicrobial use: a systematic review and the implications**. *Expert review of anti-infective therapy* 2020, **18**(9):897-910.
117. Lee Y-L, Wang W-Y, Ko W-C, Hsueh P-R: **Global epidemiology and antimicrobial resistance of Enterobacterales harbouring genes encoding OXA-48-like carbapenemases: insights from the results of the Antimicrobial Testing Leadership and Surveillance (ATLAS) programme 2018–2021**. *Journal of Antimicrobial Chemotherapy* 2024:dkae140.
118. Kimani R, Wakaba P, Kamita M, Mbogo D, Mutai W, Ayodo C, Suliman E, Kanoi BN, Gitaka J: **Detection of multidrug-resistant organisms of concern including Stenotrophomonas maltophilia and Burkholderia cepacia at a referral hospital in Kenya**. *Plos one* 2024, **19**(4):e0298873.
119. Garba Z, Kaboré B, Bonkoungou IJ, Natama MH, Rouamba T, Haukka K, Kirveskari JP, Tinto H, Sangaré L, Barro N: **Phenotypic Detection of Carbapenemase and AmpC-β-Lactamase Production among Extended Spectrum β-Lactamase (ESBL)-Producing Escherichia coli and Klebsiella spp. Isolated from Clinical Specimens**. *Antibiotics* 2023, **13**(1):31.
120. Acolatse JEE, Portal EA, Boostrom I, Akafity G, Dakroah MP, Chalker VJ, Sands K, Spiller OB: **Environmental surveillance of ESBL and carbapenemase-producing gram-negative bacteria in a Ghanaian Tertiary Hospital**. *Antimicrobial Resistance & Infection Control* 2022, **11**(1):49.
121. Kubde D, Badge AK, Ugemuge S, Shahu S: **Importance of Hospital Infection Control**. *Cureus* 2023, **15**(12).
122. Carter DL, Docherty KM, Gill SA, Baker K, Teachout J, Vonhof MJ: **Antibiotic resistant bacteria are widespread in songbirds across rural and urban environments**. *Science of the Total Environment* 2018, **627**:1234-1241.
123. Baquero F, Martínez J-L, Cantón R: **Antibiotics and antibiotic resistance in water environments**. *Current opinion in biotechnology* 2008, **19**(3):260-265.
124. Lepper HC, Woolhouse ME, van Bunnik BA: **The role of the environment in dynamics of antibiotic resistance in humans and animals: A modelling study**. *Antibiotics* 2022, **11**(10):1361.
125. Wang W, Weng Y, Luo T, Wang Q, Yang G, Jin Y: **Antimicrobial and the resistances in the environment: ecological and health risks, influencing factors, and mitigation strategies**. *Toxics* 2023, **11**(2):185.
126. Hosain MZ, Kabir SL, Kamal MM: **Antimicrobial uses for livestock production in developing countries**. *Veterinary World* 2021, **14**(1):210.
127. Ardakani Z, Canali M, Aragrande M, Tomassone L, Simoes M, Balzani A, Beber CL: **Evaluating the contribution of antimicrobial use in farmed animals to global antimicrobial resistance in humans**. *One Health* 2023, **17**:100647.
128. Ho CS, Wong CT, Aung TT, Lakshminarayanan R, Mehta JS, Rauz S, McNally A, Kintses B, Peacock SJ, de la Fuente-Nunez C: **Antimicrobial resistance: a concise update**. *The Lancet Microbe* 2024.

129. Russell NJ, Stöhr W, Plakkal N, Cook A, Berkley JA, Adhisivam B, Agarwal R, Ahmed NU, Balasegaram M, Ballot D: **Patterns of antibiotic use, pathogens, and prediction of mortality in hospitalized neonates and young infants with sepsis: A global neonatal sepsis observational cohort study (NeoOBS)**. *PLoS Medicine* 2023, **20**(6):e1004179.
130. England PH: **English surveillance programme for antimicrobial utilisation and resistance (ESPAUR)**. In.: London; 2014.
131. Ilmavirta H, Ollgren J, Räisänen K, Kinnunen T, Hakanen AJ, Jalava J, Lyytikäinen O: **Increasing proportions of extended-spectrum β-lactamase-producing isolates among Escherichia coli from urine and bloodstream infections: results from a nationwide surveillance network, Finland, 2008 to 2019**. *Eurosurveillance* 2023, **28**(43):2200934.
132. Gandra S, Mojica N, Klein EY, Ashok A, Nerurkar V, Kumari M, Ramesh U, Dey S, Vadwai V, Das BR: **Trends in antibiotic resistance among major bacterial pathogens isolated from blood cultures tested at a large private laboratory network in India, 2008–2014**. *International Journal of Infectious Diseases* 2016, **50**:75-82.
133. Tola MA, Abera NA, Gebeyehu YM, Dinku SF, Tullu KD: **High prevalence of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae fecal carriage among children under five years in Addis Ababa, Ethiopia**. *PloS one* 2021, **16**(10):e0258117.
134. Loyola S, Concha-Velasco F, Pino-Dueñas J, Vasquez-Luna N, Juarez P, Llanos C, Salvatierra G, Tamariz J, Lescano AG: **Antimicrobial resistance patterns and dynamics of extended-spectrum β-lactamase-producing uropathogenic escherichia coli in cusco, peru**. *Antibiotics* 2021, **10**(5):485.
135. Maveke SM, Aboge GO, Kanja LW, Mainga AO, Gachau N, Muchira BW, Moriasi GA: **Phenotypic and Genotypic Characterization of Extended Spectrum Beta-Lactamase-Producing Clinical Isolates of Escherichia coli and Klebsiella pneumoniae in Two Kenyan Facilities: A National Referral and a Level Five Hospital**. *International Journal of Microbiology* 2024, **2024**(1):7463899.
136. Waithiru D, Njeru JM, Maingi JM, Mulinge E, Ngugi B, Maina J, Kiiru J: **Unravelling Antimicrobial Resistance Phenotypes and Carriage of Extended-Spectrum β-Lactamase Genes in Escherichia coli Isolated from Dairy Farms in Kiambu County, Kenya**. *Advances in Microbiology* 2022, **12**(5):295-315.
137. Kajeguka DC, Nambunga PP, Kabissi F, Kamugisha B, Kassam N, Nyombi B, Mataro C, Maro V, Chilongola JO: **Antimicrobial resistance patterns of phenotype Extended Spectrum Beta-Lactamase producing bacterial isolates in a referral hospital in northern Tanzania**. *Tanzania Journal of Health Research* 2015, **17**(3).
138. Who W: **Priority Pathogens List for R&D of New Antibiotics**. *WHO, Geneva, Switzerland* 2017.
139. Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W: **The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria**. *The Lancet infectious diseases* 2013, **13**(2):155-165.
140. Ramatla T, Mafokwane T, Lekota K, Monyama M, Khasapane G, Serage N, Nkhebenyane J, Bezuidenhout C, Thekisoe O: **"One Health" perspective on prevalence of co-existing extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae: a comprehensive systematic**

review and meta-analysis. *Annals of Clinical Microbiology and Antimicrobials* 2023, **22**(1):88.
141. Dadashi M, Fallah F, Hashemi A, Hajikhani B, Owlia P, Bostanghadiri N, Farahani N, Mirpour M: **Prevalence of blaNDM− 1-producing Klebsiella pneumoniae in Asia: A systematic review and meta-analysis**. *Journal des Anti-infectieux* 2017, **19**(2):58-65.
142. Organization WH: **Antimicrobial Resistance in the WHO African Region: a systematic literature review**. *Antimicrobial resistance in the WHO African Region: a systematic literature review* 2021.
143. Davies J, Davies D: **Origins and evolution of antibiotic resistance**. *Microbiology and molecular biology reviews* 2010, **74**(3):417-433.
144. Partridge SR, Kwong SM, Firth N, Jensen SO: **Mobile genetic elements associated with antimicrobial resistance**. *Clinical microbiology reviews* 2018, **31**(4):10.1128/cmr. 00088-00017.
145. Hendriksen RS, Munk P, Njage P, Van Bunnik B, McNally L, Lukjancenko O, Röder T, Nieuwenhuijse D, Pedersen SK, Kjeldgaard J: **Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage**. *Nature communications* 2019, **10**(1):1124.
146. Seethalakshmi P, Ru VPN, Prabhakaran A, Prathiviraj R, Pamanji R, Kiran GS, Selvin J: **Genomic investigation unveils high-risk ESBL producing Enterobacteriaceae within a rural environmental water body**. *Current Research in Microbial Sciences* 2024, **6**:100216.
147. Alekshun MN, Levy SB: **Molecular mechanisms of antibacterial multidrug resistance**. *Cell* 2007, **128**(6):1037-1050.
148. Ntirenganya C: **Antimicrobial resistance profile of common bacterial pathogens and impact on patient treatment outcomes at Kigali University Teaching Hospital (KUTH)**. University of Rwanda; 2014.
149. Muriuki CW, Ogonda LA, Kyanya C, Matano D, Masakhwe C, Odoyo E, Musila L: **Phenotypic and genotypic characteristics of uropathogenic Escherichia coli isolates from Kenya**. *Microbial drug resistance* 2022, **28**(1):31-38.
150. Kiyaga S, Kyany'a C, Muraya AW, Smith HJ, Mills EG, Kibet C, Mboowa G, Musila L: **Genetic diversity, distribution, and genomic characterization of antibiotic resistance and virulence of clinical Pseudomonas aeruginosa Strains in Kenya**. *Frontiers in Microbiology* 2022, **13**:835403.
151. Woodford N, Turton JF, Livermore DM: **Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance**. *FEMS microbiology reviews* 2011, **35**(5):736-755.
152. Medugu N, Tickler IA, Duru C, Egah R, James AO, Odili V, Hanga F, Olateju EK, Jibir B, Ebruke BE: **Phenotypic and molecular characterization of beta-lactam resistant Multidrug-resistant Enterobacterales isolated from patients attending six hospitals in Northern Nigeria**. *Scientific reports* 2023, **13**(1):10306.
153. Hijazi S, Fawzi M, Ali F, Abd El Galil K: **Prevalence and characterization of extended-spectrum beta-lactamases producing Enterobacteriaceae in healthy children and associated risk factors**. *Annals of clinical microbiology and antimicrobials* 2016, **15**:1-9.
154. Control CfD, Prevention: **Core elements of hospital antibiotic stewardship programs, 2014**. *Atlanta: CDC* 2019:8-20.
155. Organization WH: **Antimicrobial stewardship programmes in health-care facilities in low-and middle-income countries: a WHO practical toolkit**: World Health Organization; 2019.

156. Organization WH: **WHO Access, Watch, Reserve (AWARE) classification of antibiotics for evaluation and monitoring of use, 2021**. In: *WHO access, watch, reserve (AWaRe) classification of antibiotics for evaluation and monitoring of use, 2021*. edn.; 2021.
157. Organization WH: **Report of the 6th meeting of the WHO advisory group on integrated surveillance of antimicrobial resistance with AGISAR 5-year strategic framework to support implementation of the global action plan on antimicrobial resistance (2015-2019), 10-12 June 2015, Seoul, Republic of Korea**: World Health Organization; 2015.
158. Davey P, Brown E, Charani E, Fenelon L, Gould IM, Holmes A, Ramsay CR, Wiffen PJ, Wilcox M: **Interventions to improve antibiotic prescribing practices for hospital inpatients**. *Cochrane database of systematic reviews* 2013(4).
159. Organization WH: **Global report on infection prevention and control**: World Health Organization; 2022.
160. Pittet D, Hugonnet S, Harbarth S, Mourouga P, Sauvan V, Touveneau S, Perneger TV: **Effectiveness of a hospital-wide programme to improve compliance with hand hygiene**. *The Lancet* 2000, **356**(9238):1307-1312.
161. Ginsburg AS, Klugman KP: **Vaccination to reduce antimicrobial resistance**. *The Lancet Global Health* 2017, **5**(12):e1176-e1177.
162. Lipsitch M, Siber G: **How can vaccines contribute to solving the antimicrobial resistance problem? MBio. 2016; 7**. In.: 7.
163. Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen J-A, Klugman K, Davies S: **Access to effective antimicrobials: a worldwide challenge**. *The Lancet* 2016, **387**(10014):168-175.
164. Sanderson ND, Street TL, Foster D, Swann J, Atkins BL, Brent AJ, McNally MA, Oakley S, Taylor A, Peto TE: **Real-time analysis of nanopore-based metagenomic sequencing from infected orthopaedic devices**. *BMC genomics* 2018, **19**:1-11.
165. Ellington M, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden M, Hopkins KL: **The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee**. *Clinical microbiology and infection* 2017, **23**(1):2-22.
166. Okeke IN, Feasey N, Parkhill J, Turner P, Limmathurotsakul D, Georgiou P, Holmes A, Peacock SJ: **Leapfrogging laboratories: the promise and pitfalls of high-tech solutions for antimicrobial resistance surveillance in low-income settings**. *BMJ global health* 2020, **5**(12):e003622.
167. Gwinn M, MacCannell D, Armstrong GL: **Next-generation sequencing of infectious pathogens**. *Jama* 2019, **321**(9):893-894.
168. Mongan AE, Tuda JSB, Runtuwene LR: **Portable sequencer in the fight against infectious disease**. *Journal of human genetics* 2020, **65**(1):35-40.
169. Wolk DM, Johnson JK: **Rapid diagnostics for blood cultures: supporting decisions for antimicrobial therapy and value-based care**. *The Journal of Applied Laboratory Medicine* 2019, **3**(4):686-697.
170. **Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2016-2017** [https://www.who.int/publications/i/item/9789241513449]
171. Köser CU, Ellington MJ, Peacock SJ: **Whole-genome sequencing to control antimicrobial resistance**. *Trends in Genetics* 2014, **30**(9):401-407.
172. Brooks BD, Brooks AE: **Therapeutic strategies to combat antibiotic resistance**. *Advanced drug delivery reviews* 2014, **78**:14-27.

173. Organization WH: **Epidemiological Bulletin WHO Health Emergencies Programme: WHO Regional Office for South-East Asia: 23 August 2023, Reporting period: 7–20 August 2023**. 2023.
174. Robinson TP, Bu D, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, Hay SI, Jiwakanon J, Kakkar M, Kariuki S: **Antibiotic resistance is the quintessential One Health issue**. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2016, **110**(7):377-380.
175. Huttner B, Saam M, Moja L, Mah K, Sprenger M, Harbarth S, Magrini N: **How to improve antibiotic awareness campaigns: findings of a WHO global survey**. *BMJ global health* 2019, **4**(3):e001239.
176. Wintaco LM, Quintero-Lesmes DC, Vargas-Soler JA, Barrera DM, Palacio LN, Granados U, Uribe LG: **Analysis of Healthcare-associated Infections before and during the COVID-19 pandemic in a Colombian hospital**. *Revista Cuidarte* 2024, **15**(1).
177. Childhood Acute Illness Nutrition Network: **Childhood Acute Illness and Nutrition (CHAIN) Network: a protocol for a multi-site prospective cohort study to identify modifiable risk factors for mortality among acutely ill children in Africa and Asia**. *BMJ Open* 2019, **9**(5):e028454.

3 HOSPITAL-ACQUIRED GRAM-NEGATIVE BLOODSTREAM INFECTIONS IN AFRICAN CHILDREN: A SYSTEMATIC REVIEW AND METANALYSIS OF PREVALENCE, PATHOGENS, AND RISK FACTORS

3.1 Background

Hospital-acquired infections (HAIs) pose a significant global threat to patient health, with particularly severe consequences in low- and middle-income countries[1, 2]. In Africa, hospital -acquired Gram-negative bloodstream infections (HA-GNBSIs) among children represent a major public health challenge due to high morbidity, mortality, and the rapid emergence of antibiotic-resistant pathogens[3]. These infections place a significant burden on healthcare systems, particularly in regions with limited resources and infection control measures.

HA-GNBSIs are among the most concerning hospital-acquired infections in paediatric settings, often caused by multidrug-resistant Gram-negative bacteria such as *E. coli*, *K. pneumoniae*, and *A. baumannii*[4]. These pathogens complicate treatment options and contribute to increased mortality. Studies indicate that HA-GNBSIs account for a substantial proportion of paediatric bloodstream infections across African hospitals. For instance, a study in a tertiary hospital in Tanzania found that Gram-negative bacteria were responsible for 58.4% of bloodstream infections in hospitalised children[5].

Methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant Gram-negative bacteria are among the most concerning hospital-acquired pathogens, increasing treatment complexity and healthcare burden. MRSA, a leading cause of hospital-acquired bloodstream and surgical site infections, has been reported with variable prevalence across African settings,

reaching up to 25% in some hospitals.[4]. However, the burden of Gram-negative infections appears to be higher, particularly among critically ill patients due to resistance to commonly used antibiotics, further complicating treatment[4]. AMR in these species in hospital-acquired infection is often reported in association with higher levels of care and underlying comorbidities such as diabetes, malignancies, chronic respiratory diseases, renal failure, and immunosuppression[5].

Despite the gravity of this issue, comprehensive data on the prevalence and risk factors of HA-GNBSIs in paediatric populations across Africa remain limited[6]. The pooled prevalence of hospital-acquired infections (HAIs) in sub-Saharan Africa has been synthesised to be approximately 12.9% (95% CI: 8.9-17.4; n = 7336), with regions like East Africa at 19.7% (n = 2142), Southern Africa at 6.5% (n = 2963), West Africa at 15.5% (n = 2107) and Central Africa at 10.3% (n = 124)[3]. While specific prevalence rates for HA-GNBSIs in children vary across different studies and regions, their overall impact on healthcare systems is considerable
[5]. Factors such as prolonged hospital stay, the use of invasive medical devices, and suboptimal infection control practices contribute to the persistence of HA-GNBSIs. Additionally, the scarcity of water, sanitation, and hygiene (WASH) facilities in nearly 50% of healthcare institutions in the region facilitates the spread of infections[7].

The lack of comprehensive surveillance data complicates the accurate determination of prevalence of HA-GNBSI burden, but available studies consistently highlight the significant role of Gram-negative bacteria in paediatric hospital-acquired bloodstream infections in African healthcare settings[8]. The rise of multidrug-resistant pathogens, including MRSA, CRE and ESBL-E, increasingly hinder effective infection management.

Although there is widespread awareness, key drivers of HAIs are that IPC implementation, and behaviours are still poorly implemented. For example, while healthcare professionals in some regions, such as Western Uganda, demonstrate awareness and positive attitudes toward HAI prevention, significant gaps persist between knowledge and practice. In this setting, only 36% of healthcare workers were found to exhibit commendable preventive behaviours[9]. These findings underscore the urgent need for bridging this gap through improved IPC practices.

This systematic review aims to fill a critical knowledge gap on the prevalence, causative organisms, and risk factors associated with resistant hospital-acquired Gram-negative BSIs in children across Africa.

3.2 Research Questions

1. What proportion of HA-BSI are caused by Gram-negative bacteria in African children?
2. What is the pooled prevalence of phenotypic ESBL-E and CRE among hospital acquired HA-GNBSI?
3. What species are causing HA-GNBSI with ESBL-E/CRE in neonates and children?
4. What genotypes of ESBL-E/occur in HA-GNBSI?
5. What factors are associated with ESBL-E and CRE in HA-GNBSI?

3.3 Materials and Methods

To understand the background context relevant to my research questions above, I have conducted a review of HA-GNBSIs, caused by extended-spectrum beta-lactamase and carbapenemase-producing Enterobacterales in Africa, performed in compliance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)

statement and checklist. The review was registered in PROSPERO under ID number CRD42024519109.

3.4 Study Area

The review provided information on published articles in English from all African countries from 22nd of November 1992 until 22nd of January 2024, 18.01hrs.

3.5 Information sources

Three electronic databases Web of Science, PubMed and Scopus were used to identify eligible articles. The databases were searched from 16/01/2024 to 22/02/2024 using the search terms described below. The reference lists of selected studies were also examined for eligible articles.

3.6 Search strategy

The keywords “nosocomial”, “hospital environment”, “antimicrobial resistance” and Africa with their synonyms were combined using Boolean indicators. The following keywords were used to conduct the search on the electronic databases: Hospital OR nosocomial AND environment OR acquisition OR transmission AND ESBL OR antimicrobial resistance OR "beta-lactamase" OR "extended spectrum" OR carbapenemase OR CPE OR CRE AND Algeria OR Angola OR Benin OR Botswana OR “Burkina Faso” OR Burundi OR “Cape Verde” OR “Cabo Verde” OR Cameroon OR “Central African Republic” OR Chad OR Comoros OR Congo OR “Democratic Republic of Congo” OR DRC OR “Cote d'Ivoire” OR “Ivory Coast” OR Djibouti OR Egypt OR Equatorial Guinea OR Eritrea OR Eswatini OR Swaziland OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR “Guinea-Bissau” OR Kenya OR Lesotho OR Liberia OR Libya OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Morocco OR

Mozambique OR Namibia OR Niger OR Nigeria OR Rwanda OR "Sao Tome" and Principe OR "São Tomé e Príncipe" OR Senegal OR Seychelles OR "Sierra Leone" OR Somalia OR South* Africa OR "South Sudan" OR Sudan OR Tanzania OR Togo OR Tunisia OR Uganda OR Zambia OR Zimbabwe.

3.7 Selection process

All articles identified from electronic databases were merged and screened using Rayyan AI to remove duplicates. Two independent reviewers (myself and Anne Amulele) screened the titles and abstracts of the remaining articles based on specific eligibility criteria: peer-reviewed studies conducted on admitted neonates and children in African countries, involving blood culture samples or isolates collected after 48 hours of hospital admission, and confirmed as hospital-acquired infections. The studies had to be published in English and available as free full text. Exclusion criteria included studies with blood samples taken only at admission, point-prevalence surveys without infection incidence over time, previous reviews, editorials, commentaries, and unpublished articles. Full texts of eligible studies were reviewed, and any disagreements between the reviewers were resolved by consensus.

3.8 Data collection process

The selected studies were reviewed, and the data extracted using a data collection tool designed in REDCap (https://redcap.medsci.ox.ac.uk). Data extraction was conducted by the wo independent reviewers (myself and Anne Amulele).

3.9 Data extraction

A data extraction checklist was developed to guide the acquisition of the information which included name of the title, author(s), year of publication, study setting (hospital set up),

study design, countries, subjects/target population, source of isolates/specimen, sample types, isolate name and their prevalence, sample size, bacteriological methods for estimating ESBL's and CRE, molecular methods used, isolate species, number of isolates analysed, ESBL and CRE positive isolates, genes encoding for ESBL and CRE identified, risk factors associated and their odds ratio with ESBL and CRE.

3.10 Data Analysis

Extracted data were entered into REDCap and statistical analysis done using R v4.0.5. Random effects meta-analysis was done to determine heterogeneity of ESBL-E and CRE prevalence in Africa and variation in study outcomes between studies was measured using the I^2 statistic. The overall prevalence of HA-GNBSIs and 95% confidence interval was determined according with the total number of sampled participants using a mixed-effects logistic regression model. Risk factors were described using forest plots and heterogeneity examined using the Higgins I^2 statistic. Meta-regression analysis was done for pooled risk estimates for the statistically significant factors associated with HA-GNBSIs.

The primary outcome was the pooled prevalence among all HA-GNBSIs among hospitalised children <18 years of age in Africa. HA-GNBSI was defined as clinical samples collected after 48 hours of admission and confirmed not to present at admission. Secondary outcomes included the risk factors associated with HA- GNBSIs and the microorganisms that caused HA-GNBSIs. The primary outcome was presented as frequency and percentage among children less that 18 years of age. The types of HA-GNBSIs were presented as frequency and percentage. The risk factors associated with all HA BSI were presented as adjusted odds ratios.

3.11 Results

3.11.1 Study selection

A total of 5761 articles were identified from the electronic databases and 779 duplicates were removed. After screening the title and abstracts of the non-duplicate articles, 4855 articles were excluded. The full text of 115 articles was reviewed and 39 eligible studies were selected for inclusion in the systematic review (Figure 3.1).

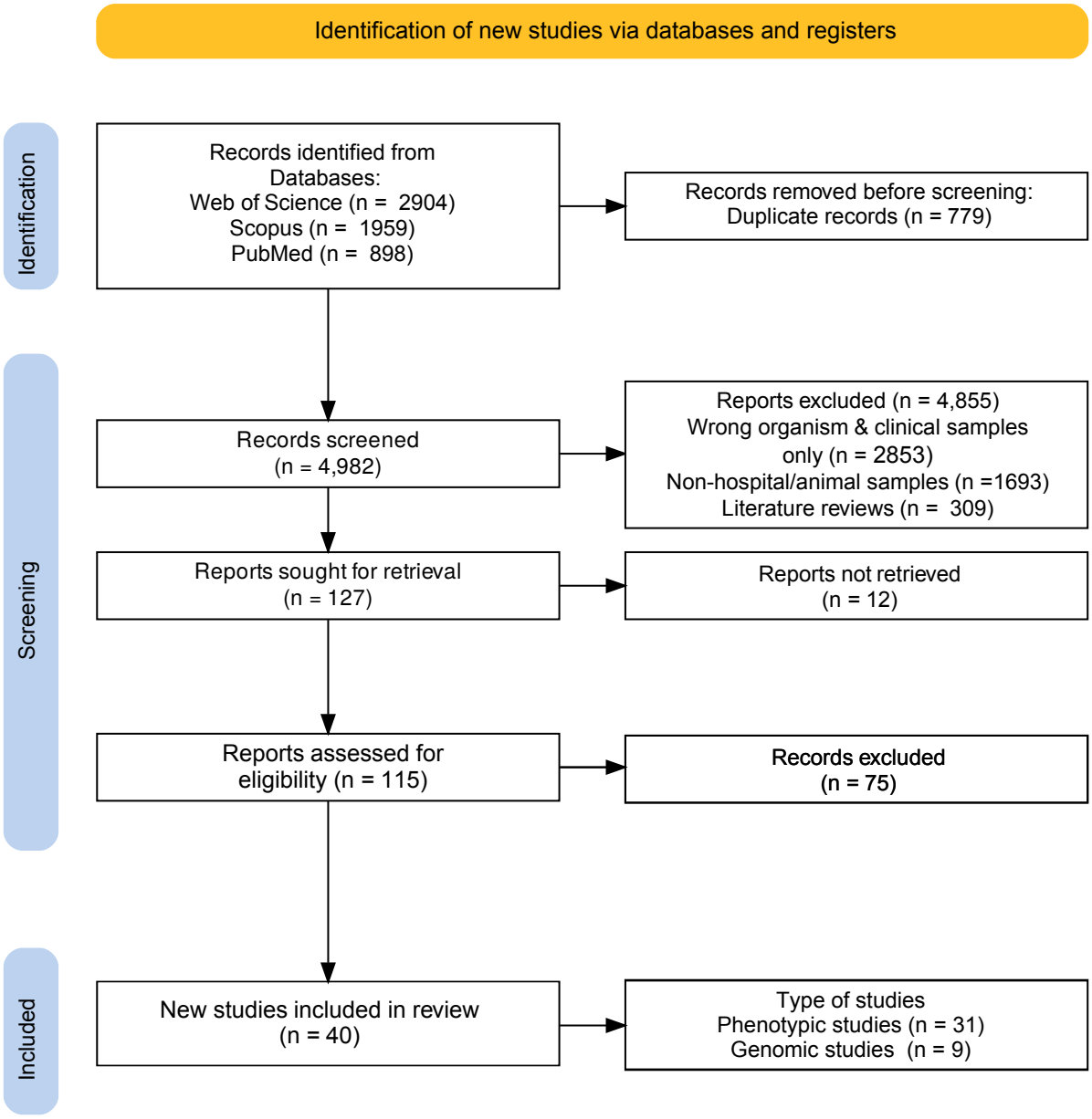


***Figure 3.1: PRISMA** Flow chart of article screening and selection*

3.11.2 Characteristics of selected studies

The studies were conducted in West Africa (9), South Africa (14), North Africa (7), East Africa (9) and Central Africa (1) countries with the highest number being South Africa (14), followed by Egypt (5), and Ghana (4) (Figure 3.2). Overall prevalence of HA-GNBSI among all HA-BSI in each country is indicated on the map.

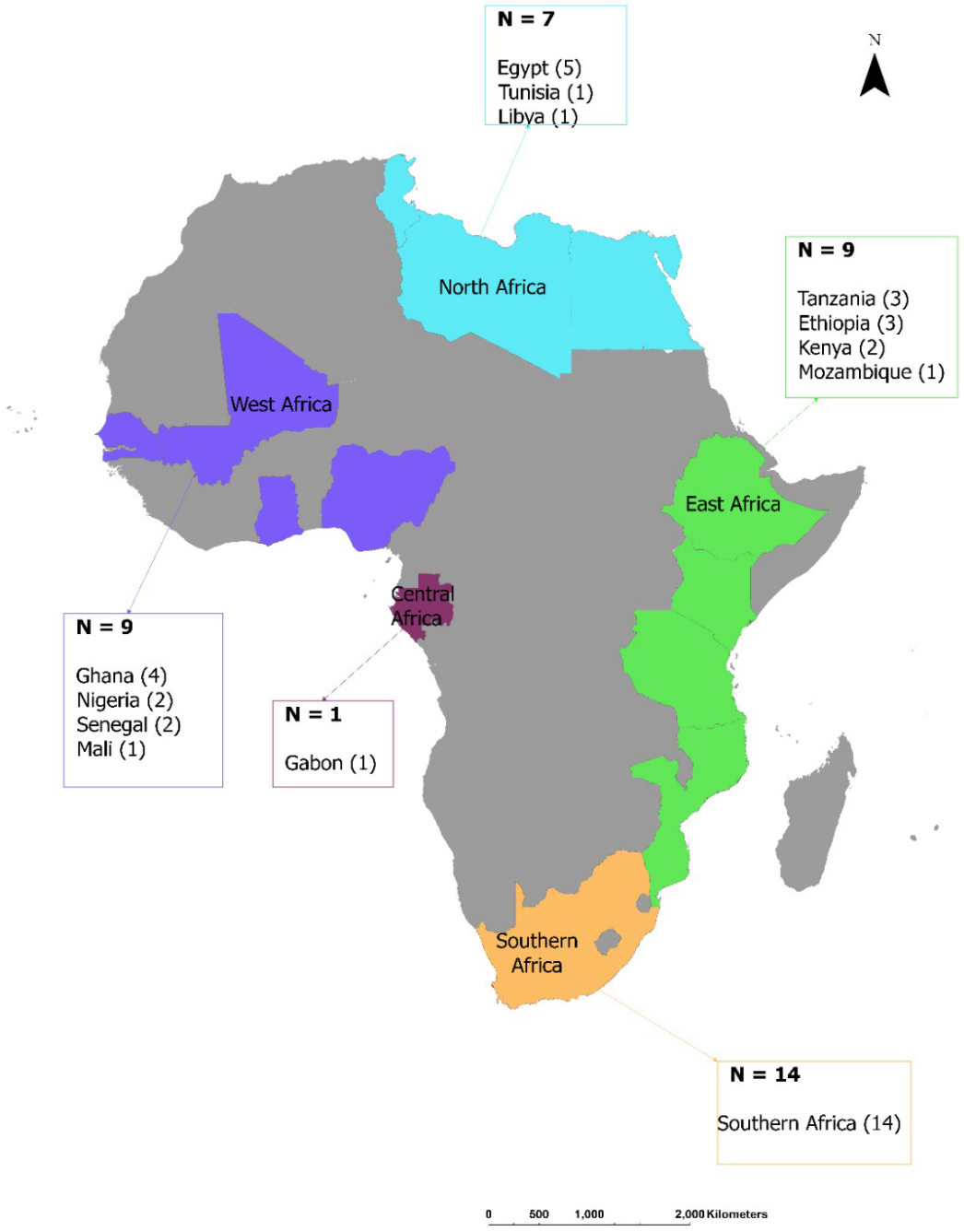


Figure 3.2: Map of Africa showing distribution of studies included in the review

3.11.3 Pooled prevalence of Hospital Acquired blood stream infections caused by HA-GNBSI

The prevalence of HA-GNBSI among all HA-BSIs could be determined in 31/40 selected papers totalling 314,321 participants [4, 5, 10-39]. Prevalence ranged from 19 to 95%, with a pooled prevalence of 63% [95%CI 56-69%] (Figure 3.3).

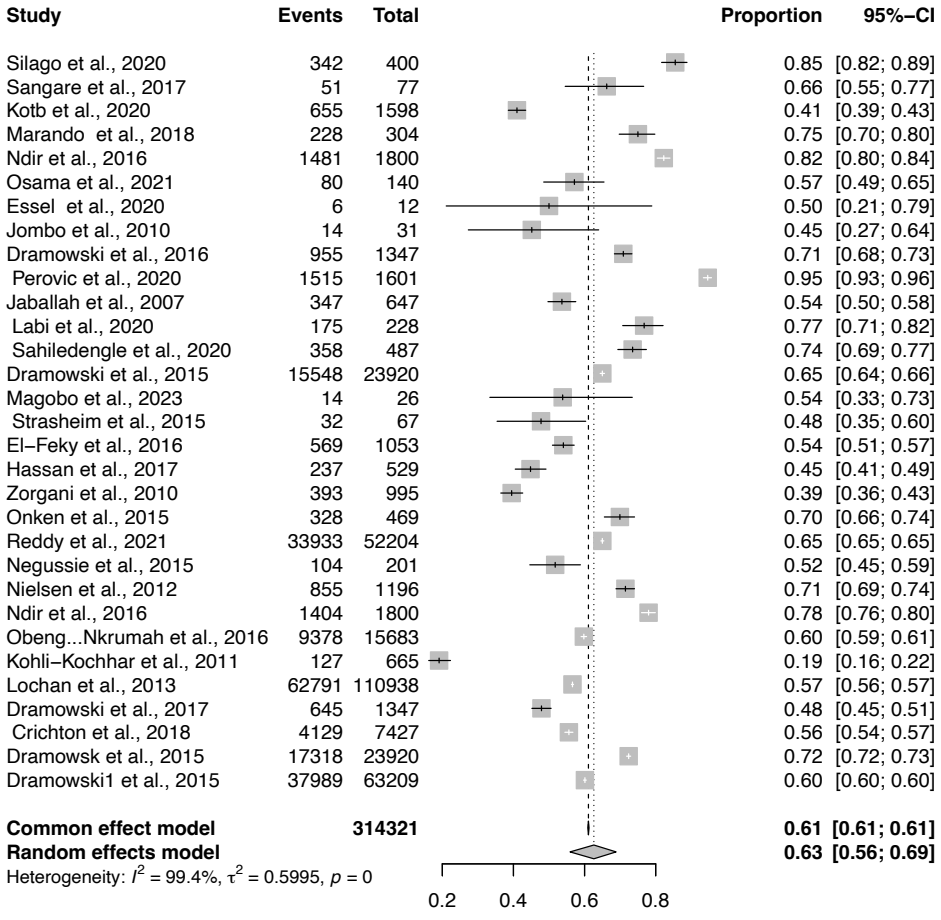


Figure 3.3: Metanalysis of the prevalence of HA-GNBSI among HA-BSI in African studies

3.11.4 Gram-negative pathogens involved in Hospital Acquired GNBSIs

Across studies in Africa *K. pneumoniae, E. coli, P. aeruginosa, A. baumannii*, and *Citrobacter* spp. were the predominant pathogens causing HA-GNBSIs in neonates and children, with prevalence varying of between 40–100% in these populations. suggesting heightened vulnerability in neonates to certain pathogens[4, 5, 14-17, 25, 28, 40-45]. Interestingly, species proportions distributions differed significantly across studies in children and neonates with *K. pneumoniae* and *E. coli* leading in children and *K. pneumoniae* and *A. baumannii* leading in neonates, while *E. coli* and *P. aeruginosa* were similar in both (Table 3.1). The pooled proportion of HA-GNBSI in children caused by *K. pneumoniae and E. coli* leading in children and *was* 17% (95% CI 6-37%), followed by *E. coli* 9% (95% CI 4-19%) and 5 (95% CI 1.15; 36.97) (Table 3.1). In neonates, *K. pneumoniae* surged to 37% (95% CI 20-39%), *E. coli* 10% (95% CI 7-15%) and *A. baumannii* 21% (95% CI 15-30%) suggesting heightened vulnerability in neonates to certain pathogens (Table 3.1).

Organism name	**Author**	**Year**	**Pooled proportion (95% CI)**	**Overall effect size**	***P* value**	***I*^{2} (%)**
	HA-GNBSI in children					
Klebsiella pneumoniae	Sangare et al., Osama et al., Dramowski et al., Negussie et al., lsendahl et al., Lochan et al., Dramowski1 et al.,	2017 2021 2016 2015 2014 2013 2015	0.17 (0.06; 0.37)	2.06	**< 0.01**	100
Escherichia coli	Sangare et al., Ndir et al., Dramowski et al., Negussie et al., Nielsen et al., Obeng et al., lsendahl et al., Lochan et al., Mandomando et al., Dramowski1 et al.,	2017 2016 2016 2015 2012 2016 2014 2013 2010 2015	0.09 (0.04–0.19)	1.84	**< 0.01**	100

Pseudomonas aeruginosa	Obeng et al., 2016 Dramowski1 et al., 2015 Ndir *et al*., 2016	0.04 (0.04–0.04)	0.0	0.44	0
Enterobacter cloacae	Sangare et al., 2017 Negussie et al., 2015 lsendahl et al., 2014 Dramowski1 et al., 2015	0.04 (0.02-0.11)	0.89	**< 0.01**	87
Acinetobacter spp.	Nielsen et al., 2012 Obeng et al., 2016	0.04 (0.01- 0.13)	0.44	0.03	80
Acinetobacter baumannii	Negussie et al., 2015 Lochan et al., 2013	0.05 (0.05-0.05)	0.00	0.07	69
	HA-GNBSI in neonates				
Klebsiella pneumoniae	Silago et al., 2020 Mzimela et al., 2021 Marando et al., 2018 Moodley et al., 2005 Dramowski1 et al., 2015 Magobo et al., 2023	0.37 (0.23- 0.53)	0.66	**< 0.01**	96
Escherichia coli	Marando et al., 2018 Mzimela et al., 2021 Silago et al., 2020 Dramowski1 et al., 2015 Reddy et al., 2021	0.10 (0.07- 0.15)	0.17	**< 0.01**	79
Pseudomonas aeruginosa	Marando et al., 2018 Reddy et al., 2021	0.03 (0.02- 0.04)	0.0	0.84	0
Acinetobacter baumannii	Marando et al., 2018 Magobo et al., 2023	0.21 (0.15-0.30)	0.09	**< 0.01**	92
Citrobacter freundii	Mzimela et al., 2021 Silago et al., 2020	0.05 (0.03-0.08)	0.0	0.65	0

Table 3.1: *Meta-analysis of prevalences of the pathogens identified as causes of HA-GNBSI in children and neonates.*

3.11.5 Meta-analysis of ESBL and CRE prevalences among paediatric HA-GNBSIs

Hospital acquired GNBSIs caused by ESBL and CRE-producing organisms across African countries vary significantly in children and neonates with proportions ranging from 45% to 84% in children and 4% to 93% in neonates depending on the settings (Figure 3.4 and 3.5)[4, 10, 15-18, 20, 24, 26, 28, 40, 44, 46-48]. The pooled prevalence of ESBL in children and neonates was 65% (95% CI: 0.55-0.74) and 53% (95% CI: 0.29-0.75%) respectively while that of CRE in neonates was 85% (95% CI: 81-88%) and reported in 2 studies only; no studies evaluated CRE prevalence in children (Figure 3.5 and 3.6)[38, 49].

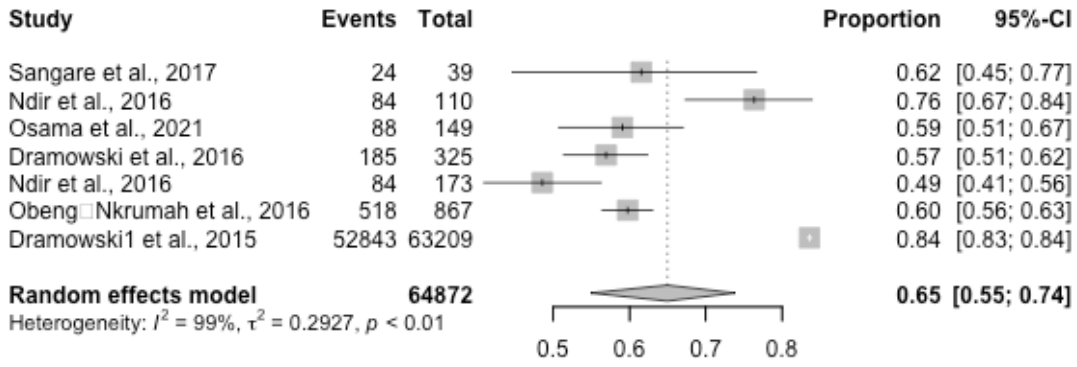


Figure 3.4: *Meta-analysis of ESBL prevalence reported in children with HA-GNBSIs*

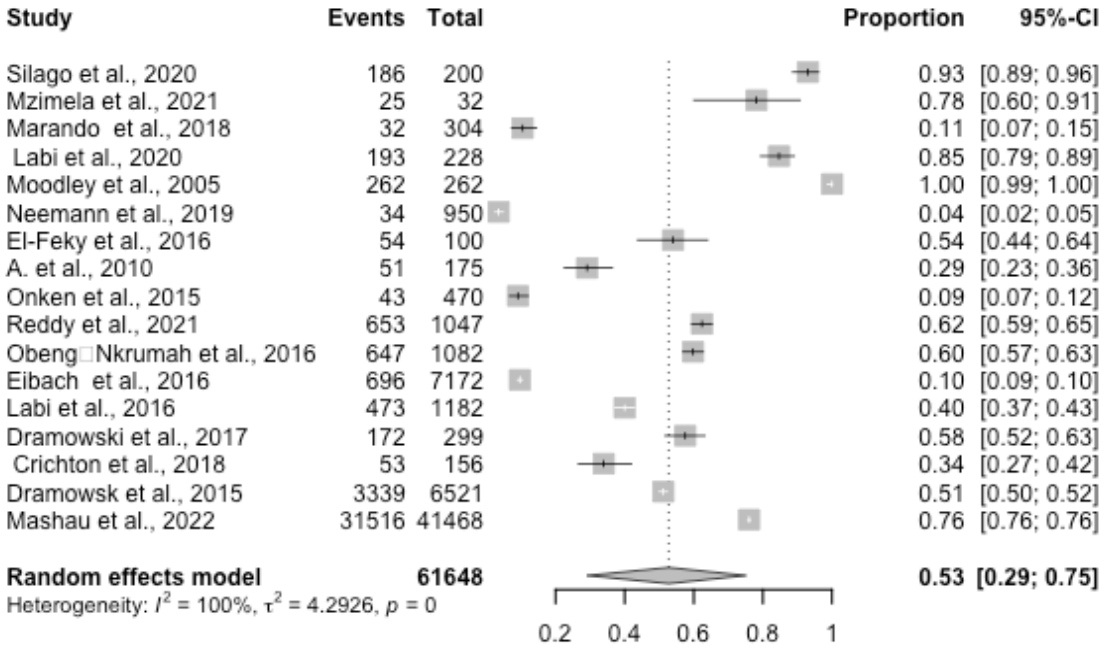


Figure 3. 5*: Meta-analysis of ESBL prevalence reported in neonates with HA-GNBSI*

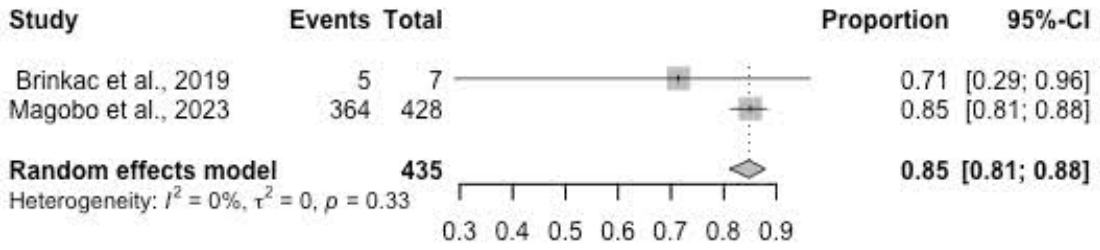


***Figure 3.6**: Meta-analysis of CRE prevalence reported in neonates with HA-GNBSIs*

3.11.6 AMR and genes reported among GNBSI.

Several antimicrobial resistance genes were reported in HA-GNBSIs in 9 out of 40 studies included in the review. ESBL-producing organisms frequently harboured the *bla*$_{\text{CTX-M-15}}$ gene, particularly in *E. coli* and *K. pneumoniae*, which was associated with resistance to third-generation cephalosporins[13, 16, 17, 27, 34, 35, 50]. The *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ genes were also prevalent in *K. pneumoniae*. Carbapenem resistance was primarily linked to the *bla*$_{\text{KPC}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{NDM 1 \& 5}}$, and *bla*$_{\text{OXA-48 \& 181}}$ genes, particularly in CRE isolates of *K. pneumoniae* and *A. baumannii*. These resistance genes often coexisted with others that confer resistance to aminoglycosides (e.g., *aac(3)-IV*) and fluoroquinolones. In a multicentre study in Kenya, 35% of *K. pneumoniae* isolates from paediatric BSIs were resistant to carbapenems, largely due to the presence of the *bla*$_{\text{KPC}}$ and *bla*$_{\text{NDM}}$ genes[51]. The figures 3.7 and 3.8 present a single count ESBL or CRE genes detected in 9 studies included in the review on HA-GNBSIs[13, 16, 17, 27, 34, 35, 39, 50, 52].

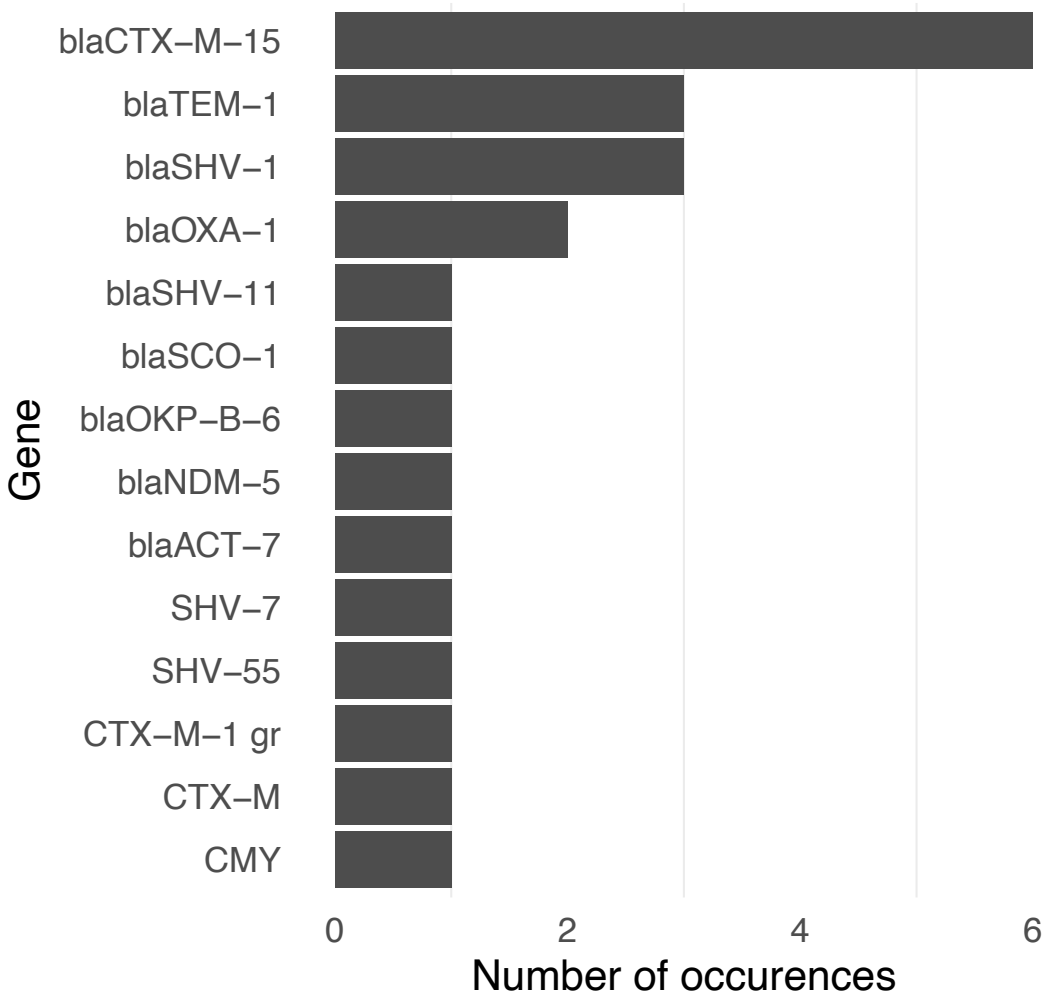


Figure 3.7: *Frequency of ESBL genes reported in HA-GNBSIs.*

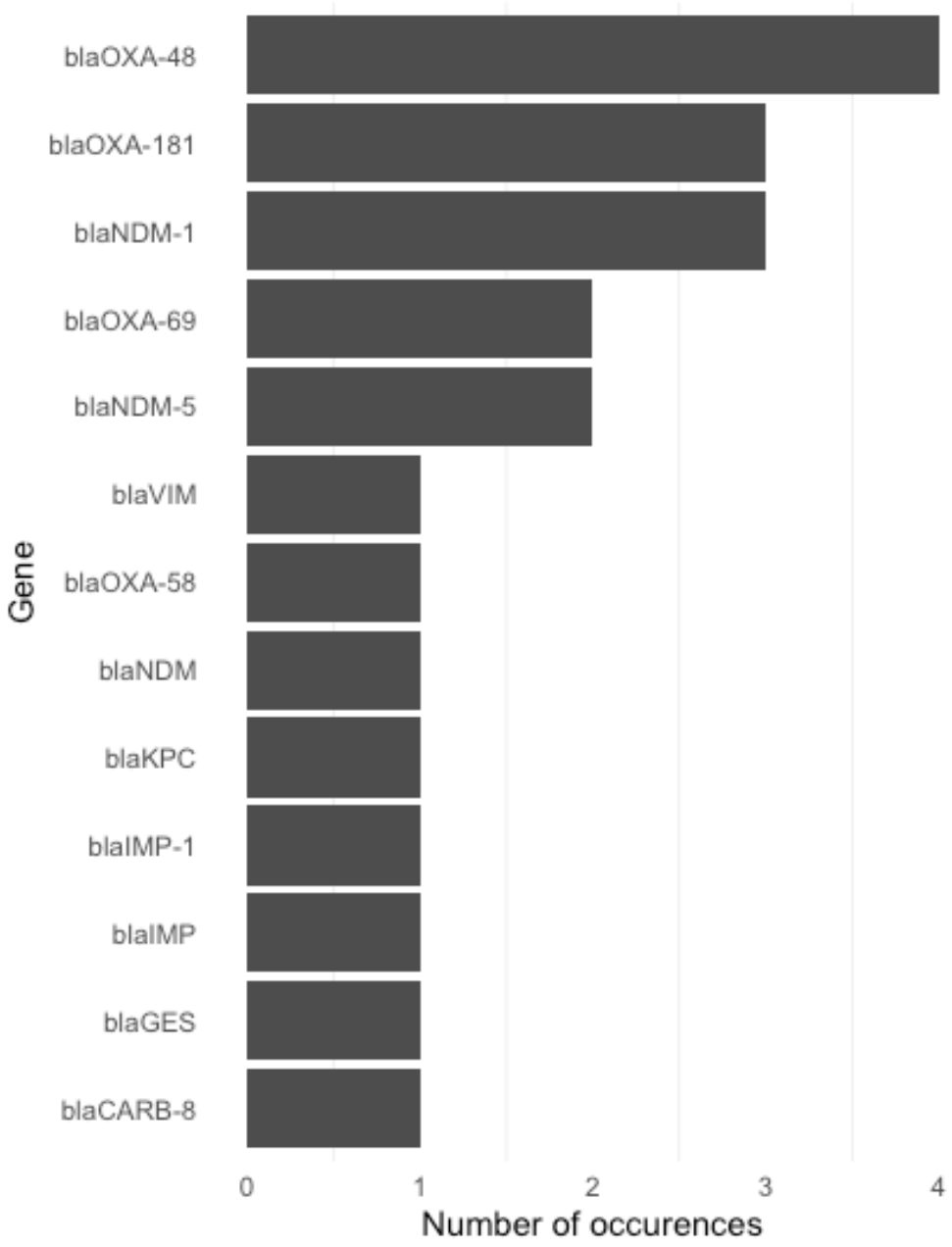


Figure 3.8: *Frequency of carbapenemase genes reported in HA-GNBSIs*

3.11.7 Factors associated with Hospital Acquired GNBSIs in Africa

Reported factors associated with HA-GNBSIs in children and neonates in HA-GNBSIs in children included prolonged hospitalisation, particularly in neonatal intensive care units (NICUs), where invasive medical devices such as central venous catheters and mechanical ventilators are commonly used[13, 15, 21, 22, 29, 36, 53]. From the meta-analysis, the major risk factors with significant adjusted odds ratios were prior and hospital antibiotic use 4.51 (95% CI 0.80; 25.51)[13, 34] and long hospital/ICU stay 2.52 (95% CI 0.84–7.59)[34, 40, 54]. Others reported and included in the analysis were having signs of acute disease (e.g. jaundice or cyanosis) 2.82 (95% CI 2.01; 3.95)[15, 55] and malnutrition 1.92 (95% CI 1.30–2.84)[29, 34, 54] as associated with HA-GNBSI(Table 3.2).

Risk factor	**Author**	**Year**	**Pooled AOR (95% CI)**	**Overall effect size**	***P* value**	**I^2 (%)**
History of antibiotic use	Marando *et al.,* Labi *et al*.,	2018 2020	4.51 (0.80- 25.51)	1.46	**< 0.01**	94
Long hospital/ICU stay	Sahiledengle *et al*., Dramowski *et al*., Labi *et al*.,	2020 2016 2020	2.52 (0.84–7.59)	0.86	**< 0.01**	93
Mechanical ventilation/invasive device	Jaballah et al., Hassan et al., Jaballah *et al*., Ndir *et al*., Zorgani. et al., Ndir *et al*., Zorgani *et al*., Ndir *et al*.,	2007 2017 2007 2016 2010 2016 2010 2016	2.82 (1.81–4.38)	0.28	**< 0.01**	79
Malnutrition	Labi *et al*., Sahiledengle *et al*., Zorgani. *et al*., Ndir *et al*., Ndir *et al.,* Hassan *et al*.,	2020 2020 2010 2016 2016 2017	1.92 (1.30–2.84)	0.14	**< 0.01**	79
Parenteral nutrition	Jaballah et al., Hassan et al., Ndir et al.,	2007 2017 2016	1.04 (0.87- 1.24)	0.160	< 0.0001	45
Admission to ICU ward	Kotb et al., Dramowski et al., Hassan et al.,	2020 2020 2020	2.26 (0.76- 6.71)	0.86	**< 0.01**	92
Signs of acute disease (jaundice/cyanosis)	Silago et al., Ndir et al., Silago et al.,	2020 2016 2020	2.82 (2.01- 3.95)	0.00	0.620	0

Maternal factors (ESBL carriage/fever)	Marando et al., Silago et al.,	2018 2020	2.23 (1.49;-3.33)	0.00	0.910	0

***Table 3. 2**: Meta-regression analysis of factors associated with paediatric HA-GNBSI in Africa*

3.12 Discussion

This systematic review revealed a substantial burden of HA-GNBSIs in the selected studies across Africa, emphasizing the need for targeted interventions. These studies covered a broad geographical range, with the highest number originating from South Africa, Egypt, and Ghana. This uneven geographic distribution may reflect disparities in research focus, healthcare infrastructure, and surveillance capacity across the continent. Notably, countries with more robust healthcare and/or research systems, such as South Africa, were better represented. However, a likely more significant bias is that most reports originate from tertiary or university centres, intensive care units (ICUs), and private hospitals, which are not representative of HA-GNBSIs occurring in secondary-level public hospitals where most admissions occur. These settings often lack microbiology capacity, making it difficult to capture the true burden and pathogen profile of HA-GNBSIs. This highlights the critical need for more inclusive research efforts that address these gaps and provide a more comprehensive understanding of HA-GNBSIs across different healthcare levels.

The pooled prevalence of HA-GNBSIs across the continent was estimated at 60%, with individual study estimates ranging from 19% to 95%. This wide variability suggests significant differences in populations targeted by studies, hospital settings, infection control measures, and antibiotic use policies across African countries. Factors such as overcrowded hospitals, limited resources, and inconsistent adherence to infection prevention protocols are recognized contributors to the disparities in HAIs across different

healthcare settings. The inclusion of nearly 314,321 participants in the meta-analysis provides a substantial evidence base, reinforcing the need for targeted interventions to mitigate HA-GNBSIs across diverse healthcare settings.

K. pneumoniae and *E. coli* were identified as the leading pathogens responsible for HA-GNBSIs in both children and neonates, with pooled prevalence rates of 17% and 9% in children, respectively. In neonates, *K. pneumoniae* accounted for 37% of infections, underscoring its critical role in neonatal sepsis[40]. The review also noted a concerning prevalence of *Acinetobacter spp.,* which accounted for over 20% of infections in some neonatal ICUs, indicating the need for focused interventions in these high-risk units. Interestingly, other studies have shown similar findings with differing species distributions differ by geographical region and hospital setting, with *A. baumannii* 2072(44%) and *K. pneumoniae* 1794(38%) being more prominent in neonatal units, likely reflecting environmental contamination and its multidrug resistance[56]. In contrast, *E. coli* and *P. aeruginosa* are more evenly distributed across paediatric populations, indicating varying infection control practices and community-acquired exposures[57].

There was high prevalence of ESBL and CRE among these pathogens of public health significance, particularly in paediatric and neonatal settings. For instance, studies from South Africa indicated that 45% of bloodstream infections in neonatal ICUs were due to *K. pneumoniae*, with more than half of these isolates being ESBL-positive[40]. In Senegal, 57% of HA-GNBSIs in children were caused by ESBL-producing Enterobacterales. These infections were associated with a significantly higher case-fatality rate compared to non-ESBL infections in analysis that was unadjusted for confounders (54.8% vs. 15.4%, $p<0.001$), with AMR being higher in neonates who have higher mortality[15]. The

prevalence of CRE-producing organisms was also alarming. In Tanzania, 20% of bloodstream infections caused by gram-negative bacteria in neonates were carbapenem-resistant, with *Acinetobacter baumannii* being the most frequent CRE organism[5].

The emergence of CRE organisms, particularly in paediatric settings, poses an alarming threat. In Kenya, 35% of *K. pneumoniae* isolates from paediatric bloodstream infections were carbapenem-resistant due to the presence of bla_{KPC} and bla_{NDM} genes[51]. Similarly, a study conducted at Tygerberg Hospital's neonatal unit in Cape Town over a 10-year period found that Gram-negative pathogens accounted for 57% of all isolates, with *Klebsiella* species being the most common. Notably, 85% of *Klebsiella* isolates were extended-spectrum beta-lactamase (ESBL) producers, and 8% were carbapenem-resistant[56].

Given the high prevalence of phenotypic resistance observed, the detection of a similarly high prevalence of AMR genes is unsurprising. Resistance determinants such as $bla_{CTX\text{-}M\text{-}15}$, bla_{SHV}, bla_{TEM}, bla_{KPC}, bla_{VIM}, bla_{NDM}, and $bla_{OXA\text{-}48/181}$ were frequently identified, often co-occurring with genes conferring resistance to aminoglycosides and fluoroquinolones, thereby significantly limiting treatment option [58]. Studies from China, Europe, and Korea have similarly reported be $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}15}$, and $bla_{CTX\text{-}M\text{-}27}$ as the most prevalent ESBL genes across all age groups, aligning with findings from Africa[58-60]. The frequent detection of these genes in HA-GNBSI pathogens highlights the critical need for enhanced surveillance, molecular diagnostics, and prompt identification of resistant strains to guide effective antimicrobial therapy[59-61].

HA-GNBSIs are influenced by several well-documented factors, particularly in paediatric populations, including prolonged hospital stays, invasive device use (e.g., central venous catheters), prior antibiotic exposure, malnutrition, and suboptimal infection control practices[13, 34]. Neonatal intensive care units (NICUs) are especially vulnerable due to the critical condition of patients and high device utilization rates. Findings align with other studies highlighting these risks. For instance, research conducted in all ages in Ethiopia, Korea and China similarly identifies central venous catheter use, previous hospitalisation, and comorbidities as significant risk factors, emphasizing the universality of invasive devices as a primary contributor to HA-BSIs across age groups[61-63].

Comparisons with studies in different regions reveal notable variations. A European investigation done in Switzerland into ESBL-producing Enterobacterales demonstrated that prior antibiotic exposure and prolonged hospital stays are consistent risk factors, but community-acquired ESBL infections are increasingly reported[64]. Similarly, a review on HAIs reported that indwelling devices, body weight, overcrowding and antibiotic use further exacerbate infection risk, in sub-Saharan Africa. In neonatal populations, poor hand hygiene compliance, malnutrition, preterm delivery and indwelling medical devices remain uniquely critical factors[65, 66]. These comparisons underscore that while certain risks, such as invasive device use and prior antibiotic exposure, are universally significant, local healthcare infrastructure, age-specific vulnerabilities, and community health dynamics shape infection patterns. This highlights the necessity of tailoring prevention strategies to the specific context, focusing on improving hand hygiene, reducing overcrowding, and minimizing invasive procedures where feasible[13, 34].

Given that antibiotic use and prolonged hospital stay were the biggest risk factors associated with HA-GNBSIs, a focus on optimising hospital hygiene, robust antimicrobial stewardship programs as well as risk stratification of patients to discharge low-risk groups early to reduce exposure to contaminated hospital environment. Strengthening diagnostic capabilities and implementing effective infection control protocols are essential steps to mitigate the rising threat of resistant infections.

A limitation of this review is that only a few studies incorporated genomic analysis, which may introduce bias. Genomic data provide critical insights into pathogen diversity, antimicrobial resistance mechanisms, and transmission dynamics factors that traditional microbiological methods may overlook. The absence of genomic data in most studies could result in an incomplete understanding of the distribution of AMR genes of interest in this review and the resistance trends driving bloodstream infections. These findings underscore the uncertainty regarding the representativeness of genomic data across different settings in Africa.

3.13 Conclusion

HA-GNBSIs represent a critical and escalating challenge in African healthcare settings, particularly among vulnerable paediatric and neonatal populations. The high prevalence of ESBL and CRE Gram negative pathogens underscores the growing threat of AMR, which significantly complicates treatment efforts and contributes to increased mortality rates. The frequent detection of resistance genes in Africa, China and Europe such as $bla_{\text{CTX-M14,15\&27}}$, bla_{KPC}, and bla_{NDM} further highlights the urgent need for effective strategies to combat AMR[5, 51, 59].

Addressing this growing threat necessitates a comprehensive approach that includes strengthening infection control measures, particularly in neonatal and intensive care units. Enhancing adherence to hand hygiene protocols, optimizing the use of invasive devices, and implementing robust antibiotic stewardship programs are crucial steps in reducing the incidence of HA-GNBSIs. Investing in improved surveillance systems and diagnostic capabilities will enable earlier detection of resistant pathogens, allowing for timely and appropriate treatment interventions.

Furthermore, targeted investments in healthcare infrastructure, research, and capacity building are essential to address the unique challenges faced by African healthcare systems. Implementing evidence-based policies and tailored interventions may improve patient outcomes but also safeguard the effectiveness of existing antibiotics[67]. Reducing risk factors, such as prolonged hospital stays and malnutrition, and enhancing support for resource-limited settings will be critical in mitigating the impact of HA-BSIs.

A coordinated, multifaceted approach is essential to address the burden of healthcare-associated Gram-negative bloodstream infections (HA-GNBSIs) and the escalating threat of AMR in Africa. While the challenges faced by paediatric populations in Africa mirror those observed globally, they are intensified by significant resource constraints, including limited laboratory capacity, underfunded healthcare systems, and restricted access to effective therapies. This review underscores critical gaps in understanding, particularly the urgent need for reliable, comprehensive data from typical healthcare facilities across Africa, including research centres in low-resource settings. Such data are pivotal for accurately characterizing infection dynamics and guiding targeted interventions. Moreover, context-specific research is essential to develop and validate treatment protocols that are

practical and effective within resource-limited settings, where standard antimicrobial options may be either unavailable or ineffective.

While these findings align with global patterns, the unique challenges in African settings exacerbate the impact of HA-GNBSIs on vulnerable paediatric populations. Infrastructure deficits and limited surveillance systems further complicate the effective management of these infections, necessitating innovative approaches tailored to these environments. By bridging these gaps through focused research and investments in infection prevention, antimicrobial stewardship, and healthcare system resilience, there is substantial potential to mitigate the dual threats of HA-GNBSIs and AMR, ultimately improving outcomes for children across the continent.

REFERENCES

1. Allegranzi B, Nejad SB, Combescure C, Graafmans W, Attar H, Donaldson L, Pittet D: **Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis**. *The Lancet* 2011, **377**(9761):228-241.
2. Organization WH: **Report on the burden of endemic health care-associated infection worldwide**. 2011.
3. Melariri H, Freercks R, van der Merwe E, Ten Ham-Baloyi W, Oyedele O, Murphy RA, Claasen C, Etusim PE, Achebe MO, Offiah S: **The burden of hospital-acquired infections (HAI) in sub-Saharan Africa: a systematic review and meta-analysis**. *Eclinicalmedicine* 2024, **71**.
4. Dramowski A, Cotton MF, Rabie H, Whitelaw A: **Trends in paediatric bloodstream infections at a South African referral hospital**. *BMC pediatrics* 2015, **15**:1-11.
5. Silago V, Kovacs D, Msanga DR, Seni J, Matthews L, Oravcová K, Zadoks RN, Lupindu AM, Hoza AS, Mshana SE: **Bacteremia in critical care units at Bugando Medical Centre, Mwanza, Tanzania: the role of colonization and contaminated cots and mothers' hands in cross-transmission of multidrug resistant Gram-negative bacteria**. *Antimicrobial Resistance & Infection Control* 2020, **9**:1-14.
6. Majigo M, Makupa J, Mwazyunga Z, Luoga A, Kisinga J, Mwamkoa B, Kim S, Joachim A: **Bacterial aetiology of neonatal sepsis and antimicrobial resistance pattern at the regional referral Hospital, Dar es Salam, Tanzania; a call to strengthening antibiotic stewardship program**. *Antibiotics* 2023, **12**(4):767.
7. Mbim E, Mboto C, Agbo B: **A review of nosocomial infections in Sub-Saharan Africa**. *British Microbiology Research Journal* 2016, **15**(1):1-11.
8. Nejad SB, Allegranzi B, Syed SB, Ellis B, Pittet D: **Health-care-associated infection in Africa: a systematic review**. *Bulletin of the World Health Organization* 2011, **89**(10):757-765.
9. Sethi AK, Acher CW, Kirenga B, Mead S, Donskey CJ, Katamba A: **Infection control knowledge, attitudes, and practices among healthcare workers at Mulago Hospital, Kampala, Uganda**. *Infection Control & Hospital Epidemiology* 2012, **33**(9):917-923.
10. Dramowski A, Madide A, Bekker A: **Neonatal nosocomial bloodstream infections at a referral hospital in a middle-income country: burden, pathogens, antimicrobial resistance and mortality**. *Paediatrics and international child health* 2015, **35**(3):265-272.
11. Essel V, Ntshoe G, Mphaphuli E, Tshabalala K: **A multisectoral investigation of a neonatal unit outbreak of Klebsiella pneumoniae bacteraemia at a regional hospital in Gauteng Province, South Africa**. *South African Medical Journal* 2020, **110**(8):783-790.
12. Kotb S, Lyman M, Ismail G, Abd El Fattah M, Girgis SA, Etman A, Hafez S, El-Kholy J, Zaki MES, Rashed H-aG: **Epidemiology of carbapenem-resistant Enterobacteriaceae in Egyptian intensive care units using National Healthcare–associated Infections Surveillance Data, 2011–2017**. *Antimicrobial Resistance & Infection Control* 2020, **9**:1-9.
13. Marando R, Seni J, Mirambo MM, Falgenhauer L, Moremi N, Mushi MF, Kayange N, Manyama F, Imirzalioglu C, Chakraborty T: **Predictors of the extended-spectrum-beta lactamases producing Enterobacteriaceae neonatal sepsis at a**

tertiary hospital, Tanzania. *International Journal of Medical Microbiology* 2018, **308**(7):803-811.
14. Mzimela BW, Nkwanyana NM, Singh R: **Clinical outcome of neonates with Carbapenem-resistant Enterobacteriaceae infections at the King Edward VIII Hospital's neonatal unit, Durban, South Africa**. *Southern African Journal of Infectious Diseases* 2021, **36**(1):223.
15. Ndir A, Diop A, Faye PM, Cissé MF, Ndoye B, Astagneau P: **Epidemiology and burden of bloodstream infections caused by extended-spectrum beta-lactamase producing Enterobacteriaceae in a pediatric hospital in Senegal**. *PLoS One* 2016, **11**(2):e0143729.
16. Osama D, El-Mahallawy H, Mansour MT, Hashem A, Attia AS: **Molecular characterization of carbapenemase-producing Klebsiella pneumoniae isolated from Egyptian pediatric cancer patients including a strain with a rare gene-combination of β-lactamases**. *Infection and Drug Resistance* 2021:335-348.
17. Sangare SA, Rondinaud E, Maataoui N, Maiga AI, Guindo I, Maiga A, Camara N, Dicko OA, Dao S, Diallo S: **Very high prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in bacteriemic patients hospitalized in teaching hospitals in Bamako, Mali**. *PLoS One* 2017, **12**(2):e0172652.
18. Crichton H, O'Connell N, Rabie H, Whitelaw A, Dramowski A: **Neonatal and paediatric bloodstream infections: Pathogens, antimicrobial resistance patterns and prescribing practice at Khayelitsha District Hospital, Cape Town, South Africa**. *South African Medical Journal* 2018, **108**(2):99-104.
19. Dramowski A, Cotton M, Whitelaw A: **Surveillance of healthcare-associated infection in hospitalised South African children: Which method performs best?** *South African Medical Journal* 2017, **107**(1):56-63.
20. El-Feky EA, Doa'a AS, El-Kholy J, Sayed AM, Mansi Y, Hashem M: **Use of personal digital assistants to detect healthcare-associated infections in a neonatal intensive care unit in Egypt**. *The Journal of Infection in Developing Countries* 2016, **10**(11):1250-1257.
21. Hassan RH, Eldegla H, Elmorsy F, Eldars WM: **Clinical and microbiological characteristics of healthcare-associated infections in a tertiary care pediatric hospital**. *Egyptian Pediatric Association Gazette* 2017, **65**(4):127-131.
22. Jaballah NB, Bouziri A, Mnif K, Hamdi A, Khaldi A, Kchaou W: **Epidemiology of hospital-acquired bloodstream infections in a Tunisian pediatric intensive care unit: a 2-year prospective study**. *American journal of infection control* 2007, **35**(9):613-618.
23. Kohli-Kochhar R, Omuse G, Revathi G: **A ten-year review of neonatal bloodstream infections in a tertiary private hospital in Kenya**. *The Journal of Infection in Developing Countries* 2011, **5**(11):799-803.
24. Labi A-K, Obeng-Nkrumah N, Bjerrum S, Enweronu-Laryea C, Newman MJ: **Neonatal bloodstream infections in a Ghanaian Tertiary Hospital: Are the current antibiotic recommendations adequate?** *BMC infectious diseases* 2016, **16**:1-12.
25. Negussie A, Mulugeta G, Bedru A, Ali I, Shimeles D, Lema T, Aseffa A: **Bacteriological profile and antimicrobial susceptibility pattern of blood culture isolates among septicemia suspected children in selected hospitals Addis Ababa, Ethiopia**. *International journal of biological and medical research* 2015, **6**(1):4709.
26. Obeng-Nkrumah N, Labi A-K, Addison NO, Labi JEM, Awuah-Mensah G: **Trends in paediatric and adult bloodstream infections at a Ghanaian referral hospital:**

a retrospective study. *Annals of clinical microbiology and antimicrobials* 2016, **15**:1-10.
27. Onken A, Said AK, Jørstad M, Jenum PA, Blomberg B: **Prevalence and antimicrobial resistance of microbes causing bloodstream infections in Unguja, Zanzibar**. *PloS one* 2015, **10**(12):e0145632.
28. Reddy K, Bekker A, Whitelaw AC, Esterhuizen TM, Dramowski A: **A retrospective analysis of pathogen profile, antimicrobial resistance and mortality in neonatal hospital-acquired bloodstream infections from 2009–2018 at Tygerberg Hospital, South Africa**. *PLoS One* 2021, **16**(1):e0245089.
29. Sahiledengle B, Seyoum F, Abebe D, Geleta EN, Negash G, Kalu A, Woldeyohannes D, Tekalegn Y, Zenbaba D, Quisido BJE: **Incidence and risk factors for hospital-acquired infection among paediatric patients in a teaching hospital: a prospective study in southeast Ethiopia**. *BMJ open* 2020, **10**(12):e037997.
30. Strasheim W, Kock MM, Ueckermann V, Hoosien E, Dreyer AW, Ehlers MM: **Surveillance of catheter-related infections: the supplementary role of the microbiology laboratory**. *BMC infectious diseases* 2015, **15**:1-10.
31. Essel V, Tshabalala K, Ntshoe G, Mphaphuli E, Feller G, Shonhiwa A, McCarthy K, Ismail H, Strasheim W, Lowe M: **A multi-sectoral investigation of a neonatal unit outbreak of Klebsiella pneumoniae bacteraemia at a regional hospital in South Africa, 2018**. *International Journal of Infectious Diseases* 2020, **101**:277-278.
32. Jombo G, Akpan S, Epoke J, Akaa P, Eyong K, Gyuse A: **Antimicrobial susceptibility profile of community acquired and nosocomial isolates of Escherichia coli from clinical blood culture specimens at a Nigerian university teaching hospital**. *ASIAN PACIFIC JOURNAL OF TROPICAL MEDICINE* 2010, **3**(8):662-665.
33. Perovic O, Ismail H, Quan V, Bamford C, Nana T, Chibabhai V, Bhola P, Ramjathan P, Swe-Han K, Wadula J *et al*: **Carbapenem-resistant Enterobacteriaceae in patients with bacteraemia at tertiary hospitals in South Africa, 2015 to 2018**. *EUROPEAN JOURNAL OF CLINICAL MICROBIOLOGY & INFECTIOUS DISEASES* 2020, **39**(7):1287-1294.
34. Labi A-K, Bjerrum S, Enweronu-Laryea CC, Ayibor PK, Nielsen KL, Marvig RL, Newman MJ, Andersen LP, Kurtzhals JA: **High carriage rates of multidrug-resistant gram-negative bacteria in neonatal intensive care units from Ghana**. In: *Open Forum Infectious Diseases: 2020*: Oxford University Press US; 2020: ofaa109.
35. Magobo RE IH, Lowe M, Strasheim W, Mogokotleng R, Perovic O, Kwenda S, Ismail A, Makua M, Bore A, Phayane R, Naidoo H, Dennis T, Ngobese M, Wijnant W, Govender NP, GERMS-SA1. fB: **Outbreak of NDM-1- and OXA-181-Producing Klebsiella pneumoniae Bloodstream Infections in a Neonatal Unit, South Africa**.
36. Zorgani A AA, Glia A, Albarbar A, Hanish S.: **Prevalence of Device-associated Nosocomial Infections Caused By Gram-negative Bacteria in a Trauma Intensive Care Unit in Libya**.
37. Nielsen MV, Sarpong N, Krumkamp R, Dekker D, Loag W, Amemasor S, Agyekum A, Marks F, Huenger F, Krefis AC: **Incidence and characteristics of bacteremia among children in rural Ghana**. 2012.
38. Brinkac LM, White R, D'Souza R, Nguyen K, Obaro SK, Fouts DE: **Emergence of New Delhi metallo-β-lactamase (NDM-5) in Klebsiella quasipneumoniae**

from neonates in a Nigerian hospital. *MSphere* 2019, **4**(2):10.1128/msphere. 00685-00618.
39. Taoufik L, Hanchi A, Fatiha B, Nissrine S, Rabou M, Nabila S: **Emergence of OXA-48 Carbapenemase Producing Klebsiella pneumoniae in a Neonatal Intensive Care Unit in Marrakech, Morocco**. *CLINICAL MEDICINE INSIGHTS-PEDIATRICS* 2019, **13**:-.
40. Dramowski A, Whitelaw A, Cotton M: **Burden, spectrum, and impact of healthcare-associated infection at a South African children's hospital**. *Journal of Hospital Infection* 2016, **94**(4):364-372.
41. Isendahl J, Manjuba C, Rodrigues A, Xu W, Henriques-Normark B, Giske CG, Nauclér P: **Prevalence of community-acquired bacteraemia in Guinea-Bissau: an observational study**. *BMC infectious diseases* 2014, **14**:1-9.
42. Lochan H, Bamford C, Eley B: **Blood cultures in sick children**. *South African Medical Journal* 2013, **103**(12):918-919.
43. Dramowski A. MA, Bekker A.: **Neonatal nosocomial bloodstream infections at a referral hospital in a middle-income country: Burden, pathogens, antimicrobial resistance and mortality**. *Paediatrics and International Child Health* 2015, **35**(3).
44. Moodley P, Coovadia YM, Sturm AW: **Intravenous glucose preparation as the source of an outbreak of extended-spectrum β-lactamase-producing Klebsiella pneumoniae infections in the neonatal unit of a regional hospital in KwaZulu-Natal**. *South African Medical Journal* 2005, **95**(11):861-864.
45. Mandomando I, Sigaúque B, Morais L, Espasa M, Vallès X, Sacarlal J, Macete E, Aide P, Quintò L, Nhampossa T: **Antimicrobial drug resistance trends of bacteremia isolates in a rural hospital in southern Mozambique**. *The American journal of tropical medicine and hygiene* 2010, **83**(1):152.
46. Mashau RC, Meiring ST, Dramowski A, Magobo RE, Quan VC, Perovic O, von Gottberg A, Cohen C, Velaphi S, van Schalkwyk E: **Culture-confirmed neonatal bloodstream infections and meningitis in South Africa, 2014–19: a cross-sectional study**. *The Lancet Global Health* 2022, **10**(8):e1170-e1178.
47. Neemann K OE, Izevbigie N, Akaba G, Olanipekun GM, Richard JC, Duru CI, Kocmich NJ, Samson KK, Rezac-Elgohary A, Anigilaje EA, Yunusa T, Megafu CON, Ajose TO, Medugu N, Meza J, Obaro S.: **Neonatal outcomes associated with maternal recto-vaginal colonization with extended-spectrum β-lactamase producing Enterobacteriaceae in Nigeria: a prospective, cross-sectional study**.
48. Eibach D, Campos CB, Krumkamp R, Al-Emran HM, Dekker D, Boahen KG, Kreuels B, Adu-Sarkodie Y, Aepfelbacher M, Park SE: **Extended spectrum beta-lactamase producing Enterobacteriaceae causing bloodstream infections in rural Ghana, 2007–2012**. *International Journal of Medical Microbiology* 2016, **306**(4):249-254.
49. Magobo RE, Ismail H, Lowe M, Strasheim W, Mogokotleng R, Perovic O, Kwenda S, Ismail A, Makua M, Bore A: **Outbreak of NDM-1–and OXA-181–producing klebsiella pneumoniae bloodstream infections in a neonatal unit, South Africa**. *Emerging Infectious Diseases* 2023, **29**(8):1531.
50. Brinkac LM WR, D'Souza R, Nguyen K, Obaro SK, Fouts DE.: **Emergence of New Delhi Metallo-β-Lactamase (NDM-5) in Klebsiella quasipneumoniae from Neonates in a Nigerian Hospital**.
51. Villinger D ST, Musyoki VM, Inwani I, Aluvaala J, Okutoyi L, Ziegler AH, Wieters I, Stephan C, Museve B, Kempf VAJ, Masika M.: **Genomic transmission analysis**

of multidrug-resistant Gram-negative bacteria within a newborn unit of a Kenyan tertiary hospital: A four-month prospective colonization study.

52. Essel V, Tshabalala K, Ntshoe G, Mphaphuli E, Ntshoe G, Shonhiwa A, McCarthy K, Ismail H, Strasheim W, Lowe M *et al*: **A multisectoral investigation of a neonatal unit outbreak of Klebsiella pneumoniae bacteraemia at a regional hospital in Gauteng Province, South Africa**. *SAMJ SOUTH AFRICAN MEDICAL JOURNAL* 2020, **110**(8):783-790.
53. Jaballah N, Bouziri A, Mnif K, Hamdi A, Khaldi A, Kchaou W: **Epidemiology of hospital-acquired bloodstream infections in a Tunisian pediatric intensive care unit: A 2-year prospective study**. *AMERICAN JOURNAL OF INFECTION CONTROL* 2007, **35**(9):613-618.
54. Sahiledengle B, Seyoum F, Abebe D, Geleta E, Negash G, Kalu A, Woldeyohannes D, Tekalegn Y, Zenbaba D, Quisido B: **Incidence and risk factors for hospital-acquired infection among paediatric patients in a teaching hospital: a prospective study in southeast Ethiopia**. *BMJ OPEN* 2020, **10**(12):-.
55. Mwakyabala J.G. MCI, Mshana S., Mwakyoma A.A., Silago V.: **Characterisation of genes encoding for extended spectrum β-lactamase in Gram-negative bacteria causing healthcare-associated infections in Mwanza, Tanzania**. *African Journal of Laboratory Medicine* 2023, **12**(1):-.
56. Thomas R, Ondongo-Ezhet C, Motsoaledi N, Sharland M, Clements M, Velaphi S: **Incidence, pathogens and antimicrobial resistance of blood and cerebrospinal fluid isolates from a tertiary neonatal unit in South Africa: A 10 year retrospective review**. *Plos one* 2024, **19**(1):e0297371.
57. Ndung'u CW: **Prevalence and Antibiotic Resistance Patterns of Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli among Hospitalized Patients at Thika Level 5 Hospital**. JKUAT-COHES; 2024.
58. Hansen DS, Schumacher H, Hansen F, Stegger M, Hertz FB, Schønning K, Justesen US, Frimodt-Møller N, Group DS: **Extended-spectrum β-lactamase (ESBL) in Danish clinical isolates of Escherichia coli and Klebsiella pneumoniae: prevalence, β-lactamase distribution, phylogroups, and co-resistance**. *Scandinavian Journal of Infectious Diseases* 2012, **44**(3):174-181.
59. Zhao S, Wu Y, Dai Z, Chen Y, Zhou X, Zhao J: **Risk factors for antibiotic resistance and mortality in patients with bloodstream infection of Escherichia coli**. *European Journal of Clinical Microbiology & Infectious Diseases* 2022, **41**(5):713-721.
60. Naas T, Cuzon G, Robinson A, Andrianirina Z, Imbert P, Ratsima E, Ranosiarisoa Z, Nordmann P, Raymond J: **Neonatal infections with multidrug-resistant ESBL-producing E. cloacae and K. pneumoniae in Neonatal Units of two different Hospitals in Antananarivo, Madagascar**. *BMC Infectious Diseases* 2016, **16**:1-10.
61. Shi N, Kang J, Wang S, Song Y, Yin D, Li X, Guo Q, Duan J, Zhang S: **Bacteriological profile and antimicrobial susceptibility patterns of gram-negative bloodstream infection and risk factors associated with mortality and drug resistance: a Retrospective Study from Shanxi, China**. *Infection and Drug Resistance* 2022:3561-3578.
62. Beshah D, Desta AF, Woldemichael GB, Belachew EB, Derese SG, Zelelie TZ, Desalegn Z, Tessema TS, Gebreselasie S, Abebe T: **High burden of ESBL and carbapenemase-producing gram-negative bacteria in bloodstream infection patients at a tertiary care hospital in Addis Ababa, Ethiopia**. *PLoS One* 2023, **18**(6):e0287453.

63. Park YS, Bae IK, Kim J, Jeong SH, Hwang S-s, Seo Y-H, Cho YK, Lee K, Kim JM: **Risk factors and molecular epidemiology of community-onset extended-spectrum β-lactamase-producing Escherichia coli bacteremia**. *Yonsei medical journal* 2014, **55**(2):467-475.
64. Kuster S, Hasse B, Huebner V, Bansal V, Zbinden R, Ruef C, Ledergerber B, Weber R: **Risks factors for infections with extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae at a tertiary care university hospital in Switzerland**. *Infection* 2010, **38**:33-40.
65. Osinupebi O, Ogunlesi T, Fetuga M: **Pattern of nosocomial infections in the special care baby unit of the Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria**. *Nigerian Journal of Paediatrics* 2014, **41**(1):54-58.
66. Rameshwarnath S, Naidoo S: **Risk factors associated with nosocomial infections in the Neonatal Intensive Care Unit at Mahatma Gandhi Memorial hospital between 2014 and 2015**. *Southern African Journal of Infectious Diseases* 2018, **33**(4):93-100.
67. Cuevas C, Batura N, Wulandari LPL, Khan M, Wiseman V: **Improving antibiotic use through behaviour change: a systematic review of interventions evaluated in low-and middle-income countries**. *Health Policy and Planning* 2021, **36**(5):754-773.

4 ANTIMICROBIAL USAGE AMONG ACUTELY ILL HOSPITALISED CHILDREN AGED 2-23 MONTHS IN 9 SITES, 6 COUNTRIES ACROSS SUB-SAHARAN AFRICA AND SOUTH ASIA

Note: This chapter has been preprinted and is under review for publication. I authored the text and figures below (which are reproduced here largely unaltered from the preprinted work) with input from supervisors commensurate with the amount of input/advice that would be considered appropriate for a DPhil thesis. All authors have agreed to the inclusion of this preprinted work in my thesis.

4.1 Introduction

Antimicrobials are the most prescribed medications in hospitals, however, AMR and the lack development of new antimicrobial agents pose serious threats to public health. Worldwide, AMR contributes to more than 1.2 million deaths annually, with the highest mortality burden in sub-Saharan Africa[1]. Up to one third of child deaths may be attributable to AMR[1, 2], however, a lack of access to antimicrobials also contributes to child deaths[3]. The WHO Global Antimicrobial Resistance And Surveillance System (GLASS) provides a standardised approach for collection, analysis, and sharing AMR data, supporting national AMR surveillance and interventions[4]. To facilitate monitoring and stewardship, WHO have classified antimicrobial agents as Access, Watch and Reserve (AWaRe) based on their intended target usage and impact on AMR[5].

Many hospitals in low- and middle-income countries (LMICs) aim to follow WHO or national guidelines, which recommend adjustment according to local susceptibilities. However, treatment decisions are mostly made without any microbiology or relevant local antibiograms. Guidelines are explicit about which antimicrobials to use as first-line treatment, and sometimes, second-line choices. However, they mostly leave open to clinicians the criteria and timing for switching up and down and what to use as other second- and third-line agents, and do not provide guidance in the common situation where children meet criteria for more than one syndrome.[6, 7] Hence there is still considerable scope for variation within guidelines. This, as well as diagnostic uncertainty, can lead to excessive broad-spectrum prescribing, prolonged treatment or inappropriate switching [8]. Concurrently, agents to treat resistant infections may be unaffordable or unavailable [9, 10].

Most studies of paediatric inpatient antimicrobial usage utilise point prevalence surveys, determining usage and indications on a single day[5]. A recent systematic review amongst African children and adults suggests overuse of Watch and relative neglect of Access agents[11]. However, such reports typically over-include referral centres and intensive care units, potentially limiting generalisability. Understanding what drives use of Watch antimicrobials may help target guidelines and stewardship programmes.

We aimed to describe daily in-hospital antimicrobial use and its clinical and demographic correlates among acutely ill children aged 2-23 months at nine hospitals in sub-Saharan Africa

and South Asia[12]. We specifically examined the variation in timing and use of second- and third-line agents for which there is limited guidance.

4.2 Methods

4.2.1 Study Design and Population

This was a secondary analysis of the prospective Childhood Acute Illness & Nutrition (CHAIN) Network stratified cohort November 2016 through January 2019. CHAIN aimed to determine the epidemiology and pathways to mortality in hospital and after discharge among acutely-ill children despite provision of care according to current guidelines at nine hospitals in sub-Saharan Africa and South Asia: Dhaka ICDDR,B (tertiary) and Matlab ICDDR,B (secondary) in Bangladesh; Banfora Referral Hospital (secondary) in Burkina Faso; Kilifi County, Mbagathi County and Migori County Hospitals (secondary) in Kenya; Queen Elizabeth Central Hospital (tertiary) in Malawi; Civil Hospital (tertiary) in Pakistan; and Mulago Hospital (tertiary) in Uganda. Sites were selected to represent a range of levels, facilities, environments, populations, and comorbidities such as malaria and HIV. Dhaka, Matlab, Kilifi County, Mbagathi County Hospitals, and Civil Hospital could do blood cultures routinely. Civil Hospital and Dhaka Hospital have paediatric intensive care units. All sites had access to the recommended first-line and second-line antibiotics.

CHAIN recruited acutely ill children aged 2-23 months, excluding children who required immediate resuscitation, were intolerant of oral feeds prior to illness, had a terminal illness, a condition requiring surgery within 6 months, a likely chromosomal abnormality, poisoning,

trauma, or surgery, unwilling to remain in follow-up within the hospital catchment area for 6 months. To ensure representation across mortality risks, children were enrolled in three strata (2:1:2) by nutritional status as shown in the table below (Table 4.1).

		Criteria	
Nutritional group	**Metric**	**≥6 months of age**	**<6 months of age**
1- Not wasted (NW)	MUAC	≥ 125 mm	≥ 120 mm
2- Moderate wasting (MW)	MUAC	<125 mm to ≥115 mm	<125 mm to ≥115 mm
3- Severe wasting or nutritional oedema/Kwashiorkor (SWK)	MUAC	<115 mm	<110 mm
	Evaluation of oedema	Mild	Mild
		Moderate	Moderate
		Severe	Severe

Mild (+) in feet only; Moderate (++) in feet and lower limbs; Severe (+++) generalized including upper body and face

Table 4.1: *Enrolment strata*

Patients were screened on specified days each week until the weekly quota for each stratum was met, controlling the enrolment rate to help ensure data completeness and quality[13]. Prior to initiation, sites were audited and supported to provide standard of care to national and/or WHO guidelines[14]. These antimicrobial prescribing guidelines are based on broad clinical syndromes and recommend adjustment according to local bacterial susceptibility patterns. Sites were given biannual reports on clinical care including antimicrobial prescription patterns.

4.2.2 Patient Consent Statement

Written informed consent was obtained from all parents or caregivers. The Oxford University Tropical Research Ethics Committee (OXTREC 34-16) and the ethics committees at all

participating institutions provided approval. The study was registered at ClinicalTrials.gov: NCT03208725.

4.2.3 Data Collection and Study Variables

Clinical definitions and data collection were harmonised across sites through training, standard operating procedures and case report forms (https://chainnetwork.org/resources/). Demographics, history, prior hospital admission, prior antimicrobials, clinical presentation, and laboratory tests were recorded at admission (Table 4.2). We have previously found that the type or duration of pre-admission antimicrobial usage are frequently inaccurately reported and so were collected as Y/N. During admission, daily clinical features and antimicrobials received were recorded on standardised proforma by trained study clinicians and entered in a database (https://www.project-redcap.org).

4.2.4 Outcomes

I examined the proportions of children and the number of child-days in hospital receiving intravenous (IV) or oral antimicrobials; use of Access, Watch and Reserve categories and antimicrobial classes received before and after 48 hours of admission. For children with sepsis, severe pneumonia or severe malnutrition, for whom recommended as first-line treatment is penicillin or ampicillin plus gentamicin, I examined the use of first-, second- or third-line antimicrobials according to WHO guidelines in these syndromes. For these children, amoxicillin plus clavulanic acid, azithromycin, amikacin, vancomycin, ceftriaxone,

ciprofloxacin, levofloxacin, and cefalexin were regarded as second-line, and ceftazidime, carbapenems, and linezolid as third-line[15].

4.2.5 Statistical Analysis

I summarised the baseline characteristics and antimicrobial use using counts and proportions. For missing data, for ≤3 days, use of the same antimicrobial before and after the gap, no change was assumed. Gaps >3 days were left unimputed. Rates were calculated per 100 inpatient child-days with 95% confidence intervals (CI). We categorized children according to the WHO syndrome groups, carefully accounting for all overlaps and identifying those who received antibiotics in cases where they were not indicated.

To identify factors associated with hospital prescriptions of Watch antimicrobials, I used multilevel binomial regression and a mixed-effects generalized linear model including site as a random intercept and inverse sampling weights using lme4, glmmTMB, and survey packages were designed based on the overall profile of admitted children, adjusting for intentional oversampling of undernutrition within the CHAIN cohort[16]. Weighted simple proportions were also calculated in this way. I used backwards stepwise selection (using exit $p<0.10$ and *MASS* R package). Final model performance was assessed using area under the receiver operating characteristic curve (ROC) using 1000 bootstrap samples using the *pROC* R package. Differences in antibiotic use between sites or groups were modelled using logistic regression with or without inverse weighting as appropriate. Marginal means were derived in

the response scale using the *emmeans* R package. Post-hoc pairwise comparisons between nutritional groups or sites were conducted and p-values adjusted following Tukey procedure.

4.3 Results

4.3.1 Participant characteristics

Overall, 3101 children (median age: 11 [IQR: 7-16] months) were enrolled in the three nutritional strata: n=1120 (36%) non-wasted (NW), 763 (25%) moderately wasted (MW), and 1218 (39%) severely wasted or kwashiorkor (oedematous malnutrition) (SWK) children (Table 4.2). The most frequent diagnoses were anaemia (n=1724 [59%]), diarrhoea (n=1721 [55%]), severe pneumonia (n=665 [21%]), malaria (n=419 [21%]), and suspected sepsis (n=436 [14%]). These conditions co-occurred in 1430 (46%) children, varying by nutritional strata and site (Table 4.2 and Figure 4.1). Inpatient case fatality and the median duration of admission were 182/3101 (5.9%, 95%CI 5.07-6.75) and 4 [IQR 2-7] days overall (Table 4.2). The frequency and proportions of children who died by syndrome combination and antibiotics is shown in Figure 4.1.

	NW N = 1,120	**MW N = 763**	**SWK N = 1,218**	**Overall N = 3,101**
Demographics				
Sex, female	432 (39%)	329 (43%)	583 (48%)	1344 (43%)
Age group				
> 6 months	205 (18%)	143 (19%)	268 (22%)	616 (20%)
6-12 months	405 (36%)	316 (41%)	432 (35%)	1153 (37%)
>12 months	510 (46%)	304 (40%)	518 (43%)	1332 (43%)
Site				
Kilifi	117 (10%)	52 (6.5%)	76 (6.2%)	245 (7.9%)
Mbagathi	88 (7.9%)	81 (11%)	110 (9.0%)	279 (9.0%)
Migori	106 (9.5%)	51 (6.7%)	123 (10%)	280 (9.0%)
Kampala	131 (12%)	109 (14%)	236 (19%)	476 (15%)
Blantyre	174 (16%)	52 (6.8%)	107 (8.8%)	333 (11%)

Karachi	134 (12%)	75 (9.8%)	140 (11%)	349 (11%)
Dhaka	125 (11%)	109 (14%)	160 (13%)	394 (13%)
Matlab	95 (8.5%)	123 (16%)	96 (7.9%)	314 (10%)
Banfora	150 (14%)	111 (15%)	170 (14%)	431 (14%)
Clinical presentation at admission				
Pneumonia*				
None	695 (62%)	519 (68%)	905 (74%)	2119 (68%)
Mild	139 (12%)	65 (8.5%)	113 (9.3%)	317 (10%)
Severe	286 (26%)	179 (23%)	200 (16%)	665 (21%)
Diarrhoea	525 (47%)	484 (63%)	712 (58%)	1721 (55%)
Sepsis†	142 (13%)	96 (13%)	198 (16%)	436 (14%)
Meningitis/Encephalopathy†	52(4.6%)	24(3.2%)	35(3.9%)	111(3.6%)
Nutritional oedema	-	-	353 (29%)	353 (11%)
Malaria rapid diagnostic test positive	202 (18%)	106 (14%)	132 (11%)	440 (14%)
Haemoglobin *g/dl*				
>110	216 (20%)	116 (16%)	196 (17%)	528 (18%)
100–110	265 (25%)	166 (23%)	252 (22%)	683 (23%)
70–100	443 (42%)	310 (43%)	530 (46%)	1283 (44%)
<70	135 (13%)	121 (17%)	185 (16%)	441 (15%)
Blood glucose mmol/L				
< 3	12 (1.1%)	7 (0.9%)	32 (2.7%)	51 (1.7%)
≥ 3 to ≤ 10	1,022 (93%)	702 (94%)	1,074 (90%)	2798 (92%)
> 10	67 (6.1%)	41 (5.5%)	85 (7.1%)	193 (6.3%)
Underlying conditions				
Stunted (LAZ <-2)	269 (24%)	348 (46%)	925 (76%)	1542 (50%)
Premature birth or low birth weight¶	132 (12%)	132 (18%)	245 (21%)	509 (17%)
HIV status				
Negative	1028 (92%)	692 (91%)	1022 (84%)	2742 (88%)
Exposed	69 (6.2%)	46 (6.0%)	96 (7.9%)	211 (6.8%)
Infected	23 (2.1%)	25 (3.3%)	100 (8.2%)	148 (4.8%)
Untested	39 (3.5%)	14 (1.8%)	23 (1.9%)	76 (2.5%)
Other chronic illness‖	64 (5.7%)	55 (7.2%)	98 (8.0%)	217 (7.0%)
Antimicrobials within last 7 days	526 (47%)	370 (48%)	526 (43%)	1422 (46%)
Prior hospitalisation				
< 1 week ago	50 (4.5%)	52 (6.8%)	73 (6.0%)	175 (5.7%)
1 week-1 month ago	56 (5.0%)	42 (5.5%)	109 (9.0%)	207 (6.7%)
>1 month ago	146 (13%)	89 (12%)	176 (15%)	411 (13%)
Outcome				
Time to discharge, days**	3.0 (2.0, 5.0)	4.0 (2.0, 6.0)	7.0 (4.0, 12)	4.0 (2.0, 7.0)
Died during index admission	22 (2.0%)	32 (4.2%)	128 (11%)	182 (5.9%)
Time to inpatient death, days	1.5 (0.0, 4.0)	2.0 (1.0, 5.3)	3.0 (1.0, 8.0)	3.0 (1.0, 8.0)
Duration of admission, days	3 (2, 5)	4 (2, 6)	7 (4, 12)	4 (2, 7)
Readmitted	173 (15%)	129 (17%)	204 (17%)	506 (16%)
Time to death, days	1·5 (0·0, 4·0)	2·0 (1·0, 5·5)	3·0 (1·0, 8·0)	3.0 (1.0, 8.0)

Results presented as frequency (%) or median (Interquartile Range (IQR)). * Cough or difficulty breathing with oxygen saturation <90%, central cyanosis, or grunting; very severe chest indrawing or inability to breastfeed or drink; or lethargy, reduced level of consciousness, or convulsions. † Clinician diagnosis. ‡ AVPU, alert, voice, pain and unresponsive; ¶ reported premature (<37 weeks) or low birthweight (<2.5 kg); || Chronic illnesses including thalassemia, cerebral palsy, sickle cell disease, congenital cardiac diseases and known tuberculosis; LAZ: length for age Z score; ** Index admission for survivors only; NW, no wasting; MW, moderate wasting; SMK, severe wasting or kwashiorkor (severe malnutrition).

Table 4.2: *Socio-demographic and clinical characteristics by enrolment strata.*

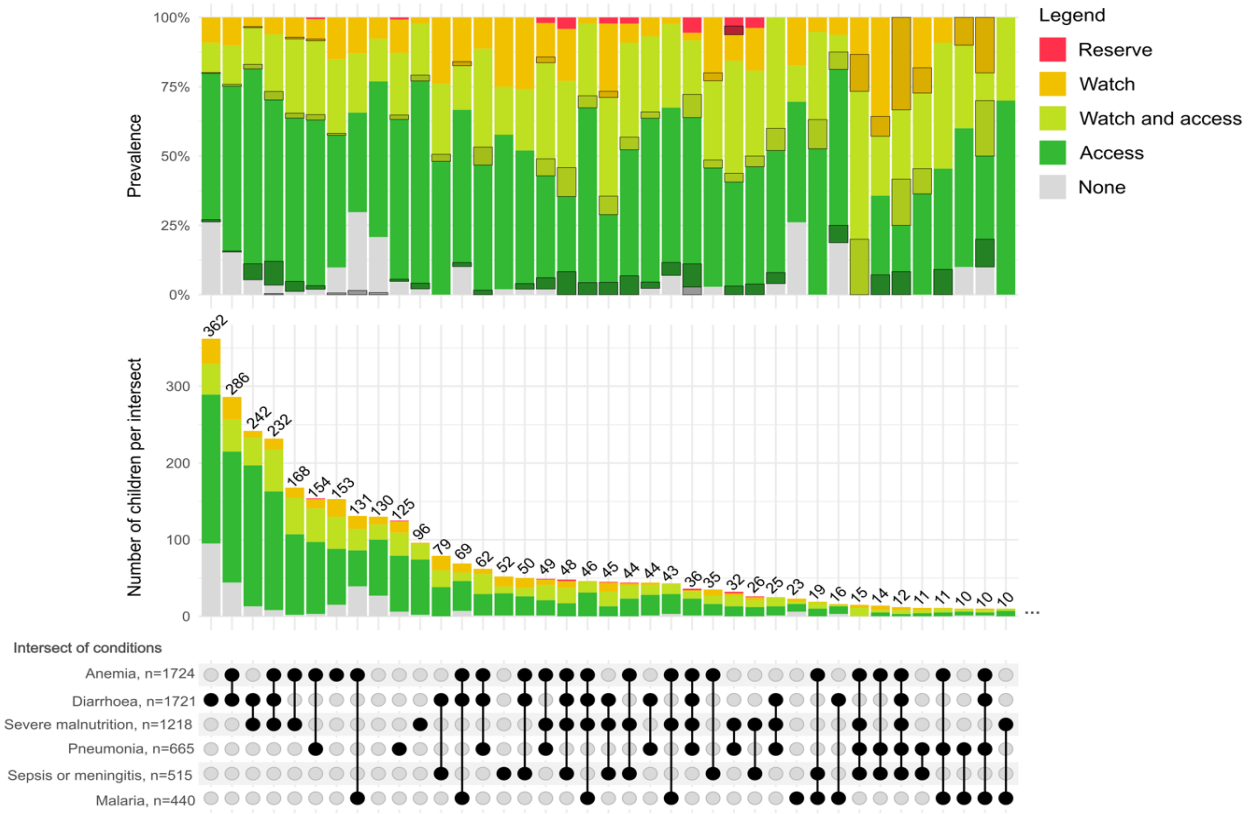
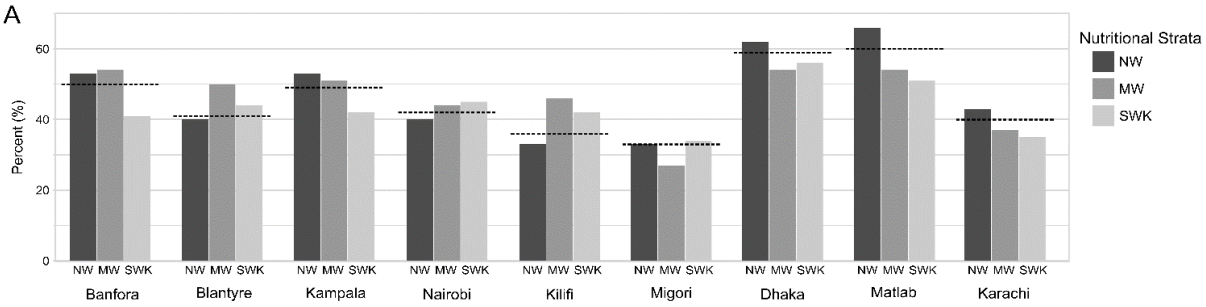


Figure 4.1: *Upset plot detailing antibiotic use in children with specific combinations of most frequent clinical syndromes. The bottom panel represents combination of clinical syndrome if found in at least 10 patients (X-axis). Combination of black dots indicate co-presence of conditions. Y-axis of middle panel presents, for each syndrome combination, the absolute count of children who received as per legend: None (grey), only Access (green), Watch and Access (luminous green), only Watch (yellow), and Reserve (alone or with other compounds) (red). The Y-axis of the top panel presents the proportion of children that receive the different antibiotics for each combination of conditions. The proportion of children who died by syndrome combination and antibiotics received is indicated by greyed shadow boxes.*

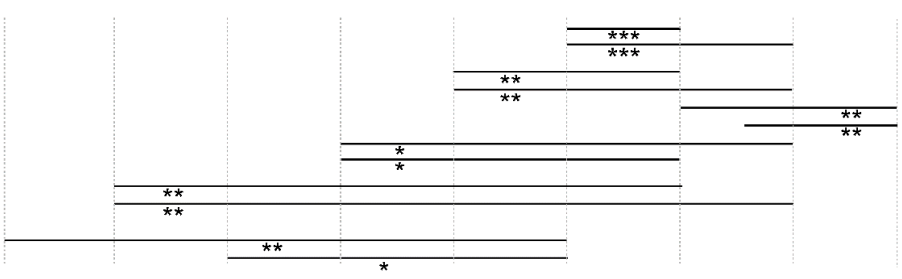
4.3.2 Prior admission and reported antimicrobial use.

Prior hospitalisation was reported in 382 (11%) children within 1 month, and 411 (13%) 1-6 months ago (Table 4.2). Antimicrobial use within 7 days prior to admission was reported in 1422 (46%) children, varying by site but not by nutritional strata (Figure 4.2).

A



B



C

Prior antibiotics	NW	MW	SWK	Overall	Weighted
Banfora	80/150 (53%)	60/111 (54%)	70/170 (41%)	210/431 (49%)	50%
Blantyre	69/174 (40%)	26/52 (50%)	47/107 (44%)	142/333 (43%)	41%
Kampala	69/131 (53%)	56/109(51%)	98/236 (42%)	223/476 (47%)	49%
Nairobi	35/88 (40%)	36/81 (44%)	49/110 (45%)	120/279 (43%)	42%
Kilifi	39/117 (33%)	24/52 (46%)	32/76 (42%)	95/245(39%)	36%
Migori	35/106 (33%)	14/51 (27%)	42/123 (34%)	91/280 (32%)	33%
Dhaka	78/125 (62%)	59/109 (54%)	90/160 (56%)	227/394 (58%)	59%
Matlab	63/95 (66%)	67/123 (54%)	49/96 (51%)	179/314 (57%)	60%
Karachi	58/134 (43%)	28/75 (37%)	49/140 (35%)	135/349 (39%)	40%
Total				1422/3101 (46%)	46%

Figure 4.2: *Percent and number of children reported to have received antibiotics in the 7 days prior to hospital admission. A) Bar chart presenting percentages split by site and nutritional strata. Dashed black line indicates the weighted overall average for each site; B) Line graph indicating significant differences between site as obtained from pairwise comparisons derived from logistic regression models, stars indicated significance threshold as follows: *p<0.05; **p<0.001, ***p<0.0001; C) Table detailing counts and percentages including unweighted- and weighted-overall values. NW, not wasting; MW, moderate wasting; SWK, severe wasting or kwashiorkor.*

4.3.3 Inpatient antimicrobial usage

A total of 19907 daily reviews were collected and 440 (2.2%) were excluded. Overall, 2818/3101 (91%) children received an antimicrobial in hospital. Intravenous antimicrobials were received by 2644/3101 (85%) children (ranging from 51% in Matlab to 100% in Nairobi). Both oral and intravenous antimicrobials concurrently were received by 1681/3101 (54%) children for at least one day (Table 4.3). Most (2787/3101 [90%]) children-initiated antimicrobials <48 hours after admission (2608/3101 [84%] intravenous antimicrobials). Very few (31/3101, [1.0%]) started antimicrobials >48 hours after admission. Daily antimicrobial data were missing for 195/21807 (0.9%) days. Overall, antimicrobials were prescribed for 19,398/21,807 (93%) inpatient child-days.

Characteristic	NW N = 1,120	MW N = 763	SWK N = 1,218	Overall N = 3,101	Weighted Proportions*
Mode of administration					
Receiving IV antibiotics	827 (74%)	647 (85%)	1170 (96%)	2644 (85%)	81%
Days on IV antibiotics	4.0 (0.00, 8.0)	6.0 (3.0, 11)	10 (6.0, 16)	7.0 (3.0, 12)	
Receiving oral antibiotics	286 (26%)	190 (25%)	473 (39%)	949 (31%)	29%
Days on oral antibiotics	1.0 (0.00, 2.0)	1.0 (0.00, 2.0)	1.0 (0.00, 4.0)	1.0 (0.00, 2.0)	
Receiving oral & IV antibiotics	552 (49%)	432 (57%)	697 (57%)	1681 (54%)	52%
WHO AWARE classification					
Access	778 (69%)	611 (80%)	1,088 (89%)	2477 (80%)	76%
Watch	373 (33%)	267 (35%)	452 (37%)	1092 (35%)	35%
Reserve	1 (<0.1%)	1 (0.1%)	10 (0.8%)	12 (0.4%)	0.3%
First-, second- and third-line regimens for sepsis†					
First-line	700 (63%)	569 (75%)	1,038 (85%)	2307 (74%)	70%
Second-line	429 (38%)	293 (38%)	469 (39%)	1191 (38%)	38%
Third-line	13 (1.2%)	9 (1.2%)	40 (3.3%)	62 (2.0%)	1.7%
Antimicrobial line, IV					
First-line IV	580 (52%)	520 (68%)	1,011 (83%)	2,111 (68%)	59%
Second-line IV	386 (34%)	272 (36%)	427 (35%)	1,085 (35%)	35%
Third-line IV	12 (1.1%)	7 (0.9%)	32 (2.6%)	51 (1.6%)	1.7%
Antimicrobial classes					

Penicillins	636 (57%)	525 (69%)	979 (80%)	2,140 (69%)	65%
Aminoglycosides	460 (41%)	477 (63%)	915 (75%)	1,852 (60%)	53%
Penicillins & aminoglycosides	439 (58%)	403 (36%)	838 (69%)	1,680 (54%)	55%
Co-amoxiclav	89 (7.9%)	47 (6.2%)	49 (4.0%)	185 (6.0%)	6.7%
Imidazoles	39 (3.5%)	32 (4.2%)	101 (8.3%)	172 (5.5%)	4.8%
Amphenicols	26 (2.3%)	5 (0.7%)	1 (<0.1%)	32 (1.0%)	1.5%
Anti-pseudomonal beta-lactams ‡	6 (0.5%)	2 (0.3%)	17 (1.4%)	25 (0.8%)	0.7%
Lincosamides	0 (0%)	0 (0%)	1 (<0.1%)	1 (<0.1%)	<0.1%
Macrolides	41 (3.7%)	35 (4.6%)	66 (5.4%)	142 (4.6%)	4.3%
Fluoroquinolones	22 (2.0%)	15 (2.0%)	54 (4.4%)	91 (2.9%)	2.6%
Glycopeptides	6 (0.5%)	2 (0.3%)	17 (1.4%)	25 (0.8%)	2.1%
Third generation cephalosporins	334 (30%)	243 (32%)	413 (34%)	990 (32%)	31%
Carbapenems	4 (0.4%)	2 (0.3%)	10 (0.8%)	16 (0.5%)	0.5%
Oxazolidinones	1 (<0.1%)	1 (0.1%)	10 (0.8%)	12 (0.4%)	0.3%
Polymyxins	1 (<0.1%)	0 (0%)	0 (0%)	1 (<0.1%)	<0.1%
Trimethoprim/sulfamethoxazole (Prophylaxis in HIV)	39 (3.5%)	42 (5.5%)	131 (11%)	212 (6.8%)	5.6%

Results presented as frequency (%), or days as median (IQR). Proportions for AWARE categories and antimicrobial classes total more than 100% because 2831/3101 (91%) of children receive more than one type of antibiotic. * Weighted proportions; † First-, second- and third-line agents for sepsis are defined as per WHO guidelines as defined in**.** WHO also recommends these agents for children with severe pneumonia and severe malnutrition. Here they are applied to data from all children. Sepsis regimens is also used for severe pneumonia and severe malnutrition. ‡ Beta-lactam anti-pseudomonal antimicrobials include some third generation cephalosporins such as ceftazidime and piperacillin/tazobactam.

Table 4.3: *Antibiotics received by children at any time during admission.*

4.3.4 Types of antimicrobials

Overall, 2477 children received (76%) Access, 1092 (35%) Watch, and 12 (0.3%) Reserve antimicrobials during admission (weighted proportions) (Figure 4.3 & 4.5). Penicillins (n=2140 [65%]), aminoglycosides (1852 [53%]) and third generation cephalosporins (990 [31%]) were most frequently used (Figure 4.4).

4.3.5 Oral antimicrobials

Overall, 949/3101 (31%) children received oral antimicrobials during admission (range across sites 4.6-62%), median duration 1 day (IQR: 0.0-2.0 days) during hospitalisation (Table 4.3).

Penicillins (522 (17%)) and prophylaxis in HIV using co-trimoxazole (212 (6.8%)) were most frequent (Table 4.3).

4.3.6 Intravenous antimicrobials

Overall, 2644 /3101 (85%) children received intravenous antimicrobials (74% of NW, 85% MW and 96% SWK groups) during admission (range across sites of 51-100%), median duration 7 days (IQR: 3.0, 12 days). Penicillins (1974 (64%)), aminoglycosides (1852 (60%)) and ceftriaxone (970 (31%)) were most frequent (Table 4.3).

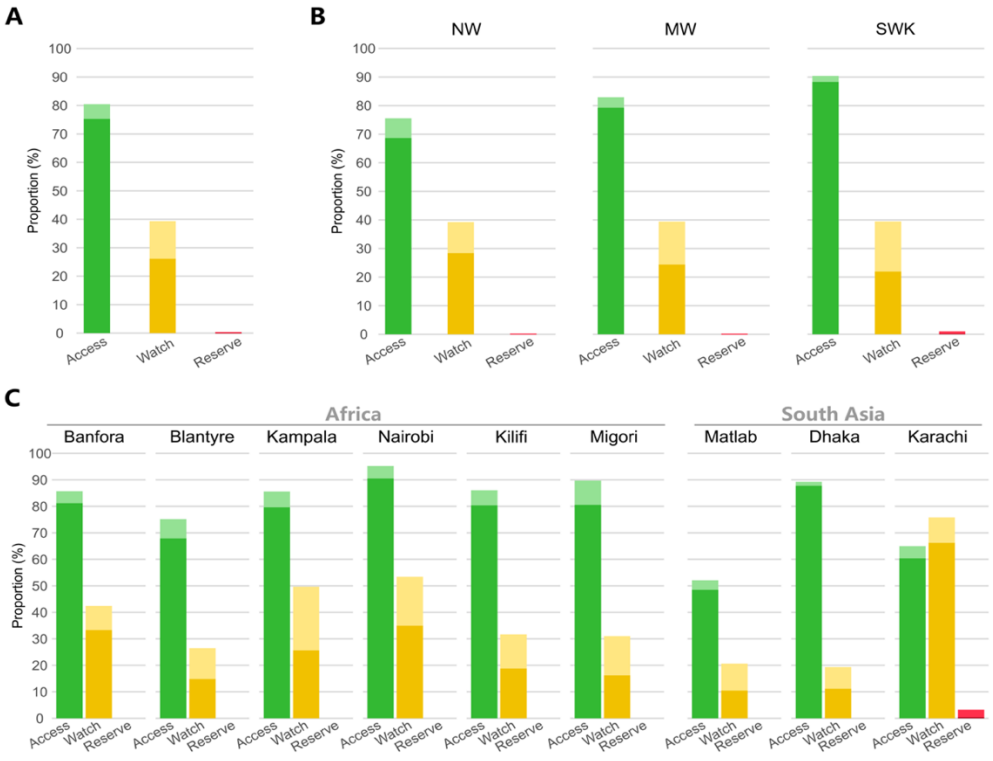


Figure 4.3: *Bar graph representing percentage of children receiving antimicrobials classified as: Access in green, Watch in yellow, and Reserve in red. Dark coloured bars represent percentage of children that received antimicrobials within 48 hours of admission while light coloured bar indicates those starting antimicrobials after 48 hours. Percentage of children who received antimicrobial classes during admission A) All children combined B) Split by nutritional strata C) Split by split by site NW not wasted; MW, moderately wasted; SWK, severely wasted or kwashiorkor (severe malnutrition).*

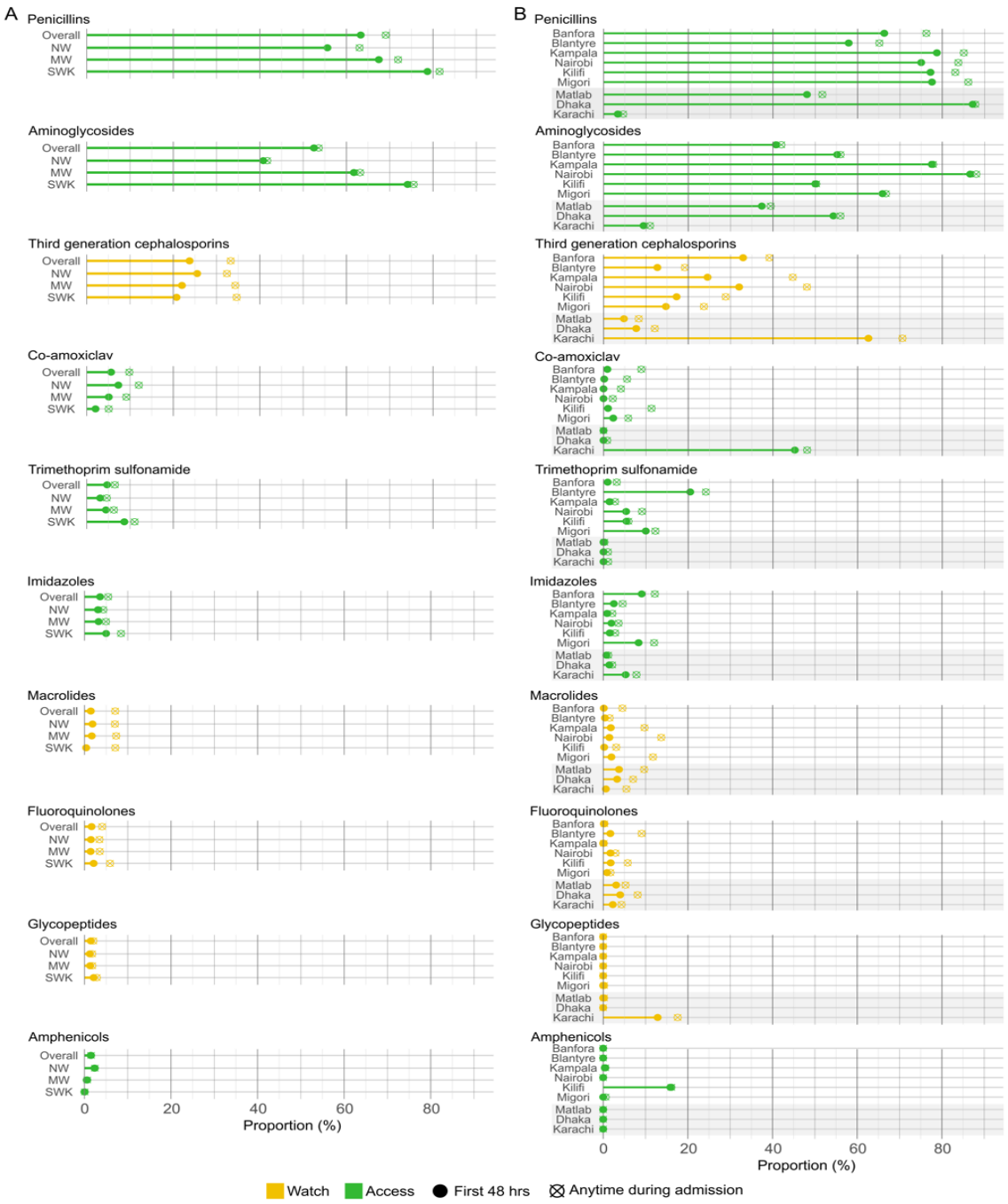


Figure 4.4. Line graph colours representing percentage of children receiving antimicrobials classified as: Access in green, watch in yellow, and Reserve in red; A) Split by nutritional strata B) Split by site. *Filled dot represents % of children that started antimicrobials within 48 hours of admission while dot with cross bar indicates those starting antimicrobials after 48 hours. Overall percentages weighted by enrolment stratum. NW not wasted; MW, moderately wasted; SWK, severely wasted or kwashiorkor (severe malnutrition). Antimicrobial groups not shown if proportions <1%.*

4.3.7 Access antimicrobials

Access antimicrobials were used for 62(95%CI: 61–63) days per 100 inpatient child-days, ranging from 38.3 to 80.7 across sites (Table 4.4). Most Access antimicrobials 1875/2884(65%) were prescribed <48 hours of admission reducing to a low of 7% at day 8-9 since most children were discharged by day 7 of (Figure 4.5). A penicillin/aminoglycoside combination was used in 1680/3101 (54%) children (1680/2425(69%) of children who started antimicrobials <48 hours) (Table 4.3). Use of penicillin and aminoglycoside combinations ranged from <0.1% in Karachi to 84% in Kampala (Figure 4.4B) and was more common in children in the SWK stratum 69% (95%CI: 66-71) than the MW (58% (95%CI: 54-61)) or SWK (36% (95%CI: 33-39)) strata (both P<0.0001), (Figure 4.4A). One site, Kilifi, prescribed chloramphenicol in 16% (95%CI: 10-22%) of children with almost no use at other sites.

Category of antimicrobial	Number of children receiving this antimicrobial classification for at least one day (%)	Total Inpatient child-days receiving this antimicrobial classification	Total Inpatient child-days of admission	Days receiving this antimicrobial classification per 100 inpatient child-days (95% CI)
Access				
Kilifi	198 (81)	964	1476	65(61–70)
Mbagathi	262 (94)	1828	2620	70(67–73)
Migori	237 (85)	1180	1832	64(61–68)
Kampala	410 (86)	2429	4847	50 (48–52)
Blantyre	242 (73)	1129	1920	59 (55–62)
Karachi	210 (60)	862	2251	38 (36–41)
Dhaka	365 (93)	2062	2759	75 (72–78)
Matlab	178 (57)	772	1357	57 (53–61)
Banfora	375 (87)	2215	2745	81 (77–84)
Total Access	2477 (80)	13441	21807	62 (61–63)
Watch				
Kilifi	79 (32)	454	1476	31(28–34)
Mbagathi	143 (51)	1008	2620	39 (36–41)
Migori	71 (25)	334	1832	18(16–20)

Kampala	170 (36)	811	4847	17 (16–18)
Blantyre	77 (23)	334	1920	17 (16–19)
Karachi	264 (76)	1691	2251	75 (72–79)
Dhaka	72 (18)	422	2759	15(14–17)
Matlab	42 (13)	157	1357	12 (9.8–14)
Banfora	174 (40)	685	2745	25 (23–27)
Total Watch	1092 (35)	5896	21807	27 (26–28)
Reserve				
Kilifi	0	0	1476	0
Mbagathi	0	0	2620	0
Migori	0	0	1832	0
Kampala	0	0	4847	0
Blantyre	0	0	1920	0
Karachi	12 (3.4)	61	2251	2.7 (2.1–3.5)
Dhaka	0	0	2759	0
Matlab	0	0	1357	0
Banfora	0	0	2745	0
Total Reserve	12 (0.4)	61	21807	0.3 (0.2–0.4)

Kilifi (n=245), Mbagathi (n=279), Migori (n=280), Kampala (n=476), Blantyre (n=333), Karachi (n=349), Dhaka (n=394), Matlab (n=314), Banfora (n=4)

Table 4.4*. Child-days spent on each WHO AWaRe classification by site.*

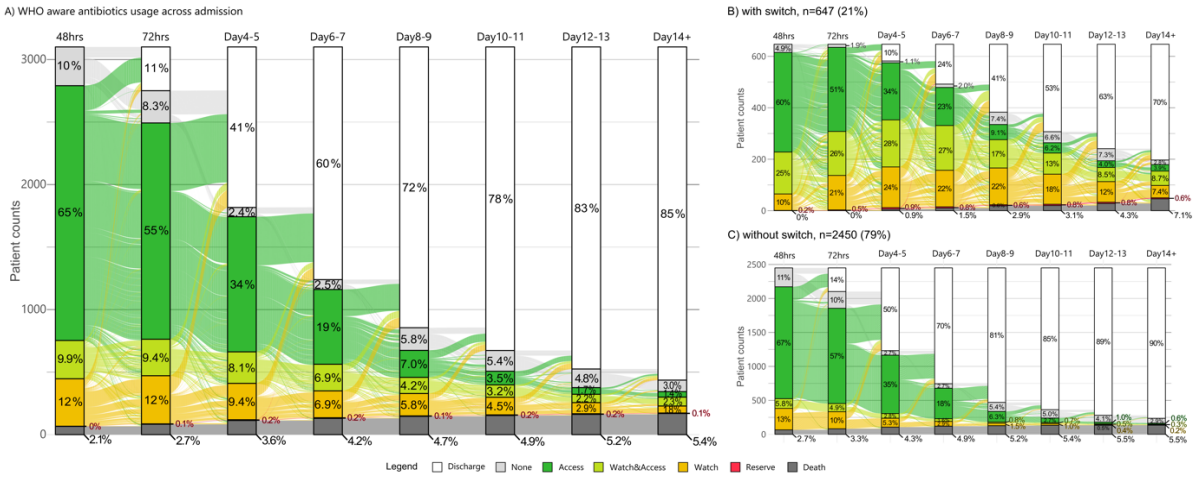


Figure 4.5: *Alluvial plots detailing the proportion of AWARE antimicrobial use in children during admission presented as sequential 48-hour periods starting from admission. Antimicrobial classes used in A) All admissions; B) children receiving antimicrobial changes (switch) and C) children with no change in antimicrobials. Each alluvial line depicts the progression of a child through admission to discharge (white) or death (grey) and the antimicrobials received colour coded as per legend. Y-axis presents stacked child counts.*

4.3.8 Watch antimicrobials

Watch antimicrobials were used for 27 [95%CI: 26–28] days per 100 inpatient child-days, among 1,092 (35%) children (Tables 4.2 & 4.3, Figure 4.3A), predominantly third-generation cephalosporins (Figures 4.4A, 4.4B). In the first 48 hours, 705 (24%) children received Watch antimicrobials. Watch antimicrobial use ranged from 12 to 75 days per 100 inpatient child-days (Table 4.3) with Civil Hospital, Karachi predominantly initiating co-amoxiclav (an Access agent) for children in the NW and MW strata, with SWK children receiving mostly third-generation cephalosporins. Carbapenems were prescribed in 16 (0.5%) children, of which 10/16 (63%) were in the SWK stratum (Figures 4.3C & 4.4B). Across nutritional strata, there was less Watch antimicrobial usage in more severely malnourished groups (Figure 4.4A): NW, 32 (IQR 30–33); MW, 29 (IQR 28–31) and SWK, 24 [95%CI, 23–25] per 100 child-days (Table 4.5). Use of Watch antibiotics across sites for at least one day ranged from 42/314 (13%) in Matlab to 264/349 (76%) of children in Karachi (Table 4.4). Combination use of Access and Watch antibiotics was highest (25%) among children with antibiotic switching during admission (Figure 4.5). Of children presenting with isolated diarrhoea and uncomplicated malaria, 64/295 (22%) and 138/369 (37%) received Watch agents respectively.

Category of antibiotic	Number of children receiving at least one day of antimicrobials	Child-days received antimicrobials	Child days of admission	Days of antimicrobials received per 100 child-days (95% CI)
First-line				
NW	700 (63)	2701	5345	51 (49–53)
MW	569 (75)	2697	4634	58 (56–60)
SWK	1038 (85)	7236	11828	61(60–64)
Second-line				
NW	429 (38)	1899	5345	36(34–37)
MW	293 (38)	1518	4634	33 (31–34)

SWK	469 (39)	2866	11828	24 (23–25)
Third-line				
NW	13 (1.2)	80	5345	1.5 (1.2–1.9)
MW	9 (1.2)	60	4634	1.3 (1.0–1.7)
SWK	40 (3.3)	252	11828	2.1 (1.9–2.4)
Access				
NW	778 (69)	3004	5345	56(54–58)
MW	611 (80)	2923	4634	63(61–65)
SWK	1088 (89)	7514	11828	64 (62–65)
Watch				
NW	373 (33)	1686	5345	32 (30–33)
MW	267 (35)	1357	4634	29 (28–31)
SWK	452 (37)	2853	11828	24 (23–25)
Reserve				
NW	1 (0.09)	3	5345	0.0 (0.0–0.1)
MW	1 (0.13)	7	4634	0.2 (0.1–0.3)
SWK	10 (0.82)	53	11828	0.5 (0.3–0.6)

NW: not wasted N=1120; MW: moderately wasted N=763; SWK: severely wasted or kwashiorkor N=1218

***Table 4.5**: Antimicrobials by cohort nutritional status strata*

4.3.9 Reserve antimicrobials

Reserve antimicrobials were used in 12 (0.4%) children (specifically, 11 linezolid and 1 polymyxin) for 61 inpatient child-days (0.3 [95%CI, 0.2–0.4] days per 100 inpatient child-days) (Table 4.3 and 4.4). Reserve antimicrobials were only used at Civil Hospital: 2.7 [95%CI, 2.1–3.5] days per 100 inpatient child-days at that hospital. Eleven of the twelve started Reserve antimicrobials > 48 hours after admission and 10/12 were in the SWK stratum (Table 4.3 and Figure 4.3).

4.3.10 Overuse of antimicrobials

We examined antimicrobial use in children with diarrhoea, malaria, moderate/severe anaemia and other diagnoses presenting in the absence of concurrent features of sepsis, meningitis/encephalopathy, pneumonia, severe malnutrition or other conditions for which antibiotics are indicated. Overall, 341/3101 (11%) of admissions received an antibiotic without

an indication, 117/341 (34%) of whom received a Watch agent, representing 3.8% of all admissions. Among the 509/3101 (16%) without any indication for an antibiotic, 341 (70%) received an antibiotic and this was similar across different diagnoses (Table 4.6).

Diagnosis not indicating antimicrobials	Children with no other indication for Abx	Proportion of children with no other indication for antimicrobials who received antimicrobials	Proportion of all admissions (N=3101) who received antimicrobials without a clinical indication
Diarrhoea	374/1721 (22%)	Any Abx = 252 (67%) Access =211 (56%) Watch = 81 (22%) Reserve = 0 None= 122 (33%)	Any = 252 (8.1%) Access = 211 (6.8%) Watch = 81 (2.6%) Reserve = 0 None = 122 (3.9%)
Malaria	142/440 (32%)	Any = 94 (66%) Access =74 (52%) Watch = 36 (25%) Reserve = 0 None = 48 (34%)	Any = 94 (3.0%) Access =74 (2.4%) Watch = 36 (1.1%) Reserve = 0 None = 48 (1.5%)
Moderate/severe anaemia	309/1827 (17%)	Any = 222 (72%) Access =182 (59%) Watch = 85 (28%) Reserve = 0 None = 87 (28%)	Any = 222 (7.2%) Access = 182 (5.9%) Watch = 85 (2.7%) Reserve = 0 None = 87 (2.8%)
None of the above diagnosis	37/433 (8.5%)	Any = 27 (73%) Access =22 (59%) Watch =8 (22%) Reserve = 0 None = 10 (27%)	Any = 27 (0.87%) Access = 22 (0.71%) Watch = 8 (0.26%) Reserve = 0 None = 10 (0.32%)
Overall	509/3101 (16%)	Any = 341 (67%) Access =280 (55%) Watch =117 (23%) Reserve = 0 None = 168 (33%)	Any = 341 (11%) Access = 280 (9.0%) Watch = 117 (3.8%) Reserve = 0 None = 168 (5.4%)

Note: There is overlap in the groups because a child can have more than one diagnosis.

Table 4.6. *Diagnosis not indicating antimicrobials.*

4.3.11 Children with sepsis, severe malnutrition, and severe pneumonia

Of 1,886 children with either sepsis, severe malnutrition and/or severe pneumonia at admission, 1,509 (80%) received first-line antimicrobials at any time, by strata 270 (66%) NW; 201 (77%) MW; and 1,038 (85%) SWK (Table 4.7). These were prescribed for 58 days per 100 inpatient child-days (Table 4.4). First-line agents were introduced within 48 hours of admission in 1,468 (78%) children, the remaining 22% >48 hours after admission (Table 4.7). Penicillins (74%) and aminoglycosides (70%) were most frequent (Table 4.3). Second-line antimicrobials were given to 806/1866 (43%) children during admission, 533 (66%) within 48 hours (Table 4.7). These were prescribed for 29 days per 100 inpatient child-days (Table 4.5): NW, MW and SWK receiving 36, 33 and 24 days per 100 inpatient child-days respectively (Table 4.7). The commonest second-line antimicrobials were third-generation cephalosporins (36%), beta-lactam/beta-lactamase inhibitor combinations (co-amoxiclav) (8.7%) and macrolides at (5.4%) (Table 4.4). Third-line antimicrobials were given to 53/1886 (2.8%) children, 1.8 [95%CI, 1.6–2.0] days per 100 inpatient child-days (Table 4.4 & 4.6), mostly >48 hours after admission Table 4.7).

	Entire admission				Within 48 hours of admission			
Characteristic	**NW** N = 408	**MW** N = 260	**SWK** N = 1,218	**Overall** N = 1,886	**NW** N = 408	**MW** N = 260	**SWK** N = 1,218	**Overall** N = 1,886
Prior to hospitalization								
Prior antibiotics taken, yes	194(48%)	134(52%)	526 (43%)	854 (45%)	194(48%)	134(52%)	526 (43%)	854 (45%)
AWARE categories								
Access	327(80%)	231(89%)	1,088(89%)	1,646(87%)	317(78%)	225(87%)	1,064(87%)	1,606(85%)
Watch	169(41%)	114(44%)	452 (37%)	735 (39%)	119(29%)	72 (28%)	248 (20%)	439 (23%)
Reserve	1 (0.2%)	1 (0.4%)	10 (0.8%)	12 (0.6%)	0 (0%)	0 (0%)	1 (<0.1%)	1 (<0.1%)
Antibiotic line								
First-line	270(66%)	201(77%)	1,038(85%)	1,509(80%)	261(64%)	193(74%)	1,014(83%)	1,468(78%)
Second-line	202(50%)	135(52%)	469 (39%)	806 (43%)	165(40%)	99 (38%)	269 (22%)	533 (28%)
Third-line	8 (2.0%)	5 (1.9%)	40 (3.3%)	53 (2.8%)	1 (0.2%)	2 (0.8%)	9 (0.7%)	12 (0.6%)
Antibiotic line, IV								
First-line, IV	251(62%)	193(74%)	1,011(83%)	1,455(77%)	245(60%)	188(72%)	987 (81%)	1,420(75%)
Second-line, IV	197(48%)	131(50%)	427 (35%)	755 (40%)	163(40%)	96 (37%)	258 (21%)	517 (27%)
Third-line, IV	7 (1.7%)	4 (1.5%)	32 (2.6%)	43 (2.3%)	1 (0.2%)	2 (0.8%)	8 (0.7%)	11 (0.6%)
Antibiotic line, oral								
First-line oral	58 (14%)	49 (19%)	422 (35%)	529 (28%)	30 (7.4%)	21 (8.1%)	146 (12%)	197 (10%)
Second-line oral	20 (4.9%)	8 (3.1%)	80 (6.6%)	108 (5.7%)	4 (1.0%)	4 (1.5%)	12 (1.0%)	20 (1.1%)
Third-line oral	1 (0.2%)	0 (0%)	1 (<0.1%)	2 (0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Received antibiotics								
Received antibiotics, yes	393(96%)	258(99%)	1,188(98%)	1,839(98%)	391(96%)	257(99%)	1,185(97%)	1,833(97%)
Received IV antibiotics, yes	382(94%)	254(98%)	1,170(96%)	1,806(96%)	380(93%)	254(98%)	1,165(96%)	1,799(95%)
Received oral antibiotics, yes	76 (19%)	57 (22%)	473 (39%)	606 (32%)	34 (8.3%)	25 (9.6%)	157 (13%)	216 (11%)
Antibiotic types								
Penicillins	242(59%)	184(71%)	979 (80%)	1,405(74%)	235(58%)	177(68%)	946 (78%)	1,358(72%)
Aminoglycosides	228(56%)	184(71%)	915 (75%)	1,327(70%)	220(54%)	176(68%)	889 (73%)	1,285(68%)
Imidazoles	15 (3.7%)	11 (4.2%)	101 (8.3%)	127 (6.7%)	11 (2.7%)	9 (3.5%)	58 (4.8%)	78 (4.1%)
Amphenicols	3 (0.7%)	2 (0.8%)	1 (<0.1%)	6 (0.3%)	2 (0.5%)	2 (0.8%)	0 (0%)	4 (0.2%)
Beta-lactamase inhibitor	62 (15%)	32 (12%)	49 (4.0%)	143 (7.6%)	57 (14%)	30 (12%)	24 (2.0%)	111 (5.9%)
Anti-pseudomonal beta-lactams	3 (0.7%)	1 (0.4%)	17 (1.4%)	21 (1.1%)	0 (0%)	1 (0.4%)	2 (0.2%)	3 (0.2%)
Lincosamides	0 (0%)	0 (0%)	1 (<0.1%)	1 (<0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
3rd Gen. cephalosporins	160(39%)	110(42%)	413 (34%)	683 (36%)	115(28%)	69 (27%)	237 (19%)	421 (22%)
Fluoroquinolones	9 (2.2%)	4 (1.5%)	54 (4.4%)	67 (3.6%)	4 (1.0%)	2 (0.8%)	18 (1.5%)	24 (1.3%)
Glycopeptides	11 (2.7%)	9 (3.5%)	34 (2.8%)	54 (2.9%)	5 (1.2%)	7 (2.7%)	23 (1.9%)	35 (1.9%)
Macrolides	19 (4.7%)	16 (6.2%)	66 (5.4%)	101 (5.4%)	3 (0.7%)	3 (1.2%)	3 (0.2%)	9 (0.5%)
Carbapenems	2 (0.5%)	1 (0.4%)	10 (0.8%)	13 (0.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Oxazolidinones	1 (0.2%)	1 (0.4%)	10 (0.8%)	12 (0.6%)	0 (0%)	0 (0%)	1 (<0.1%)	1 (<0.1%)
Trimethoprim/sulfonamide	14 (3.4%)	20 (7.7%)	131 (11%)	165 (8.7%)	12 (2.9%)	15 (5.8%)	97 (8.0%)	124 (6.6%)
Antibiotic types, IV								
Penicillins, IV	230(56%)	180(69%)	957 (79%)	1,367(72%)	225(55%)	175(67%)	933 (77%)	1,333(71%)
Aminoglycosides, IV	228(56%)	184(71%)	915 (75%)	1,327(70%)	220(54%)	176(68%)	889 (73%)	1,285(68%)
Imidazoles, IV	11 (2.7%)	8 (3.1%)	59 (4.8%)	78 (4.1%)	8 (2.0%)	8 (3.1%)	36 (3.0%)	52 (2.8%)

Amphenicols, IV	3 (0.7%)	2 (0.8%)	1 (<0.1%)	6 (0.3%)	2 (0.5%)	2 (0.8%)	0 (0%)	4 (0.2%)
Anti-pseudomonal beta-lactams, IV	3 (0.7%)	1 (0.4%)	17 (1.4%)	21 (1.1%)	0 (0%)	1 (0.4%)	2 (0.2%)	3 (0.2%)
3rd gen. cephalosporins, IV	160(39%)	109(42%)	403 (33%)	672 (36%)	115(28%)	68 (26%)	235 (19%)	418 (22%)
Glycopeptides, IV	11 (2.7%)	9 (3.5%)	34 (2.8%)	54 (2.9%)	5 (1.2%)	7 (2.7%)	23 (1.9%)	35 (1.9%)
Carbapenems, IV	2 (0.5%)	1 (0.4%)	10 (0.8%)	13 (0.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Oxazolidinones, IV	1 (0.2%)	1 (0.4%)	10 (0.8%)	12 (0.6%)	0 (0%)	0 (0%)	1 (<0.1%)	1 (<0.1%)
Antibiotic types, oral								
Penicillins, oral	32 (7.8%)	17 (6.5%)	250 (21%)	299 (16%)	14 (3.4%)	5 (1.9%)	30 (2.5%)	49 (2.6%)
Trimethoprim sulfonamide, oral	14 (3.4%)	20 (7.7%)	131 (11%)	165 (8.7%)	12 (2.9%)	15 (5.8%)	97 (8.0%)	124 (6.6%)
Imidazoles, oral	4 (1.0%)	3 (1.2%)	49 (4.0%)	56 (3.0%)	3 (0.7%)	1 (0.4%)	24 (2.0%)	28 (1.5%)
Beta-lactamase inhibitor, oral	4 (1.0%)	2 (0.8%)	19 (1.6%)	25 (1.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Lincosamides, oral	0 (0%)	0 (0%)	1 (<0.1%)	1 (<0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Third generation cephalosporins, oral	4 (1.0%)	1 (0.4%)	16 (1.3%)	21 (1.1%)	0 (0%)	1 (0.4%)	2 (0.2%)	3 (0.2%)
Fluoroquinolones, oral	4 (1.0%)	0 (0%)	22 (1.8%)	26 (1.4%)	2 (0.5%)	0 (0%)	8 (0.7%)	10 (0.5%)
Macrolides, oral	19 (4.7%)	16 (6.2%)	66 (5.4%)	101 (5.4%)	3 (0.7%)	3 (1.2%)	3 (0.2%)	9 (0.5%)
Oxazolidinones, oral	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 4.7: *Antibiotic prescriptions for 1886 children with clinical diagnosis/syndromes (sepsis, severe pneumonia, and severe malnutrition) categorised as per WHO guidelines or the Management of Common Childhood Illnesses.*

Factors associated with the use of Watch and second line antimicrobials.

Among all children admitted, prior hospital admission, chronic illness, clinician diagnosis as sepsis or meningitis/encephalopathy, blood glucose <3mmol/L and duration of admission of ≥5 days were positively associated with the use of Watch antimicrobials in a multivariable model which was similar to those of second-line antimicrobials. Use of Watch antimicrobials was not independently associated with nutritional strata, use of antimicrobials before admission, WHO danger signs, HIV status or white blood cell count at admission (coefficients and details of all variables tested in Table 4.8 also similar to second line antimicrobials).

		Univariate analysis		Multivariable analysis	
Admission characteristics	**Children who received Watch antimicrobials (N=1092)**	**Crude risk ratios**	**P-value**	**Adjusted risk ratios**	**P-value**
Sex, female	480 (44)	0.98 (0.75–1.27)	0.86	¶	

Age in months (log)	-	1.08 (0.93–1.26)	0.32	¶	
Enrolment strata					
NW	373 (34)	Reference		¶	
MW	267 (25)	1.12 (0.82–1.53)	0.49	¶	
SWK	452 (41)	1.15 (0.78–1.69)	0.49	¶	
Residence				¶	
Rural	209 (19)	Reference		¶	
Peri-urban	159 (15)	1.28 (0.96–1.70)	0.09	¶	
Urban	724 (66)	1.01 (0.57–1.79)	0.96	¶	
Tertiary hospital level	583 (53)	1.34 (0.43–4.16)	0.62	¶	
Prior hospital admission	341 (31)	1.59 (1.25–2.04)	<0.001	1.48 (1.14–1.93)	0.003
Antimicrobial <7 days prior to admission	514 (47)	1.32 (1.18–1.49)	<0.001	¶	
Stunted (LAZ <-2)	553 (51)	1.08 (0.84–1.38)	0.56	¶	
HIV status					
Negative	959 (88)	Reference		¶	
Exposed	54 (4.9)	1.12 (0.66–1.92)	0.67	¶	
Infected	43 (3.9)	1.75 (1.24–2.46)	0.002	¶	
Untested	36 (3.3)	1.68 (0.77–3.68)	0.19	¶	
Features at admission					
Diarrhoea	550 (50)	1.11 (0.65–1.89)	0.70	¶	
Sepsis†	224 (21)	2.52 (1.70–3.74)	<0.001	2.64 (1.83–3.80)	0.001
Meningitis/encephalopathy†	95 (8.7)	14.1 (5.71–34.7)	<0.001	14.2 (4.75–42.5)	<0.001
Severe pneumonia	317 (29)	1.08 (0.62–1.88)	0.78	1.22 (0.77–1.93)	0.41
Other chronic illness‖	133 (12)	2.07 (1.21–3.54)	0.008	1.97 (1.10–3.51)	0.02
WHO danger signs§	810 (74)	1.30 (0.92–1.84)	0.14	1.22 (0.90–1.66)	0.20
Malaria rapid diagnostic test					
Negative	911 (83)	Reference		¶	
Positive	162 (14)	1.01 (0.83–1.22)	0.94	¶	
Not done	19 (1.7)	1.96 (0.64–5.99)	0.24	¶	
Haemoglobin g/dl					
>110	182 (17)	Reference		Reference	
100–110	205 (19)	0.95 (0.77–1.17)	0.62	0.88 (0.70–1.09)	0.25
70–100	523 (48)	1.13 (0.93–1.37)	0.23	0.98 (0.78–1.22)	0.83
<70	182 (17)	1.11 (0.88–1.41)	0.38	0.91 (0.70–1.19)	0.49
Blood glucose mmol/L					
<3	28 (2.6)	2.40 (1.44–4.01)	0.001	2.17 (1.21–3.88)	0.009
3 to 10	1000 (92)	Reference		Reference	
>10	64 (5.9)	0.86 (0.57–1.30)	0.46	0.70 (0.45–1.09)	0.11
WBC $x10^9/L$					
<5	30 (2.8)	1.86 (0.97–3.56)	0.06	¶	
5-17.5	723 (66)	Reference		¶	
>17.5	339 (31)	1.18 (0.88–1.58)	0.27	¶	
Duration of admission					
<5 days	407 (37)	Reference		Reference	
≥5 days	685 (63)	3.06 (1.82–5.15)	<0.001	3.11 (1.82–5.32)	<0.001

Results presented as frequency (%), or risk ratios. † clinician diagnosis; || Chronic illnesses including thalassemia, cerebral palsy, sickle cell disease, congenital cardiac diseases and known tuberculosis; LAZ: length for age Z score; § presence of any WHO danger signs: obstructed breathing, respiratory distress, cyanosis, shock, severe anaemia, convulsions, severe dehydration, profuse watery diarrhoea, vomiting everything, Impaired consciousness, temperature >38°C in last 24 hrs or <36°C in last 24h; LAZ = length-for-age Z score; ¶ variables not selected for inclusion in multivariable model using backward stepwise selection; NW, no wasting; MW, moderate wasting; SWK, severe wasting or kwashiorkor (severe malnutrition). Risk ratios from multilevel mixed-effects generalized linear model with site as random intercept and sample weights, multivariable Area under the Curve (AUC) 0.78 (95%CI 0.76–0.80)

***Table 4.8**. Factors associated with receiving Watch antimicrobials during admission.*

4.4 Discussion

In this prospective cohort conducted in nine sites across six countries in sub-Saharan Africa and South Asia, almost all children admitted with an acute illness received antimicrobials, most often Access antimicrobials, typically penicillin or ampicillin plus gentamicin. Overall, 35% of children received Watch antimicrobials, two-thirds of which were started within 48 hours of admission. Antimicrobial use patterns were broadly similar, apart from one site.

A previous point prevalence survey in 56 countries observed substantial variation between countries in the paediatric use of Access, Watch, and Reserve antibiotics, with ceftriaxone being the most commonly prescribed Watch antimicrobial to hospitalised children in Africa, the Eastern Mediterranean, Europe, and South-East Asia.[17] The minimal use of Reserve antimicrobials we observed is similar to results from that survey and a wholesale antibiotic sales analysis from 70 middle-income and high-income countries in 2015, where Reserve antimicrobial use was low in all countries[5, 17]. Surprisingly, in these studies, Access antimicrobials were more often given to neonates than older children, despite their higher mortality risk and duration of exposure to the hospital environment in maternity and neonatal wards[18].

Known discrepancies between local and published AMR data and current antibiotic usage recommendations[19, 20] suggest that the coverage of currently recommended Access (most WHO first-line) and some Watch (most WHO second-line) regimens may not be appropriate. Lack of paediatric formulations and sometimes non-availability or high cost (to health services

or families) of carbapenems and Reserve antimicrobials may also limit appropriate use[21]. Using available susceptibility data from LMIC settings to inform empiric prescribing often lacks granularity, for example not distinguishing community from hospital-acquired infections; over-representing cultures that are biased towards treatment failures and critical care admissions; or extrapolating data from cultures taken in one clinical syndrome or age group, such as adult urinary tract infection, to another[1, 22],[23]. In this study, the Karachi site had the highest use of Watch and Reserve antibiotics (typically third-generation cephalosporins and/or glycopeptides) which aligned with national data suggesting high rates of resistance to Access and Watch antibiotics[23]. The use of such "pooled antibiograms", even in high resource settings where microbiological data may be much more complete, can have poor predictive value for individual patient care, and stratification by clinical syndromes and context is likely to be beneficial[24, 25]. In addition, the limited availability of antibiograms and their appropriate utilization remains a challenge in LMICs where prescribing may be more strongly influenced by the individual clinician's choice and patient demand[26].

The usage patterns of antibiotics in the African sites studied may also suggest issues with adherence to current antibiotic recommendations. Two thirds of first-line ceftriaxone use in Africa has been described as inappropriate[27, 28]. While we identified some likely inappropriate antimicrobial use in our study, including in uncomplicated diarrhoea and malaria, we also found limited escalation of antimicrobial therapy among children with severe malnutrition (20% of all admissions in these hospitals)[29] and a lack of association with Watch antimicrobial use of WHO danger signs (e.g. feeding problems, difficult breathing) and

pre-hospital antibiotic exposure (i.e. consistent with treatment failure or risk of antibiotic resistance selection). This is concerning given that these children have exceptionally high mortality risk, longer stays in hospital, and are highly vulnerable to hospital-acquired infections, including Gram-negative bacteraemia with high consequent mortality risk[18, 29, 30]. Current guidelines generally do not provide guidance based on prior exposure or underlying risk, or robust guidance on antimicrobial escalation and de-escalation, and thus may be limiting the appropriate use of Watch and Reserve antimicrobials. Clearer guidance on escalation and de-escalation is essential to ensure both appropriate broad-spectrum and narrow-spectrum antibiotic use. For guidelines revision, clinical trials testing treatment pathways rather than individual drugs head-to-head are likely to be most informative[16, 31].

Strengths of this study are that it represents a wide range of hospital settings in sub-Saharan Africa and South Asia and detailed clinical data and daily antimicrobial use were recorded. Limitations include a lack of detail on the types of pre-hospital antimicrobials taken, some missing data at weekends (<1% of days), a lack of data on reasons for antibiotic switching, and no analysis of microbiological data unavailable in these settings. In the future, better documentation of pre-hospital antimicrobial use, reasons for antibiotic switching, and clinical outcomes will be valuable.

4.5 Conclusion

In conclusion, our findings indicate that, with training, clinicians across a broad range of geographies in Africa and South Asia mostly adhere to WHO guidelines in using Access group

antimicrobials in hospitalised children. Whilst there was some overuse, in general, Watch and Reserve antimicrobials appeared under-used in groups at elevated risk for death and/or resistant infections, such as those with severe malnutrition, chronic illness including HIV, previous antibiotic treatment and/or prior healthcare exposure. In general, there are limited high quality data on relevant bacterial susceptibilities or clinical efficacy to inform guidelines on the empiric treatment of previously antibiotic-treated admissions, non-responders or hospital-acquired infection. Most government-run hospitals in low-resource settings do not have capacity to offer reliable culture and sensitivity testing and even where capacity exists, many inpatient paediatric deaths occur within 48 hours, before microbiological results are available. We argue that in such settings, empiric prescribing should be based on individual risk of mortality and the likelihood of having a resistant infection. For example, severely underweight children with severe pneumonia have a 5-fold increased inpatient mortality compared to non-underweight children[32]. Such an approach would enable reductions in inappropriate exposure to antimicrobials (including broad-spectrum antimicrobials) for lower-risk groups and timely, targeted initiation or escalation to Watch or Reserve antibiotics in higher-risk groups.

REFERENCES

1. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, Han C, Bisignano C, Rao P, Wool E: **Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis**. *The Lancet* 2022, **399**(10325):629-655.
2. Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, Knight GM, Robotham JV: **Estimating the burden of antimicrobial resistance: a systematic literature review**. *Antimicrobial Resistance & Infection Control* 2018, **7**(1):58.
3. Sulis G, Gandra S: **Access to antibiotics: not a problem in some LMICs**. *The Lancet Global Health* 2021, **9**(5):e561-e562.
4. **Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2016-2017** [https://www.who.int/publications/i/item/9789241513449]
5. Hsia Y, Lee BR, Versporten A, Yang Y, Bielicki J, Jackson C, Newland J, Goossens H, Magrini N, Sharland M: **Use of the WHO Access, Watch, and Reserve classification to define patterns of hospital antibiotic use (AWaRe): an analysis of paediatric survey data from 56 countries**. *The Lancet Global Health* 2019, **7**(7):e861-e871.
6. English M, Berkley J, Mwangi I, Mohammed S, Ahmed M, Osier F, Muturi N, Ogutu B, Marsh K, Newton CR: **Hypothetical performance of syndrome-based management of acute paediatric admissions of children aged more than 60 days in a Kenyan district hospital**. *Bull World Health Organ* 2003, **81**(3):166-173.
7. Berkley JA, Maitland K, Mwangi I, Ngetsa C, Mwarumba S, Lowe BS, Newton CR, Marsh K, Scott JA, English M: **Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area: observational study**. *BMJ* 2005, **330**(7498):995.
8. Iskandar K, Molinier L, Hallit S, Sartelli M, Hardcastle TC, Haque M, Lugova H, Dhingra S, Sharma P, Islam S: **Surveillance of antimicrobial resistance in low-and middle-income countries: a scattered picture**. *Antimicrobial Resistance & Infection Control* 2021, **10**(1):1-19.
9. Paterson DL: **Resistance in gram-negative bacteria: Enterobacteriaceae**. *American journal of infection control* 2006, **34**(5):S20-S28.
10. Smith R, Coast J: **The true cost of antimicrobial resistance: Richard Smith and Joanna Coast argue that current estimates of the cost of antibiotic resistance are misleading and may result in inadequate investment in tackling the problem**. *Bmj* 2013, **346**.
11. Abubakar U, Salman M: **Antibiotic Use Among Hospitalized Patients in Africa: A Systematic Review of Point Prevalence Studies**. *Journal of Racial and Ethnic Health Disparities* 2023:1-22.
12. Childhood Acute Illness Nutrition Network: **Childhood Acute Illness and Nutrition (CHAIN) Network: a protocol for a multi-site prospective cohort study to identify modifiable risk factors for mortality among acutely ill children in Africa and Asia**. *BMJ Open* 2019, **9**(5):e028454.

13. Childhood Acute Illness & Nutrition Network: **Childhood mortality during and after acute illness in Africa and south Asia: a prospective cohort study**. *Lancet Glob Health* 2022, **10**(5):e673-e684.
14. Tickell KD, Mangale DI, Tornberg-Belanger SN, Bourdon C, Thitiri J, Timbwa M, Njirammadzi J, Voskuijl W, Chisti MJ, Ahmed T: **A mixed method multi-country assessment of barriers to implementing pediatric inpatient care guidelines**. *PloS one* 2019, **14**(3):e0212395.
15. The World Health Organization: **Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources**. In. Geneva: World Health Organization; 2013.
16. Turner RM, Omar RZ, Yang M, Goldstein H, Thompson SG: **A multilevel model framework for meta-analysis of clinical trials with binary outcomes**. *Statistics in medicine* 2000, **19**(24):3417-3432.
17. Hsia Y, Sharland M, Jackson C, Wong IC, Magrini N, Bielicki JA: **Consumption of oral antibiotic formulations for young children according to the WHO Access, Watch, Reserve (AWaRe) antibiotic groups: an analysis of sales data from 70 middle-income and high-income countries**. *The Lancet Infectious Diseases* 2019, **19**(1):67-75.
18. Aiken AM, Mturi N, Njuguna P, Mohammed S, Berkley JA, Mwangi I, Mwarumba S, Kitsao BS, Lowe BS, Morpeth SC: **Risk and causes of paediatric hospital-acquired bacteraemia in Kilifi District Hospital, Kenya: a prospective cohort study**. *The Lancet* 2011, **378**(9808):2021-2027.
19. Balachandra SS, Sawant PS, Huilgol PG, Vithya T, Kumar G, Prasad R: **Antimicrobial resistance (AMR) at the community level: An urban and rural case study from Karnataka**. *Journal of Family Medicine and Primary Care* 2021, **10**(3):1404.
20. Woerther P-L, Burdet C, Chachaty E, Andremont A: **Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M**. *Clinical microbiology reviews* 2013, **26**(4):744-758.
21. Frost I, Craig J, Joshi J, Faure K, Laxminarayan R: **Access barriers to antibiotics**. *Center for Disease Dynamics, Economics & Policy* 2019.
22. Williams PC, Isaacs D, Berkley JA: **Antimicrobial resistance among children in sub-Saharan Africa**. *The Lancet Infectious Diseases* 2018, **18**(2):e33-e44.
23. Bilal H, Khan MN, Rehman T, Hameed MF, Yang X: **Antibiotic resistance in Pakistan: a systematic review of past decade**. *BMC infectious diseases* 2021, **21**(1):1-19.
24. MacDougall C: **A Cloudy Crystal Ball: Critically Assessing and Rethinking the Antibiogram**. *Clin Infect Dis* 2023, **77**(11):1501-1503.
25. Hasegawa S, Livorsi DJ, Perencevich EN, Church JN, Goto M: **Diagnostic Accuracy of Hospital Antibiograms in Predicting the Risk of Antimicrobial Resistance in Enterobacteriaceae Isolates: A Nationwide Multicenter Evaluation at the Veterans Health Administration**. *Clin Infect Dis* 2023, **77**(11):1492-1500.
26. Saleem Z, Saeed H, Hassali MA, Godman B, Asif U, Yousaf M, Ahmed Z, Riaz H, Raza SA: **Pattern of inappropriate antibiotic use among hospitalized patients in**

Pakistan: a longitudinal surveillance and implications. *Antimicrobial Resistance & Infection Control* 2019, **8**(1):1-7.
27. Meresa Bishaw B, Tegegne GT, Berha AB: **Appropriate Use of Ceftriaxone in Sub-Saharan Africa: A Systematic Review**. *Infect Drug Resist* 2021, **14**:3477-3484.
28. Otuko R, Maina M, Mwaniki P, Mweu M, Akech S: **Patterns and determinants of antibiotic prescription among children admitted to public hospitals in Kenya: A cross-sectional observational study**. *Wellcome Open Research* 2023, **8**(454):454.
29. Woerther P-L, Angebault C, Jacquier H, Hugede H-C, Janssens A-C, Sayadi S, El Mniai A, Armand-Lefevre L, Ruppé E, Barbier F: **Massive increase, spread, and exchange of extended spectrum β-lactamase–encoding genes among intestinal Enterobacteriaceae in hospitalized children with severe acute malnutrition in Niger**. *Clinical infectious diseases* 2011, **53**(7):677-685.
30. Williams PC, Berkley JA: **Guidelines for the treatment of severe acute malnutrition: a systematic review of the evidence for antimicrobial therapy**. *Paediatrics and international child health* 2018, **38**(sup1):S32-S49.
31. Williams PCM, Isaacs D, Berkley JA: **Antimicrobial resistance among children in sub-Saharan Africa**. *Lancet Infect Dis* 2018, **18**(2):e33-e44.
32. Kirolos A, Blacow RM, Parajuli A, Welton NJ, Khanna A, Allen SJ, McAllister DA, Campbell H, Nair H: **The impact of childhood malnutrition on mortality from pneumonia: a systematic review and network meta-analysis**. *BMJ Glob Health* 2021, **6**(11).

5 DYNAMICS OF GASTROINTESTINAL ENTEROBACTERALES PHENOTYPIC ESBL-E/CPE ACQUISITION AND CARRIAGE AMONGST HOSPITALISED AND WELL COMMUNITY KENYAN CHILDREN

5.1 Introduction

Antimicrobial resistance (AMR) represents a critical and escalating global health challenge, particularly in resource-constrained settings such as sub-Saharan Africa (sSA). Among the most concerning pathogens associated with AMR are extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) and carbapenemase-producing Enterobacterales (CPE)[1]. These mechanisms confer resistance to frontline antibiotics, including third-generation cephalosporins, and often render infections difficult or impossible to treat in low-resource settings. Gut mucosal colonisation with ESBL-E, a precursor to invasive infections and a reservoir for transmission, represents an important target for intervention[2]. However, the epidemiology and dynamics of ESBL-E colonisation in sSA remain poorly characterized, particularly in paediatric populations[2].

A systematic review and meta-analysis by Lewis et al., 2019 examined ESBL-E colonisation across 32 studies involving 8,619 participants in sSA, revealing substantial variation in prevalence across settings[2]. Community-based studies had a pooled ESBL-E colonisation prevalence of 18% (95% CI 12–28%), which increased to 32% (95% CI 24–41%) at hospital admission and peaked at 55% (95% CI 49–60%) among hospitalised inpatients[2]. Prevalence ranged widely, from 5% to 84%, with a median prevalence of 31%, underscoring the heterogeneity of the ESBL-E burden in the region[2], depending on the context and population[3]. These findings highlight the heightened risk of colonisation in healthcare settings and the critical role of hospitals as amplifiers of AMR transmission.

Risk factors for ESBL-E colonisation identified in this review included prior antimicrobial use, increasing age, hospitalisation, and HIV positivity. These risk factors were observed in both adult and paediatric populations[2]. Protective factors, such as access to treated water or water that was boiled before use, were also identified. Despite the substantial cross-sectional data available, the review emphasized significant gaps in understanding the longitudinal dynamics of ESBL-E acquisition, particularly following hospital discharge, and how colonisation patterns evolve in community settings.

Several studies provide further insights into these dynamics. In Malawi, Lewis *et al*., 2022, demonstrated that the baseline prevalence (day 0 of admission) was 42% (178/420). Following enrolment, there was rapid increase in ESBL-E colonisation prevalence among inpatients exposed to antimicrobials (109/222 (49%) at day 0 to 127/162 (78%) at day 7) compared to those that did not receive antimicrobials (41/99 (41%) at day 0 to 32/62 (51%) at day 7)[4]. A study done among adult patients hospitalised at the orthopaedic department of tertiary hospital in Uganda reported that 61% (105/172) of hospitalised patients carried ESBL-E during their hospital stay, with 49% (26/53) becoming colonised during hospitalisation[5]. Another study done in Tanzania reported an overall ESBL-E carriage prevalence of 34.3% (207/603), with hospitalised children having higher prevalence of 50.4%, compared to community children at 11.6%; $P < 0.001$; OR = 7.75; 95% CI: 4.99–12.03). ESBL prevalence was significantly higher among HIV positive children, with use of antibiotics during the past 14 days and age below 1 year identified as significant predictors ESBL carriage[6]. These findings emphasize the complex interplay between hospital and community transmission and the persistence of colonisation, which could sustain the dissemination of AMR in sSA.

Hospitalised children are particularly vulnerable to hospital-acquired infections due to factors like frequent antibiotic use, suboptimal IPC measures, and prolonged exposure to contaminated hospital environments promote colonisation[7]. Similarly, community-associated ESBL-E carriage is influenced by shared antimicrobial exposures, inadequate sanitation, and crowded living conditions, further underscoring the interconnectedness of hospital and community transmission pathways.

In Kenya, few studies have been conducted examining the longitudinal dynamics of ESBL-E and CPE acquisition and persistence among hospitalised children, during follow up and in the community. However, the dynamics of post-discharge carriage, community transmission, and associated risk factors remain poorly understood, limiting the effectiveness of targeted interventions such as IPC strategies and antimicrobial stewardship programs.

In this study, I aimed to characterise the prevalence of, and risk factors associated with ESBL-E and CPE colonisation in Kenyan children aged 2 to 23 months at admission to hospital and healthy children of the same age in the same communities. I examined the dynamics and risk factors associated with ESBL-E and CPE acquisition and loss during hospital admission and for 180 days post-discharge.

5.2 Materials and methods

5.2.1 Study setting and design

This is an analysis of children admitted to the three Kenyan sites (Kilifi County Hospital; Mbagathi Sub-County Hospital, Nairobi; Migori County Hospital) in the CHAIN cohort study (Figure 5.1) and a parallel community cross-sectional study at between 20th

November 2016 and 31st January 2019. CHAIN was a stratified prospective cohort study of acutely ill children in vulnerable populations reflecting different socio-demographic catchments, degrees of healthcare access, and levels of background comorbidities such as malaria and HIV.

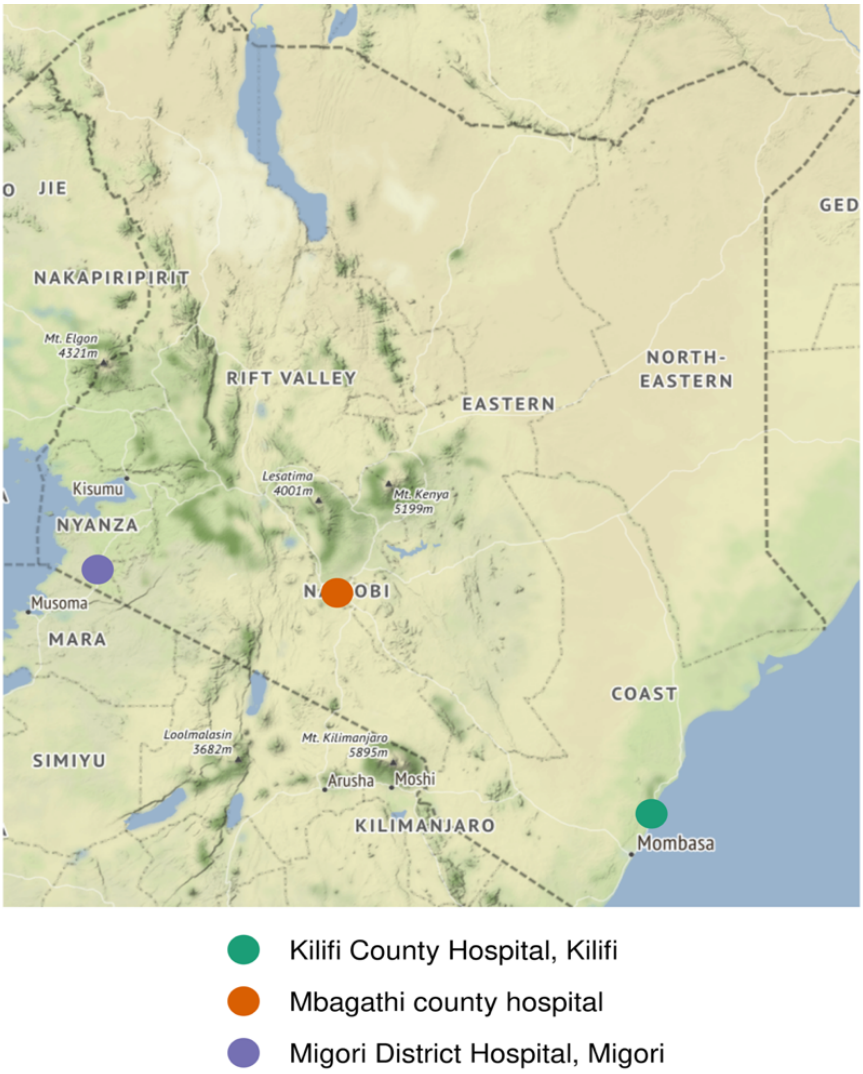


Figure 5.1: *Kenyan sites involved in the study.*

Children were screened for eligibility at admission for non-surgical, non-traumatic conditions and included or excluded as described in the CHAIN cohort study[8]. Participants were identified by choosing the first admissions from a specified day each week until the weekly quota for each stratum was met. The cohort was stratified by anthropometry at hospital admission, using mid-upper arm circumference (MUAC), into

three strata reflecting nutritional status, classified as severe wasting/kwashiorkor (SWK, moderate wasting (MW) and not wasted (NW) in a 2:1:2 ratio. This was in order to achieve a spectrum of mortality risk in the cohort. Enrolled children were under observation during their index hospital admission with a variable duration of hospital stay and were then followed up for a fixed duration of 180 days post-discharge (followed up at 45, 90 and 180 days after discharge) [11]. Participants were treated according to current national and international clinical guidelines (WHO).

Community participants were identified from the same communities as hospitalised participants representing the normal children from that community. Community participants were recruited if they were aged 7 days to 23 months and living in the same community as the acutely ill children recruited, did not have an acute illness requiring hospital admission within the previous 2 weeks, had an absence of known, untreated HIV or TB, had not been previously included in the CHAIN cohort, and if a parent or guardian consented on child's behalf. They were then invited to the study clinic for the same assessment, anthropometry, and sample collection as hospitalised children in the cohort. Each site aimed to recruit 130 community participants evenly across the period of the study.

5.2.2 Data and sample collection

Data collected included detailed demographic, clinical (clinical history and features, diagnoses, progress, and treatment received) and household sanitation and other socioeconomic data using structured case report forms (CRFs) at admission and discharge, whilst clinical danger signs and antimicrobials received were collected daily during hospitalisation[9, 10]. Rectal swabs were taken at five time points (admission, discharge,

day 45 post-enrolment, day 90 post-enrolment and day 180 post-enrolment) and, if relevant, on re-admission. The community- participants were swabbed once only on enrolment.

5.2.3 Laboratory procedures

Rectal swabs were cultured overnight at 37^{o}C in three agar plates: MacConkey agar, Gent-MacConkey agar supplemented with 8% gentamicin, and blood agar plates with cefotaxime (30µg) and ceftazidime (30µg) antibiotic disks (Oxoid, United Kingdom). All potential ESBL-E colonies were picked from Gent-MacConkey agar plate (selective media for gentamicin resistant colonies) and blood agar plate with cefotaxime and ceftazidime for identification. Three colonies of different morphologies were also picked from MacConkey agar and identified as such using BioMérieux's Analytical Profile Index (API) 20E strips for Migori and Mbagathi and a MALDI-ToF (MALDI BioTyper) in Kilifi. Antimicrobial susceptibility testing was performed using the Kirby Bauer disc diffusion method[11] and interpreted according to the Clinical and Laboratory Standards Institute 2016 guidelines[12]. ESBL production testing was inferred when the zone of inhibition around the ceftazidime-clavulanate or cefotaxime-clavulanate discs was expanded by >5mm compared to the respective ceftazidime or cefotaxime discs alone. Carbapenem-resistant Enterobacterales were determined using disk diffusion method, where a bacterial isolate was considered resistant if it showed a zone of inhibition around the imipenem and meropenem antibiotic discs that falls below the standard susceptible breakpoint. External quality assurance was done on a quarterly basis under the UK National External Quality Assessment Service scheme (UK NEQAS).

5.2.4 Study size

All the available data for 1211 children recruited into the CHAIN cohort were used and those who had a rectal swab collected at admission (802) inpatient and in the community

(405) at the three Kenyan sites was included in the analysis. Using an estimated ESBL-E carriage prevalence of 10% at admission and 55% at discharge[13], the overall number of recruited participants (N=802) was adequate to detect a relative risk ratio for ESBL-E carriage of at least 2.0 between participants admission and discharge (>90% power, two-tailed alpha=0.05).

5.2.5 Data analysis

Analyses were conducted as pre-specified in a statistical analysis plan accounting for the stratified design, specifying handling of missing data and imputation methods following STROBE guidelines. The proportion of children carrying ESBL-E/CRE at each time point, acquiring ESBL-E or CRE between admission and discharge, and retaining or losing their ESBL-E or CRE carriage status between discharge and 180 post-discharge was determined and compared using Pearson's Chi-squared test.

Putative demographic and clinical (including antimicrobial use, assessing WHO AWaRE antibiotic classifications) risk factors for ESBL/CRE carriage at admission, discharge and persistence after discharge, risk factors for ESBL/CRE carriage in community children, and association of ESBL-E/CRE acquisition with mortality within 180 days were initially tested using univariate logistic regression. Independent risk factors for ESBL-E/CRE carriage and mortality within 180 days were then assessed using multivariable, stepwise, binomial logistic regression models based on complete cases and initially including all factors significant in the univariable analyses with backwards elimination using exit $p< 0.1$ to reduce over-fitting.

To examine the post-discharge outcomes associated with ESBL-E carriage time to first hospital readmission and post-discharge death was evaluated using Cox proportional

regression models. The proportional hazard assumption was tested using Schoenfeld residuals test (there was no evidence of violation). All the regression models included recruitment site as a random intercept to address unmeasured differences and inverse sampling weights to account for the stratified cohort nutritional status profile that deliberately differed from that of all ward admissions[14]. Model performance was compared using area under the receiver operating characteristic curves (AUROCs). Statistical analyses were conducted using STATA v17 (StataCorp, College Station, TX, USA) and R version 3.6.2 (R Foundation for Statistical Computing).

5.2.6 Ethical considerations

Ethical approval was obtained from the Oxford University Tropical Research Ethical Committee and the Kenya Medical Research Institute (KEMRI) Scientific Ethical and Research Unit (SERU no. 3318). Written informed consent was obtained from parents or guardians for all enrolled children.

5.3 Results

5.3.1 Participants

In total, 1211 children were enrolled (median age: 11 [IQR 6.6−17] months), namely 804 (66%) at the time of admission to hospital (inpatients) and 407 (34%) well community participants (Figure 5.2). Of the 1211 children, 403 were enrolled in Kilifi County hospital, 405 at the Mbagathi sub-county hospital and 403 from Migori county hospital. Amongst inpatients, 311 (39%) had no wasting, 184 (23%) had moderate wasting, and 309 (38%) had severe wasting or kwashiorkor (Table 5.1). 674/1211 (56%) children were male, 349/804 (43%) and 252/804 (31%) of the children were admitted with to hospital with diarrhoea and severe pneumonia, respectively (Table 5.1a).

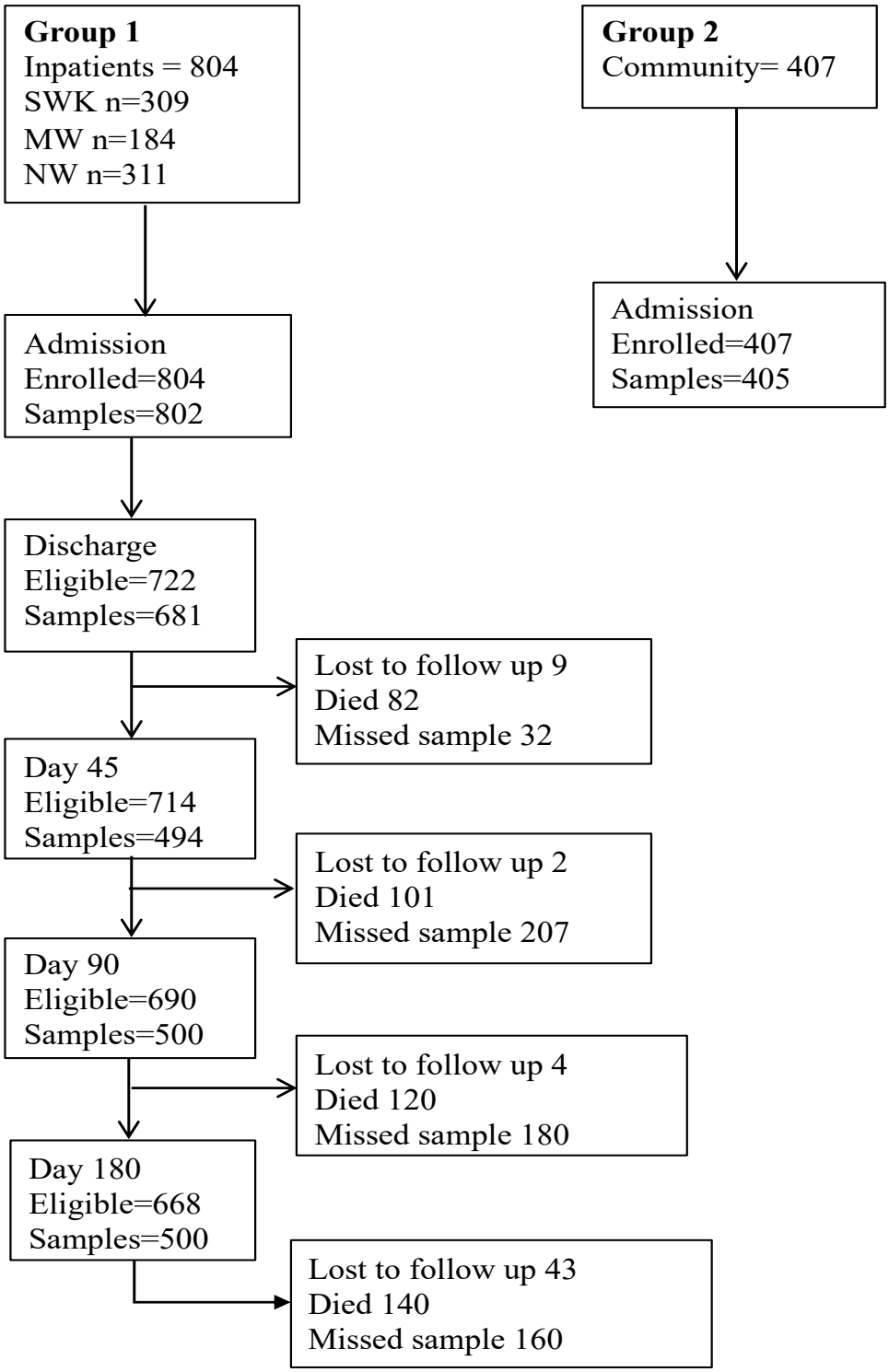


***Figure 5.2**: Participants enrolled where group 1 were inpatients stratified by nutritional status (severe wasting/kwashiorkor (SWK), moderate wasting (MW) and not wasted (NW)), while group 2 were well community participants aged 7 days to 23 months recruited if they were living in the same community as the acutely ill children recruited.*

	Strata (hospitalised children)			All participants	
	NW (N=310)	MW (N=184)	SWK (N=308)	Hospitalised (N=804)	Community (N=405)
Demographics					
Age — months median (IQR)	11.2 (6.5-17)	9.7 (6.0-15)	11.1 (6.6-17)	10.7 (6.5-16)	11.8 (8.0-17)
Sex (female) — no. (%)	113 (36)	85 (46)	157 (51)	355 (44)	182 (45)
Clinical signs at admission					
Signs of shock[†]					
None	154 (50)	88 (48)	133 (43)	375 (47)	298 (73)
Some signs	157 (50)	96 (52)	176 (57)	429 (53)	109 (27)
SIRS — no. (%)[‡]	125 (40)	91(49)	137 (44)	353 (44)	-
Conscious level (AVPU >A) — no. (%)	21 (7)	12 (7)	19 (6)	52 (6)	0
Severe Pneumonia — no. (%)[¶]	102 (33)	77 (42)	73 (24)	252 (31)	0
Diarrhoea — no. (%)	111(37)	86 (48)	152 (49)	349 (43)	0
Malaria (RDT positive) — no. (%)	63 (20)	27(15)	24 (8)	114 (14)	17 (4.2)
Anaemia — no. (%)[+]					
None	38(12)	12(7)	31(10)	81(10)	83 (20)
Mild	58(19)	38(21)	50(16)	146(18)	129 (32)
Moderate	174(56)	106(58)	178(58)	458(57)	189 (46)
Severe	41(13)	28(15)	50(16)	119(15)	6 (1.5)
Anthropometry at admission					
Nutritional oedema— no. (%)	0(0)	0(0)	90(29)	90(11)	0
MUAC (cm) — mean (sd)	13.8 (1.1)	11.9 (0.4)	10.5 (1.4)	12.1 (1.9)	13.9 (1.2)
Stunting — no. (%)§					
None	242(78)	123(67)	86(28)	451(56)	300 (74)
Moderate	53(17)	41(22)	88(28)	182(23)	71 (17)
Severe	16(5.1)	20(11)	135(44)	171(21)	36 (8.9)
Underlying conditions					
HIV status — no. (%)					
Negative	263(85)	160(87)	288(74)	651(81)	338 (83)
HIV infected	3(0.9)	4(2.1)	39(12)	56(5.7)	8 (2.0)
HIV exposed	24(8)	15(8)	34(11)	73(9)	41 (10)
Refused/untested	21(7)	5(3)	8(3)	34(4)	20 (4.9)
Small birth size — no. (%)[#]					
Normal	283(91)	162(88)	259(84)	704(88)	360 (89)
Preterm/low birth weight	28(9)	22(12)	50(16)	100(12)	44 (11)
Other chronic conditions — no. (%)[+++]	14(4.5)	13(7.1)	18(5.8)	45(5.6)	4 (1.0)
Prior hospitalisation — no. (%)					
No prior admission	259(83)	153(83)	238(77)	650(80)	382 (94)
< 1 week	8(2.5)	5(2.7)	7(2.3)	20(2.5)	3 (0.7)
1 week to 1 month ago	10(3.2)	8(4.4)	23(7.4)	41(5.1)	22 (5.4)
>1 month ago	34(10.9)	18(9.8)	41(13)	93(12)	0
Caregiver characteristics					
Biological mother is caregiver — no. (%)	298(96)	173(94)	287(93)	758(94)	383 (95)
Mother currently sick — no. (%)	13 (4.2)	6(3.3)	16(5.2)	35(4.4)	10 (2.5)
Caregiver education — no. (%)					
None	25(8.0)	13(7.1)	26(8.4)	64(8.0)	38 (9.5)
Primary	179(58)	100(54)	184(60)	463(58)	241 (60)
Secondary/Tertiary	85(27.3)	55(29.9)	86(27.8)	226(28.1)	122 (30)
Maternal mental health (PHQ9) score — no. (%)					

None to mild	254(81.7)	123(66.9)	188(60.8)	565(70.3)	337 (83)
Moderate to severe	57(18.3)	61(33.2)	121(39.2)	239(29.7)	69 (17)
Mother working — no. (%)					
Self-employed	119(38.3)	6133.2)	137(44.3)	317(39)	187 (47)
Employed	34(10.9)	18(9.8)	36(12)	88(11)	33 (8.2)
No reported income	158(51)	105(57)	136(44)	399(50)	181 (45)
Household-level exposures					
*Population density# (*1000 per km^2) median (IQR)*	0.69 (0.2- 7.5)	2.11 (0.3-18)	0.91 (0.2-17)	0.93 (0.2-13)	0.58 (0.2-9.3)
Assets index — no. (%)					
Quintile 1 (Least assets)	107 (34)	55 (30)	119 (39)	281 (35)	158 (39)
Quintile 2	64(21)	21(11)	46 (15)	131 (16)	69 (17)
Quintile 3	52(17)	38 (21)	61 (20)	151 (19)	70 (17)
Quintile 4	50(16)	38 (21)	50 (16)	138 (17)	65 (16)
Quintile 5 (Most assets)	38(12)	32 (17)	33(11)	103 (13)	45 (11)
Improved toilet — no. (%)	201(65)	119(65)	172(56)	492(61)	¶
Improved water source — no. (%)	224(72.0)	116(63.0)	187(60.5)	527(66)	¶

IQR; Interquartile range, † Signs of shock: capillary refill time >3 seconds, upper limb temperature gradient, weak pulse. ‡ SIRS-Systemic Inflammatory Response Syndrome: presence of two of the following four criteria; heart rate low (<90) or high (>180)/min; temperature low (<36°C) or high (≥38.5°C); respiratory rate high (>34 breaths per minute) and WBC low (<5 x10^9/l) or high (>17.5 x10^9/l); AVPU; Alert, Voice, pain and Unresponsive. ¶ Severe pneumonia: Cough or difficulty in breathing with oxygen saturation <90% or central cyanosis, or grunting, very severe chest indrawing, or inability to breastfeed or drink, lethargy or reduced level of consciousness, convulsions. RDT; rapid diagnostic test, MUAC; mid-upper arm circumference. + Anaemia by haemoglobin defined as None: >11, Mild:10 to11, Moderate: 7 to 10 and Severe: <7.0 g/dl, ++ Abnormal blood glucose defined as blood glucose <3 or 10mmol/l, * children with oedema excluded from these values, § Stunting: none, length-for-age z score ≥-2; moderate, length-for-age z score –2 to -3; severe, length-for-age z score <-3. # small birth size was defined as reported low birth weight (<2.5kg) or born premature, +++ Chronic conditions include thalassemia, cerebral palsy, sickle cell disease, congenital cardiac disease and known TB. Population density# is the population in the community they live in n methods, say this was assigned by the CHAIN team using remote sensing data.

Table 5.1a: *Baseline characteristics for all participants with rectal swabs*

5.3.2 Bacterial species identified in all cultured rectal swabs

The most predominant bacterial species identified were *E. coli*, *K. pneumonia* and *Enterobacter* spp., at all-time points as shown in Table 5.1b.

Species name	Admission	Discharge	Day 45	Day 90	Day 180	Community	Readmission
Escherichia coli	622	478	398	422	444	335	63
Klebsiella pneumoniae	74	140	45	35	20	11	20
Citrobacter freundii		3					
Citrobacter species	4	3	5	6	5	3	
Enterobacter cloacae	7	9	2	2	2	2	
Enterobacter aerogenes		1					
Enterobacter species	7	2	6	8	6	15	
Klebsiella oxytoca	5	6	1	1			
Klebsiella species	1						
Kluyvera species	3		2			2	
Morganella morganii	1		1	1			
Pantoea species	1	1	1		1		
Raoultella ornithinolytica	6	6	2	2	1		
Salmonella species	2	1	3	1	1	7	1
Salmonella typhi	1	1	2				
Shigella flexneri	3						
No significant growth	65	30	26	22	20	29	2
Total	802	681	494	500	500	404	86

Table 5.1b: Enterobacterales species identified in all rectal swabs and either confirmed as ESBL-producers or carbapenem-resistant.

5.3.3 ESBL-E and CPE faecal carriage prevalence

ESBL-E carriage prevalence in the community group was 65/404 (16%, 95%CI: 13-20%). Of the 802 participants sampled at admission to hospital, 291/802 (36%, 95%CI: 33-40%) carried ESBL-E. Among 681 children alive and sampled at discharge, 469 (69%, 95%CI: 67-74%) carried ESBL-E (Figure 5.3a & 5.3c). At days 45, 90 and 180 post-discharge, 199/455 (42%, 95%CI: 39-48%) 152/457 (32%, 95%CI: 29-38%) and 120/452 (25%, 95%CI: 23-31%) sampled children carried ESBL-E (Figure 5.3a & 5.3c).

Carriage of ESBL-E across nutritional groups at admission was similar. However, at discharge and readmission, SWK children had significantly higher proportions of ESBL-E carriage (80% and 79% respectively) compared to normally wasted children (Figure 5.3b). ESBL-E carriage varied across the sites with Nairobi having the highest prevalence across all time points (Figure 5.3b).

Carriage of CPE was uncommon with 4/802 (0.5%) participants being positive at admission, rising to 23/681 (3.4%) at discharge, 3/494 (0.6%) at day 45, 5/500 (1.0%) at day 90 and 1/500 (0.2%) at day 180. The Nairobi site had the highest participants carrying CPE at any time point, with a total of 26, followed by Kilifi (9) and Migori (5) (Figure 5.3d). Among children who were readmitted, after a median time of 42 days, 4/86 (4.7%) carried CPE.

Figure 5.3a: Overall ESBL-E carriage prevalence at admission (adm), discharge(disch), day 45, day 90, day 180, readmission (readm) and community participants.

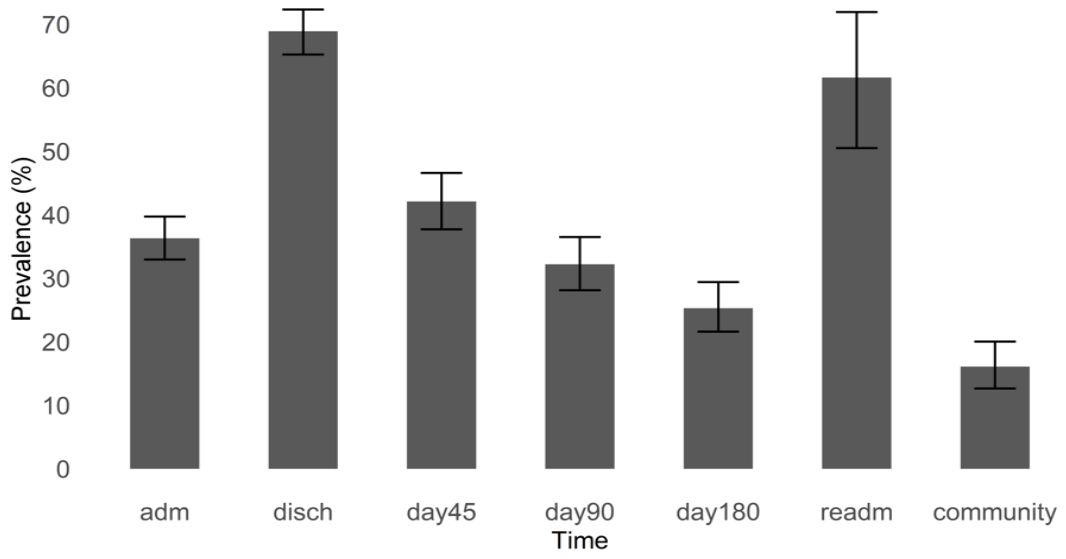


Figure 5.3b: *ESBL-E carriage prevalence by nutritional strata at admission (adm), discharge(disch), day 45, day 90, day 180, readmission (readm) and community participants*

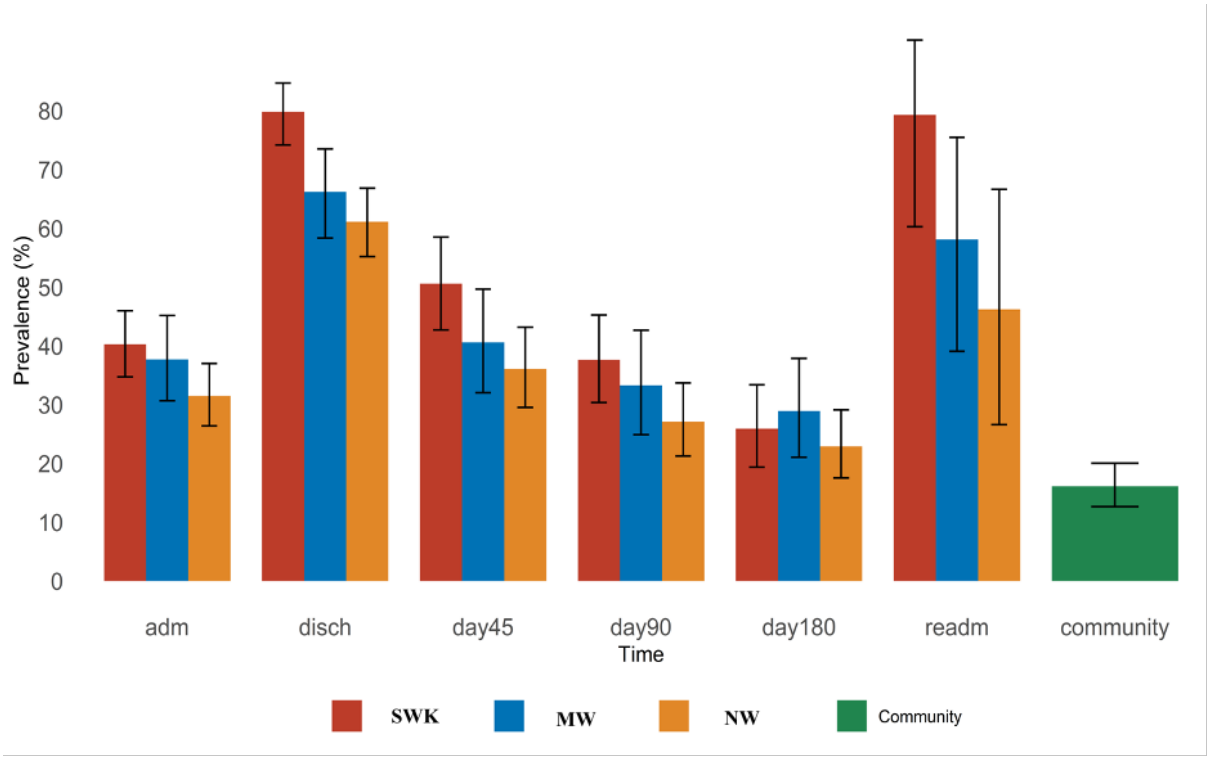


Figure 5.3c: *ESBL-E carriage prevalence by site at admission (adm), discharge(disch), day 45, day 90, day 180, readmission (readm) and community participants*

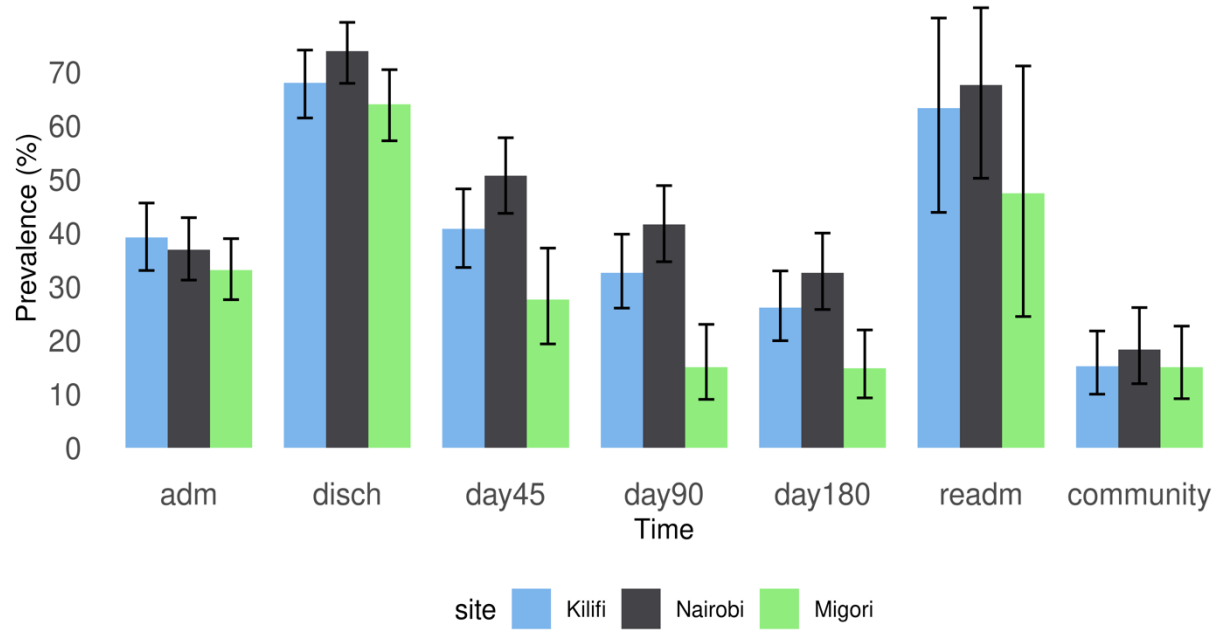
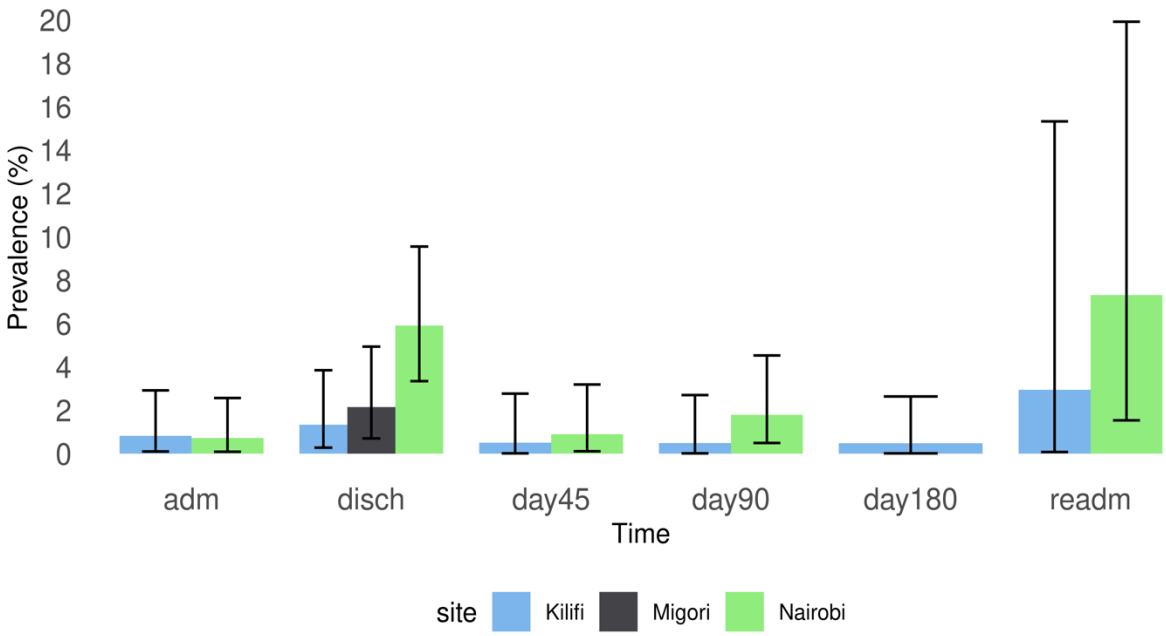


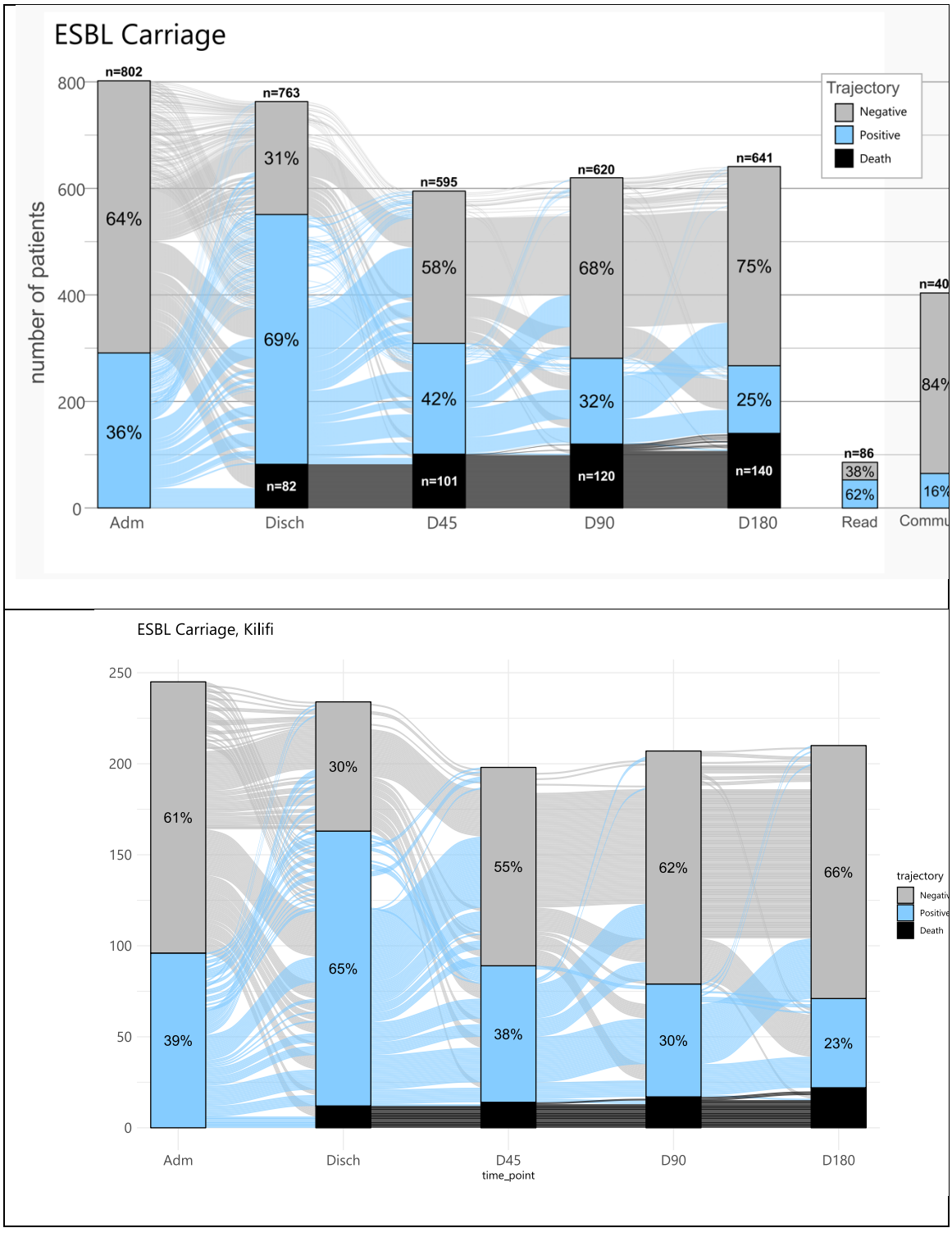
Figure 5.3d: *Prevalence of CRE among participants at admission (adm), discharge(disch), day 45, day 90, day 180 and readmission (readm)*



5.3.4 Longitudinal acquisition and loss of ESBL-E carriage

There was both acquisition and loss during admission to discharge from hospital and follow-up to day 180 post discharge (Figures 5.4a and 5.4b). There was a steady decline or loss of ESBL-E carriage after discharge from hospital with those alive at day 180 at 25% to almost community level at 16% (Figure 5.4a). Site variability in carriage was seen with Migori having an apparently more rapid loss of ESBL-E carriage at day 45 compared to other sites and carriage goes back to community level by day 180 (Figure 5.4a). Out of 271 (100%) participants who were ESBL-E negative at admission and acquired carriage in hospital at discharge, 72/198 (38%) remained positive at day 45, 62/198 (31%) at day 90 and 52/189 (28%) at day 180. 37/114 (32%) of those who were negative at discharge acquired in the community and with 27/126 (21%) retaining acquired carriage up day 180. Thirty-four (100%) participants who were positive at admission and were sampled at discharge, lost carriage at discharge, however 14/34 (41%) acquired at day 45, out of which 10/34 (29%) retained the carriage to day 180. 197 (100%) who were positive at both admission and discharge had 67/151 (44%) losing carriage by day 45 and 103/139 (74 %)

by day 180. Nine-point two percent (47/511) remained negative and 5% (14/291) retained positive to day 180 post discharge from hospital.



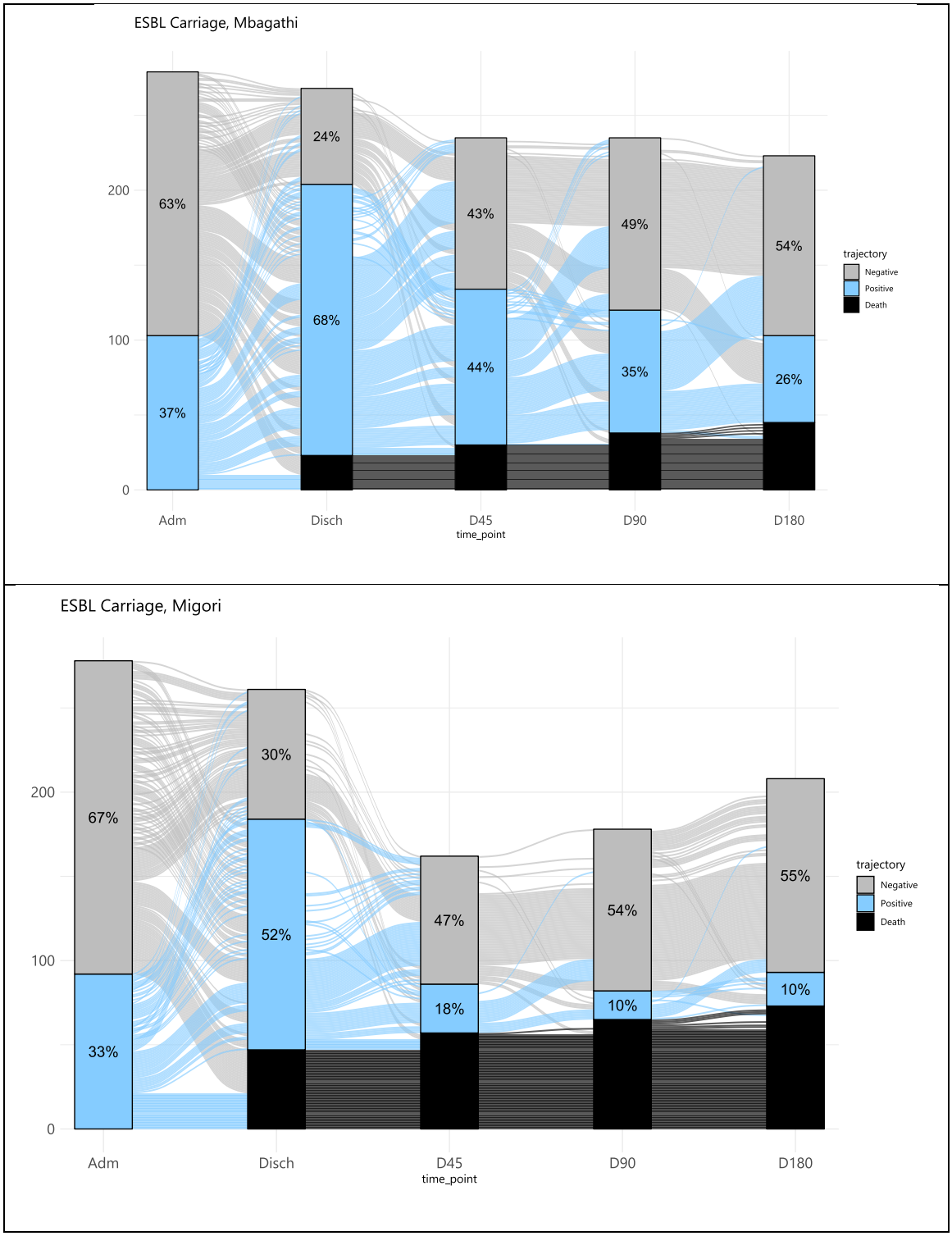


Figure 5.4a: *Alluvial plots detailing the longitudinal acquisition and loss of ESBL-E carriage in children during admission to hospital, discharge and up to day 180 post discharge from hospital combined for all children and split by site (Kilifi, Mbagathi, Migori). Each alluvial line depicts the progression of carriage for each child through admission to discharge and during follow-up colour coded as per legend. Y-axis presents stacked child counts. Readmissions and community participants captured separately. Read = readmission.*

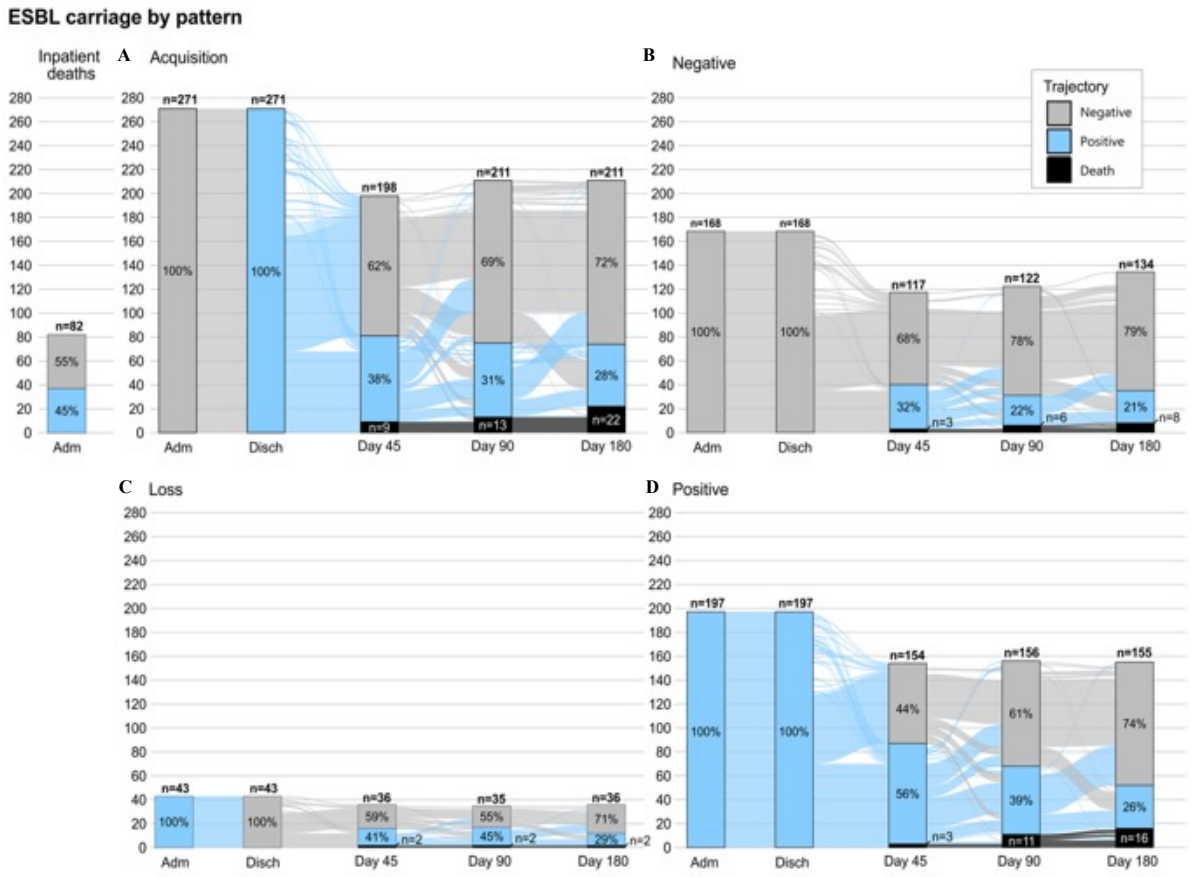


Figure 5.4b: Alluvial plots detailing the longitudinal acquisition and loss of ESBL-E carriage in children during admission to hospital, discharge and up to day 180 post discharge from hospital for those that come in negative and acquire carriage at discharge and those that come in positive and lose or retain carriage*. Panel A shows those who were ESBL-E negative at admission and acquire in hospital (n=271), panel B shows those who were ESBL-E negative at admission and discharge (n=168), but acquire in the community during follow-up, panel C shows those who were ESBL-E positive at admission (n=43) and negative at discharge with some reacquisition in the community, and panel D shows those positive at admission and discharge(n=197) but steady decline seen post discharge from hospital. Each alluvial line depicts the progression of carriage for each child through admission to discharge and during follow-up accounting for each child's events including missed samples and death, colour coded as per legend. Y-axis presents stacked child counts.*

5.3.5 Factors associated with ESBL-E carriage at different time points

Independent factors associated with ESBL-E carriage at the different timepoint (baseline colonisation, acquisition in hospital, positivity at day 180 and carriage in the community) included: age in months, gender, previous hospital admission, readmission, prior use of antibiotics (within 7 days before admission), nutritional status, clinical diagnosis at

admission, environmental/household exposures, caregiver exposures and type of antibiotics used during hospitalisation (Table 5.1).

5.3.5.1 Factors associated with ESBL-E carriage at admission

In the multivariable analysis (presenting Adjusted Risk Ratios (aRR)), independent factors positively associated with ESBL-E carriage at index admission to hospital were: previous hospitalisation within one month (aRR 2.00 [95% CI: 1.54–2.60], *P*<0.001), prior use of any antibiotics within 7 days of admission (aRR 1.20 [95% CI: 1.07–1.34], *P*=0.002), clinical diagnosis of severe pneumonia (aRR 1.11 [95% CI: 1.03–1.20], *P*=0.005), and having an unknown HIV status (aRR 1.67 [95% CI: 1.37–2.04], *P*<0.001). Clinical diagnosis of diarrhoea, livestock ownership and residence in household with fewer assets were negatively associated with ESBL-E carriage. The model bootstrapped AUC (95%CI) was 0.62 (0.58–0.66) (Table 5.2a).

Exposure	***Univariate analysis***		***Multivariable analysis***	
	Crude Risk Ratio(cRR)	**P-value**	**Adjusted Risk Ratio (aRR)**	**P-value**
Age in months	0.99 (0.97-1.01)	0.32	0.99 (0.97-1.02)	0.66
Gender (female)	1.10 (0.97–1.25)	0.14	1.08 (0.89-1.30)	0.43
Previous hospital admission				
No	Reference		Reference	
< 1 month	2.09 (1.67–2.62)	<0.001	2.00 (1.54–2.60)	<0.001
≥ 1 month	1.02 (0.75–1.38)	0.91	0.94 (0.69–1.27)	0.68
Prior use of antibiotics	1.29 (1.15–1.45)	<0.001	1.20 (1.07–1.34)	0.002
Nutritional status				
NW	Reference		¶	
MW	1.20 (0.91–1.58)	0.20	¶	
SWK	1.28 (0.77–2.13)	0.35	¶	
Clinical diagnosis				
Severe pneumonia	1.20 (1.06-1.36)	0.003	1.11 (1.03-1.20)	0.005
Diarrhoea	0.81 (0.64–1.03)	0.08	0.80 (0.65–0.99)	0.04
Systemic inflammatory	0.96 (0.81–1.15)	0.68	¶	
Malaria (RDT positive)	0.79 (0.56–1.12)	0.18	¶	
HIV status				
HIV negative	Reference		Reference	
HIV infected/exposed	0.99 (0.86–1.13)	0.86	1.10 (0.98–1.24)	0.12

HIV unknown	1.40 (1.10–1.77)	0.006	1.67 (1.37–2.04)	<0.001
Other Chronic	1.01 (0.65–1.57)	0.96		
Environmental/household				
Type of toilet				
Not improved	0.84 (0.71–0.98)	0.03	¶	
Improved	Reference		¶	
Household water/quality of				
Not improved	0.85 (0.59–1.21)	0.37	¶	
Improved	Reference		¶	
Livestock ownership	1.01 (0.88–1.16)	0.87	0.84 (0.78–0.91)	<0.001
*Population density (log) (*1000 per km^2) #*	1.03 (1.01–1.07)	0.03	¶	
Asset quantiles				
Quintile 1 (Least assets)	0.69 (0.58–0.81)	<0.001	0.63 (0.59-0.68)	<0.001
Quintile 2	0.84 (0.73–0.97)	0.02	0.59 (0.33-1.03)	0.07
Quintile 3	0.58 (0.37–0.91)	0.02	0.93 (0.80-1.07)	0.29
Quintile 4	0.67 (0.66–0.68)	<0.001	0.72 (0.61-0.85)	<0.001
Quintile 5 (Most assets)	Reference		Reference	
Multivariable model				
Bootstrapped AUC (95%CI)			0.62 (0.58–0.66)	

¶; variables not included in multivariable model, RR; Risk ratios from log binomial regression models. Source: *Environmental/household exposures; Source: WHO/UNICEF sanitation classification, 2005; www.wssinfo.org. Population density# is the population in the community they live in n methods, say this was assigned by the CHAIN team using remote sensing data.

Table 5.2a: *Factors associated with ESBL-E carriage at admission*

5.3.5.2 Factors associated with ESBL-E carriage acquisition between admission and discharge

Independent factors associated with acquisition of ESBL-E in hospital included: being hospitalised for 3 to 6 days (aRR 1.56 [95% CI: 1.53–1.59], *P*<0.001), being hospitalised for ≥7 days (aRR 1.27 [95% CI: 1.13–1.43], *P*<0.001), HIV positive/exposed (aRR 1.07 [95% CI: 1.05–1.09], *P*<0.001) chronic conditions/disease (aRR 1.24 [95% CI: 1.00–1.52], *P*=0.04), and use of antibiotics i.e. cephalosporins (aRR 1.84 [95% CI: 1.18–2.86], 0.007), penicillin (aRR 1.51 [95% CI: 1.23–1.86], *P*<0.001) and quinolones (aRR 1.96 [95% CI:

1.90–2.02], *P*<0.001)> The model bootstrapped AUC (95%CI) was 0.64 (0.60–0.68) (Table 5.2b).

	Univariate analysis		***Multivariable analysis***	
	Crude RR (95%CI)	**P-value**	**Adjusted RR (95% CI)**	**P-value**
Age in months	1.01 (0.99-	0.29	1.02 (.99-1.02)	0.24
Gender (female)	1.08 (1.01-	0.03	1.08 (1.02-1.14)	0.01
Length of hospital stay (days)				
≤2	Reference		Reference	
3 to 6	1.50 (1.43-	<0.001	1.56 (1.53-1.59)	<0.001
≥7	1.32 (1.09-	0.004	1.27 (1.13-1.43)	<0.001
Previous hospital admission				
No	Reference		Reference	
Yes	0.67 (0.43-	0.09	0.63 (0.391.01)	0.06
Prior use of antibiotics	0.98 (0.78-	0.53	¶	
Nutritional status				
NW	Reference			
MW	1.06 (0.75-	0.72	¶	
SWK	1.22 (1.03-	0.02	¶	
Clinical diagnosis at admission				
Severe pneumonia	104 (0.79-1.37)	0.77		
None	Reference			
Diarrhoea	1.20 (0.91-	0.20	1.17 (0.98-1.40)	0.08
Systemic inflammatory response syndrome†	1.16 (0.95-	0.15	¶	
Malaria (RDT positive)	0.80 (0.48-	0.40	¶	
HIV status				
HIV negative	Reference		Reference	
HIV positive/exposed	1.12 (1.10-	<0.001	1.07 (1.05-1.09)	<0.001
HIV unknown	1.01 (0.83-	0.91	1.09 (0.72-1.67)	0.68
Chronic conditions/disease	1.01 (0.77-	0.95	1.24 (1.00-1.52)	0.04
*Environmental/household exposures**				
Type of toilets				
Not improved	1.13 (1.02-	0.02	¶	
Improved toilet	Reference		¶	
Household water/quality of drinking water				
Not improved	1.05 (0.87-	0.60	¶	
Improved water source	Reference			
Livestock ownership	1.06 (0.80-	0.70	¶	
*Population density (log) (*1000 per km2)*	1.01 (0.99-	0.21	¶	
Asset quantiles				
(Patient poverty and health system poverty).				
Quintile 1 (Least assets)	1.41 (0.92-	0.12	¶	

Quintile 2	1.21 (0.69-	0.51	¶	
Quintile 3	1.12 (0.97-	0.11	¶	
Quintile 4	1.26 (0.74-	0.40	¶	
Quintile 5 (Most assets)	Reference			
Antibiotics given				
Cephalosporins	1.29 (0.93-	0.13	1.84 (1.18-2.86)	0.007
Penicillins	1.17 (1.05-	0.006	1.51 (1.23-1.86)	<0.001
Quinolones/fluoroquinolones	1.46 (1.01-	0.04	1.96 (1.90-2.02)	<0.001
Others	1.13 (0.91-	0.27		
Multivariable model performance				
Bootstrapped AUC (95%CI)			0.64 (0.60-0.68)	

¶; variables not included in multivariable model, RR; Risk ratios from log binomial regression models. †SIRS-Systemic Inflammatory Response Syndrome: presence of two of the following four criteria; heart rate low (<90) or high (>180)/min; temperature low (<36°C) or high (≥38.5°C); respiratory rate high (>34 breaths per minute) and WBC low (<5 x10^9/l) or high (>17.5 x10^9/l); *Environmental/household exposures; Source: WHO/UNICEF sanitation classification, 2005; www.wssinfo.org

Table 5.2b: *Factors associated with ESBL-E carriage acquisition between admission and discharge*

5.3.5.3 Factors associated with ESBL-E carriage persistence from discharge to day 180

A total of 88/804 children we positive at discharge and had persistence ESBL carriage up to day 180, 11% (95% CI 8.9–13%). Independent factors positively associated with persistence of ESBL-E carriage post-discharge included: Hospital readmission (aRR 2.15 [95% CI: 1.07-4.33], *P*=0.002), Systemic inflammatory response syndrome (aRR 1.75 [95% CI: 1.39-2.20], *P*<0.001), HIV positive or exposed (aRR 1.59 [95% CI: 1.03-2.44], *P*=0.04), HIV unknown (aRR 1.96 [95% CI: 1.09-3.51], *P*=0.02), and having other chronic co-morbidities (aRR 1.55 [95% CI: 1.02-2.36], 0.04). Length of hospitalisation, previous hospitalisation, Malaria infection, and residence in household with fewer assets were negatively associated with ESBL-E carriage. The model bootstrapped AUC (95%CI) was 0.71 (0.65-0.77) (Table 5.2c).

Risk factor	***Univariate analysis***		***Multivariable analysis***	
	Crude RR (95%CI)	**P-value**	**Adjusted RR (95% CI)**	**P-value**
Age in months	0.97 (0.96–0.99)	0.001		
Gender (female)	1.23 (0.86–1.74)	0.25		

Length of hospital stay (days)				
≤2	Reference		Reference	
3 to 6	0.84 (0.56–1.27)	0.42	0.79 (0.65–	0.03
≥7	1.36 (1.19–1.55)	<0.001	0.93 (0.90–	<0.001
Previous hospitalisation before index				
No	Reference		Reference	
Yes	1.11 (0.84–1.47)	0.45	0.75 (0.68–	<0.001
Hospital readmission during follow-up				
No	Reference		Reference	
Yes	2.79 (1.22−6.40)	0.03	2.15 (1.07–	0.03
Prior use of antibiotics	0.93 (0.79-1.10)	0.40	¶	
Nutritional status				
NW	Reference		¶	
MW	1.46 (0.63–3.42)	0.38	¶	
SWK	1.07 (0.81–1.42)	0.62	¶	
Clinical diagnosis at admission				
Severe Pneumonia	1.27 (0.49–3.27)	0.62	¶	
Diarrhoea	0.73 (0.52–1.04)	0.09	¶	
Systemic inflammatory response syndrome†	1.67 (1.36–2.04)	<0.001	1.75 (1.39–	<0.001
Malaria (RDT positive)	0.34 (0.19–0.59)	<0.001	0.43 (0.26–	0.001
HIV status				
HIV negative	Reference		Reference	
HIV positive/exposed	1.16 (0.67–2.03)	0.60	1.59 (1.03–	0.04
HIV unknown	2.13 (0.91–5.01)	0.08	1.96 (1.09–	0.02
Chronic conditions/disease	1.97 (1.56–2.49)	<0.001	1.55(1.02-2.36)	0.04
SIRS at index discharge	0.89 (0.72–1.11)	0.30	0.79 (0.62–	0.05
*Environmental/household exposures**				
Type of toilets				
Not improved	0.70 (0.51–0.96)	0.03	¶	
Improved toilet	Reference		¶	
Household water/quality of drinking				
Not improved	0.78 (0.44–1.38)	0.39	¶	
Improved water source	Reference			
Livestock ownership	1.37 (1.12–1.69)	<0.001		
*Population density (log) (*1000 per*	1.13 (1.02–1.24)	0.02	1.07 (0.99–	0.04
Asset quantiles				
Quintile 1 (Least assets)	0.49 (0.25-0.94)	0.03	0.68 (0.44-1.06)	0.09
Quintile 2	0.42 (0.32–0.56)	<0.001	0.50 (0.33–	0.001
Quintile 3	0.78 (0.64–0.97)	0.02	0.88 (0.76-1.02)	0.11

Quintile 4	0.88 (0.67–1.15)	0.36	0.74 (0.53-1.02)	0.07
Quintile 5 (Most assets)	Reference		Reference	
Antibiotics given during hospitalisation				
No	Reference			
Yes	1.80 (1.03–3.14)	0.04	¶	
Multivariable model performance				
Bootstrapped AUC (95%CI)			0.71 (0.65−0.77)	

¶; variables not included in multivariable model, RR; Risk ratios from log binomial regression models.
*Environmental/household exposures; Source: WHO/UNICEF sanitation classification, 2005; www.wssinfo.org

Table 5.2c: *Factors associated with ESBL-E carriage persistence between discharge and day 180 compared to being positive at discharge and negative at day 180*

5.3.5.4 Factors associated with ESBL-E carriage ESBL-E carriage among community participants

Independent factors positively associated with carriage of ESBL-E in the community included: improved house walls (aRR 1.80 [95% CI: 1.02–3.19], *P*=0.04), livestock ownership (aRR 2.10 [95% CI: 1.02–4,33], 0.04), caregiver reported as sick (aRR 3.89 [95% CI: 1.09–13.8], 0.04) and population density (number of households per km^2) (aRR 1.20 [95% CI: 1.01–1.41], 0.04). The bootstrapped AUC (95%CI) was0.68 (0.61–0.74) (5. 2d).

Risk factor	***Univariate analysis***		***Multivariable analysis***	
	Crude RR (95%CI)	**P-value**	**Adjusted RR (95% CI)**	**P-value**
Age in months	0.97 (0.93-1.02)	0.25	0.98 (0.93-1.03)	0.39
Gender (female)	1.45 (0.85-2.47)	0.17	1.28 (0.73-2.25)	0.39
Small birth size				
No	Reference		¶	
Yes	2.03 (0.82–5.05)	0.13	¶	
Currently breastfeeding				
No	Reference		Reference	
Yes	1.72 (0.78–3.79)	0.18	1.71 (0.76–3.86)	0.20
Previous hospital admission				
No	Reference		¶	
Yes	1.71 (0.66-4.47)	0.27	¶	
Recent use of antibiotics (within 7 days before)	0.84 (0.51-1.38)	0.49	¶	

No	Reference			
Yes	0.81 (0.39–1.68)	0.58		
Anthropometry			¶	
WAZ per z-score	1.04 (0.84-1.29)	0.72	¶	
HAZ per z-score	1.08 (0.90-1.29)	0.43	¶	
Reported illness			¶	
Malaria			¶	
Negative	Reference		¶	
Positive	0.72 (0.49-1.07)	0.10	¶	
Not done	0.94 (0.31-2.88)	0.92	¶	
HIV status				
HIV negative	Reference		Reference	
HIV infected/exposed	0.32 (0.09-1.05)	0.06	0.30 (0.09-1.01)	0.05
HIV unknown	2.33 (0.53-2.89)	-0.21	2.80 (0.42-18.7)	0.29
Chronic conditions/disease	1.56 (0.18-13.5)	0.69		
*Environmental/household exposures**				
Type of toilets				
Not improved	Reference		Reference	
Improved toilet	0.94 (0.55-1.61)	0.82	0.70 (0.37–1.30)	0.26
Household water/sanitation				
Not improved	Reference		¶	
Improved toilet	0.74 (0.42-1.29)	0.29	¶	
Type of house walls				
Not improved	Reference		Reference	
Improved	1.66 (0.97–2.86)	0.07	1.80 (1.02–3.19)	0.04
Type of house floor				
Not improved	Reference			
Improved	1.26 (0.74–2.15)	0.39		
Livestock ownership	1.15 (0.76-1.73)	0.51	2.10 (1.02-4.33)	0.04
Asset index (log transformed)	1.16 (0.84–1.59)	0.37		
Population density per 1000 km^2 (log transformed)	1.11 (0.99–1.25)	0.09	1.20 (1.01–1.41)	0.04
Distance to the nearest health facility (km log transformed)	0.89 (0.71–1.12)	0.31	¶	
Caregiver sick				
No	Reference		Reference	
Yes	3.95 (1.21–12.7)	0.02	3.89 (1.09–13.8)	0.04
Multivariable model performance				
Bootstrapped AUC (95%CI)			0.68 (0.61-0.74)	

¶; variables not included in multivariable model, RR; Risk ratios from log binomial regression models.
*Environmental/household exposures; source: WHO/UNICEF sanitation classification, 2005 www.wssinfo.org

Table 5.2d: *Risk factors for ESBL-E carriage among community participants*

5.3.6 ESBL acquisition in hospital - association with post-discharge death

There were 58 deaths during 3947 child-months of post-discharge follow-up representing a mortality rate of 14.7 (95%CI 11.4-19.0) deaths/1000 child-months. There were 23 deaths among 272 children who acquired ESBL-E between admission and discharge during the index admission (negative at admission, positive at discharge) versus 35 among 442 who did not acquire ESBL-E during hospitalisation, reflecting mortality rates of 15.2 (95%CI 10.1-22.9) and 14.3 (95%CI 10.3-20.0) deaths/1000 child-months respectively. The median [IQR] time to death was 69 [33 -115] days.

In a univariate model, the crude HR for death of association between acquiring ESBL-E in hospital and post-discharge mortality was 1.23 (0.74 - 2.02). After controlling for confounders with biological plausibility (table 5.3), acquisition of ESBL-E was similarly not associated with post-discharge mortality (aHR 1.22; 95%CI: 0.72 - 2.09) (Figure 5.5).

Covariates	***Multivariable analysis***	
	Adjusted HR (95% CI)	P-value
Acquired ESBL-E between admission and discharge	1.22 (0.72-2.09)	0.46
Age in months	0.99 (0.96-1.02)	0.53
Gender (female)	1.54 (1.08-2.21)	0.02
Length of hospital stay (days)		
≤2	Reference	
3 to 6	0.76 (0.15-3.84)	0.74
≥7	1.29 (0.12-14.4)	0.84
Previous hospital admission		
No	Reference	
Yes	2.01 (1.34-3.01)	0.001
Prior use of antibiotics (7 days before)	1.02 (0.38-2.72)	0.97
Nutritional status		
NW	Reference	
MW	4.72 (3.69-6.02)	<0.001
SWK	5.84 (3.75-9.08)	<0.001
Clinical diagnosis at admission		
Severe pneumonia	1.34 (0.59-3.03)	0.48

Diarrhoea	1.04 (0.961.12)	0.33
SIRS	1.02 (0.73-1.42)	0.91
Malaria (RDT positive)	0.72 (0.08-6.36)	0.77
HIV status		
HIV negative	Reference	
HIV positive/exposed	1.36 (0.49-3.81)	0.55
HIV unknown	1.74 (0.15-19.9)	0.66
Chronic conditions/disease	0.82 (0.65-1.04)	0.10
SIRS at index discharge	1.95 (1.05-3.63)	0.04
Environmental/household exposures		
Type of toilets		
Not improved	1.11 (0.33-3.75)	0.87
Improved toilet	Reference	
Household water/quality of drinking water		
Not improved	1.00 (0.95-1.06)	0.91
Improved water source	Reference	
Livestock ownership	2.02 (0.59-6.92)	0.26
*Population density (log) (*1000 per km^2)*	0.90 (0.85-0.94)	<0.001
Asset quantiles (Patient poverty and health		
Quintile 1 (Least assets)	3.02 (2.22-4.12)	<0.001
Quintile 2	4.02 (1.46-11.1)	0.007
Quintile 3	3.03 (2.07-4.44)	<0.001
Quintile 4	3.30 (2.36-4.62)	<0.001
Quintile 5 (Most assets)	Reference	
Antibiotics given		
Antibiotics given at admission		
Cephalosporins	0.95 (0.74-1.21)	067
Penicillins	1.09 (0.75-1.58)	0.65
Quinolones or fluoroquinolones	0.85 (0.07-10.9)	0.90
Others	0.88 (0.48-1.62)	0.67
Antibiotics given at discharge		
Cephalosporins	2.96 (1.79-4.89)	<0.001
Penicillins	1.07 (0.76-1.51)	0.69
Quinolones or fluoroquinolones	0.58 (0.20-1.71)	0.33

Table 5.3: *ESBL-E acquisition, covariates and post-discharge mortality.*

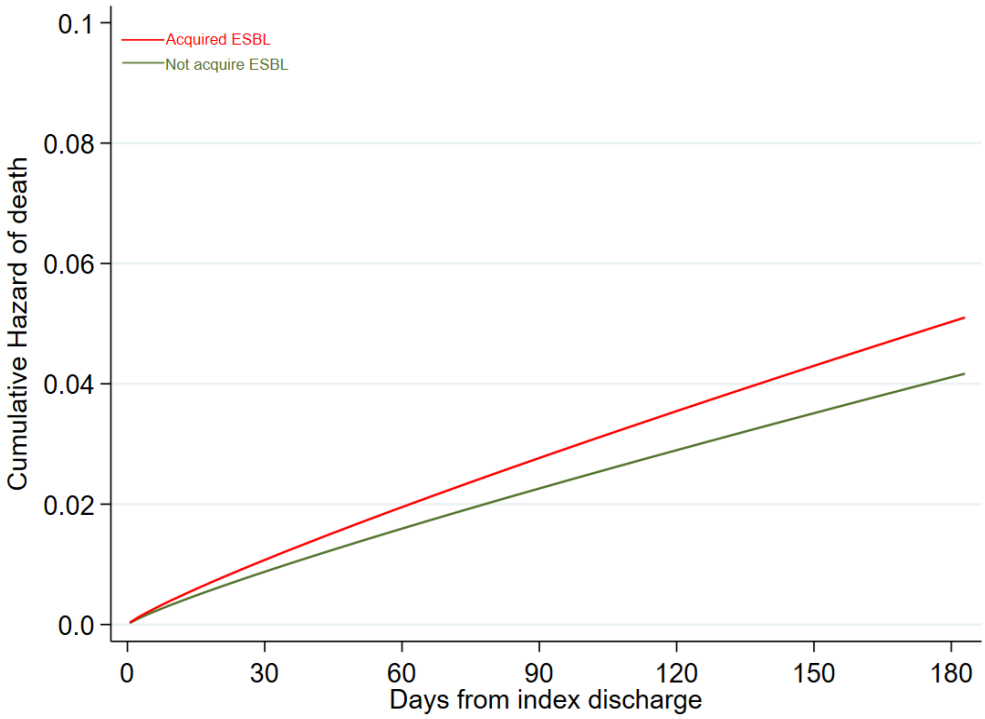


Figure 5.5: *Predicated cumulative hazard from the multivariable model curve of time to death stratified by ESBL-E acquisition at index discharge, adjusted for confounders shown in table 5.3.*

5.3.7 ESBL-E acquisition in hospital - association with hospital readmission

There were total of 141 episodes of hospital readmission during 3944.1 child-months of observation, representing an incidence rate of 35.8 (95%CI 30.3-42.2) per 1000 child-months. A total of 59 hospital readmissions episodes occurred among 271 children who acquired ESBL-E between admission and discharge during the index admission (negative at admission, positive at discharge) while 82 occurred among 443 who did not acquire ESBL-E reflecting incidence rates of 39.1 (95%CI 30.3-50.5) and 33.7 (95%CI 27.1-41.8) per 1000 child-months respectively.

In a univariate model, the crude HR of association between acquiring ESBL-E and episodes of hospital readmission was 1.38 (95%CI: 0.93-2.04), P=0.11, After controlling for confounders with biological plausibility (table 5.4), acquisition of ESBL-E was associated with an aHR 1.56 (95%CI: 1.10- 2.22) (Figure 5.6).

Covariates	***Multivariable analysis***	
	Adjusted HR (95% CI)	P-value
Acquired ESBL between admission and discharge	1.56 (1.10–2.22)	0.01
Age in months	0.84 (0.72–0.98)	0.03
Gender (female)	0.87 (0.65–1.17)	0.35
Length of hospital stay (days)		
≤2	Reference	
3 to 6	0.97 (0.78–1.21)	0.79
≥7	1.46 (0.70–3.06)	0.32
Previous hospital admission		
No	Reference	
Yes	2.08 (1.62–2.66)	<0.001
Prior use of antibiotics (7 days before)	0.88 (0.63–1.21)	0.43
Nutritional status		
NW	Reference	
MW	2.47 (2.04–3.00)	<0.001
SWK	1.34 (0.87–2.06)	0.18
Clinical diagnosis at admission		
Severe pneumonia	1.14 (0.98–1.32)	0.08
Diarrhoea	0.72 (0.37–1.40)	0.33
SIRS	1.37 (0.77–2.41)	0.28
Malaria (RDT positive)	0.67 (0.35–1.26)	0.21
HIV status		
HIV negative	Reference	
HIV positive/exposed	0.75 (0.42–1.35)	0.34
HIV unknown	2.71 (2.22–3.30)	<0.001
Chronic conditions/disease	2.30 (1.61–3.29)	<0.001
SIRS at index discharge	1.03 (0.84–1.28)	0.76
Environmental/household exposures		
Type of toilets		
Not improved	0.86 (0.47–1.58)	0.64
Improved toilet	Reference	
Household water/quality of drinking water		
Not improved	0.84 (0.74–0.96)	0.01
Improved water source	Reference	
Livestock ownership	1.02 (0.91–1.14)	0.79
*Population density (log) (*1000 per km^2)*	0.96 (0.91–1.01)	0.14
Asset quantiles (Patient poverty and health		
Quintile 1 (Least assets)	2.05 (1.63–2.58)	<0.001
Quintile 2	2.72 (1.80–4.12)	<0.001
Quintile 3	1.80 (1.42–2.27)	<0.001
Quintile 4	2.83 (2.23–3.58)	<0.001
Quintile 5 (Most assets)	Reference	

Antibiotics given		
Antibiotics given during index admission	0.78 (0.67–0.90)	0.001

Table 5.4: *ESBL-E acquisition, covariates and hospital readmission.*

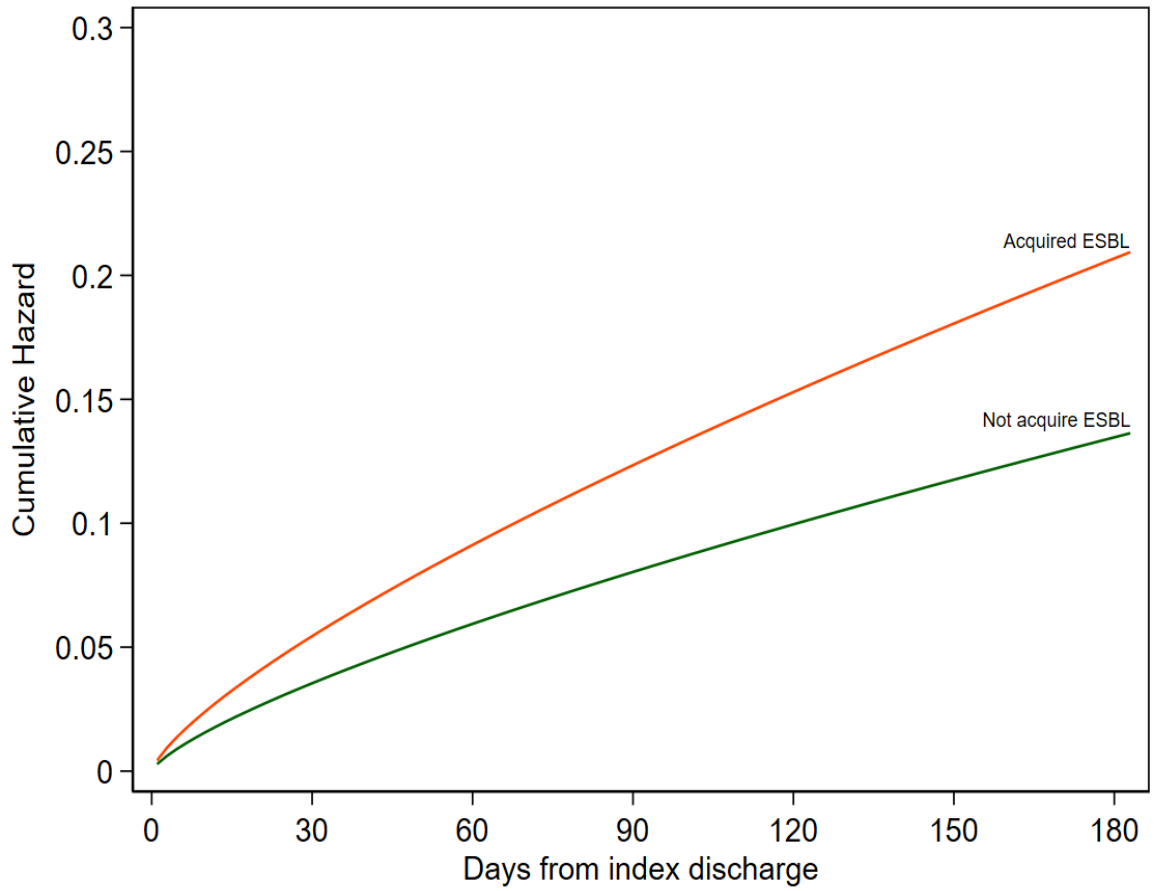


Figure 5.7: *Predicated and smoothed cumulative hazard curve from the multivariable model curve of time to hospital readmissions stratified by ESBL-E acquisition at index discharge*

5.4 Discussion

This longitudinal study on the carriage of ESBL-E among children from hospital admission through discharge and up to six months post-discharge offers critical insights into the progression and persistence of AMR within a vulnerable paediatric population. The data, now five years old remains relevant but should be interpreted in the context of evolving resistance patterns. At admission, ESBL-E carriage was observed in 36% of children, higher than the 16% seen in the community, likely due to pre-hospital antibiotic exposure

or ESBL-E's association with illness necessitating hospitalization. The sharp increase to 71% at discharge underscores the role of hospital environments and antibiotic pressure in acquisition. Post-discharge, carriage declined to 27% by day 180 (Figure 5.3a) but remained above community levels, suggesting a sustained contribution to community transmission. Site-specific trends, such as Migori's rapid decline by day 45, may reflect lower antibiotic exposure, background prevalence in rural settings and loss of carriage due to post discharge mortality seen.

Comparative studies reinforce these findings. In Kilifi, Kenya, neonatal ESBL-E carriage was 10% at admission, with a 55% in-hospital acquisition rate[13]. However, paediatric wards might have been expected to have less transmission than more crowded neonatal units.

A study in Western Kenya reported 44% carriage at discharge, strongly linked to antibiotic use[15]. Urban settings, such as Nairobi's informal settlements, report higher ESBL-E prevalence of 30.9% among children, reflecting the interplay of poor hygiene, antibiotic misuse, and transmission risks in densely populated urban settings [15, 16]. Environmental reservoirs further complicate AMR dynamics, as seen in Uganda, where ESBL-E was found in 25% of animal and environmental samples, highlighting One Health considerations[17].

Seasonal factors also play a critical role, as highlighted by a study in Malawi which reported increased ESBL-E carriage during the wet season, particularly in urban environments with higher exposure to livestock and water sources[18]. The study further emphasized the importance of addressing environmental contamination and urban-rural disparities in resistance control strategies[18]. Similarly, a study in rural Ghana among

children <5 years attending the study hospital reported 41% faecal carriage rate of ESBL-producing *E. coli* and *K. pneumoniae,* similar to the 38% prevalence of carriage reported in my study [19]. These studies underscore the substantial burden of ESBL-E in paediatric populations within the region, but without genotypic information, it is hard to understand the underlying dynamics of this variation in carriage prevalence

Causality remains complex: does ESBL-E carriage contribute to illness, or do pre-existing health conditions predispose children to colonization? Factors such as prior hospitalization, antibiotic use, HIV exposure, and malnutrition were strongly associated with both baseline carriage and hospital acquisition, aligning with previous findings, notably in East African studies, that underscore the role of antibiotic exposure and compromised immunity in AMR risk[20]. The 'HIV unknown' group was significantly associated - our experience is that families refusing testing for their child know they are positive and are in denial, avoiding treatment. The persistence of ESBL-E post-discharge suggests an ongoing cycle of transmission between hospitals and communities.

Furthermore, CRE prevalence remained low, absent in the community and declining after discharge despite hospital acquisition.[16], suggests that CRE carriage remains limited within this cohort. However, persistent CRE carriage in Nairobi and Migori raises concerns about hospital reservoirs and antibiotic selection pressures, particularly due to ceftriaxone overuse
[7].

The analysis of associations between ESBL-E acquisition during hospitalisation and subsequent health outcomes reveals that ESBL-E carriage acquisition between admission

and discharge notably is associated with the risk of hospital readmissions over the next six months. Interestingly, carefully controlled studies have shown no significant association between AMR and mortality when potential confounding factors were accounted for, which was also what I saw in my analysis. [21]. Similar findings have been observed in other rigorously designed studies, which demonstrate that while AMR, such as ESBL-E, contributes to increased healthcare utilization, it does not necessarily lead to higher mortality[22]. For instance, in African hospitals, third-generation cephalosporin (3GC) resistance in *E. coli* and *K. pneumoniae* bloodstream infections (BSI) did not significantly influence mortality rates[23]. However, ESBL-E carriage was associated with significantly higher rates of hospital readmission. In contrast, less well-controlled studies have suggested an association between AMR and increased mortality, and a systematic review has identified significant links between AMR carriage and adverse clinical outcomes, particularly in settings with limited resources and constrained treatment options[24]. This suggests that ESBL-E colonisation likely contributes to prolonged illness, increased healthcare utilization, and potential treatment challenges, reinforcing the need for robust IPC measures and antimicrobial stewardship.

A key limitation of this study is the imperfect sensitivity of the phenotypic methods used for detecting ESBL-E carriage. While phenotypic tests are robust for identifying the presence of ESBL resistance, specific acquisition dynamics may be potentially masked by potential for false negative results. Additionally, gain-loss events in ESBL-E carriage may occur between the sampled timepoints, leading to underestimation of transient or intermittent colonization. This limitation underscores the importance of integrating detailed genotypic data, to identify of specific genetic profiles, offering insights into whether observed changes in ESBL-E carriage status are linked to hospital- or community-acquired

strains. By revealing the genomic diversity and transmission pathways of ESBL-E, this approach could provide a more comprehensive understanding of the acquisition and persistence dynamics, guiding targeted interventions to mitigate the spread of resistant strains between healthcare settings and the community. The analysis also did not also include antimicrobial sensitivity results for other antibiotics, which would have provided additional information into the broader AMR landscape in these settings. The absence of ESBL-E and carbapenemase-selective media as well as use of MacConkey agar with 8% gentamicin may not effectively isolate all CRE or ESBL-E organisms, as it primarily selects for aminoglycoside-resistant strains while potentially missing carbapenem-resistant or ESBL-E isolates that remain susceptible to gentamicin; however, these were recovered from blood agar and plain MacConkey agar plates using the three-colony morphology pick.

5.5 Conclusions

In conclusion, this study reveals the impact of hospital-acquired ESBL-E acquisition among children in Kenya, with high rates of carriage at discharge and significant persistence over the following months. The findings underscore the role of the hospital environment as a major source of AMR acquisition and/or amplification, particularly for vulnerable paediatric patients with compromised health or malnutrition. ESBL-E acquisition in hospital was linked to increased readmission rates, indicating a lasting healthcare burden from AMR that extends well beyond the initial hospital stay. Although ESBL-E carriage was not directly associated with increased mortality, its correlation with recurrent hospitalisations highlights the critical need for enhanced infection control practices and diligent post-discharge follow-up, especially for high-risk groups. Key interventions such as robust antibiotic stewardship, caregiver education on hygiene

practices, and structured follow-up care could mitigate readmission risks and limit the spread of AMR within communities.

The study also identifies a complex, multifactorial risk profile for ESBL-E acquisition and persistence, including factors like prior hospitalisation, recent antibiotic exposure, HIV status, and household environment. This complexity suggests that a comprehensive approach, incorporating both hospital-based infection prevention and community-targeted strategies, is essential to effectively curb AMR transmission. Addressing these diverse risk factors in resource-limited settings will require coordinated efforts that include patient education, environmental sanitation, and accessible healthcare services to reduce AMR's lasting impact. However, a key limitation of this study is the lack of integration of detailed genotypic data, discussed in the next chapter, which could help determine whether switches in ESBL-E carriage status are more likely to be associated with hospital or community-acquired strains. Understanding the genotypic profiles of ESBL-E isolates would provide deeper insights into the dynamics of acquisition and persistence, informing targeted interventions to address hospital-to-community transmission pathways.

Ultimately, this research underscores the urgent need for sustained public health focus on paediatric AMR, with an emphasis on scalable, context-specific interventions that address AMR’s socioeconomic and environmental determinants. Future studies incorporating genotypic analysis of ESBL-E isolates should evaluate the effectiveness of targeted interventions in different resource contexts and assess long-term outcomes of paediatric AMR colonisation. By prioritizing these strategies, healthcare systems can better protect vulnerable paediatric populations, reduce the healthcare burden of AMR, and curb its transmission within communities.

REFERENCES

1. Onduru OG, Mkakosya RS, Rumisha SF, Aboud S: **Carriage prevalence of extended-spectrum β-lactamase producing enterobacterales in outpatients attending community health centers in Blantyre, Malawi**. *Tropical Medicine and Infectious Disease* 2021, **6**(4):179.
2. Lewis JM, Lester R, Garner P, Feasey NA: **Gut mucosal colonisation with extended-spectrum beta-lactamase producing Enterobacteriaceae in sub-Saharan Africa: a systematic review and meta-analysis**. *Wellcome open research* 2019, **4**.
3. Tompkins K, Juliano JJ, Van Duin D: **Antimicrobial resistance in Enterobacterales and its contribution to sepsis in sub-Saharan Africa**. *Frontiers in medicine* 2021, **8**:615649.
4. Lewis JM, Mphasa M, Banda R, Beale MA, Heinz E, Mallewa J, Jewell C, Faragher B, Thomson NR, Feasey NA: **Colonization dynamics of extended-spectrum beta-lactamase-producing Enterobacterales in the gut of Malawian adults**. *Nature microbiology* 2022, **7**(10):1593-1604.
5. Bizimana J, Ndayisenga J, Kajumbura H, Mulepo P, Christine NF: **Colonization of patients hospitalized at orthopedic department of tertiary hospital in Uganda with extended-spectrum beta-lactamase-producing enterobacterales**. *Antimicrobial Resistance & Infection Control* 2023, **12**(1):26.
6. Tellevik MG, Blomberg B, Kommedal Ø, Maselle SY, Langeland N, Moyo SJ: **High prevalence of faecal carriage of ESBL-producing Enterobacteriaceae among children in Dar es Salaam, Tanzania**. *PloS one* 2016, **11**(12):e0168024.
7. Omulo S, Ita T, Mugoh R, Ayodo C, Luvsansharav U, Bollinger S, Styczynski A, Ramay BM, Caudell MA, Palmer GH: **Risk factors for colonization with extended-spectrum cephalosporin-resistant and carbapenem-resistant Enterobacterales among hospitalized patients in Kenya: an antibiotic resistance in communities and hospitals (ARCH) study**. *Clinical Infectious Diseases* 2023, **77**(Supplement_1):S97-S103.
8. Childhood Acute Illness Nutrition Network: **Childhood Acute Illness and Nutrition (CHAIN) Network: a protocol for a multi-site prospective cohort study to identify modifiable risk factors for mortality among acutely ill children in Africa and Asia**. *BMJ Open* 2019, **9**(5):e028454.
9. Berkley J: **Childhood mortality during and after acute illness in Africa and S. Asia: a cohort study**. *The Lancet Global Health* 2022, **10**(5).
10. Illness CA, Network N: **Childhood Acute Illness and Nutrition (CHAIN) Network: a protocol for a multi-site prospective cohort study to identify modifiable risk factors for mortality among acutely ill children in Africa and Asia**. *BMJ open* 2019, **9**(5):e028454.
11. Bauer A, Kirby W, Sherris JC, Turck M: **Antibiotic susceptibility testing by a standardized single disk method**. *American journal of clinical pathology* 1966, **45**(4_ts):493-496.
12. PA W: **Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 20th informational supplement**. *CLSI document M100-S20* 2010.
13. Kagia N, Kosgei P, Ooko M, Wafula L, Mturi N, Anampiu K, Mwarumba S, Njuguna P, Seale AC, Berkley JA: **Carriage and acquisition of extended-spectrum β-lactamase–producing Enterobacterales among neonates admitted to hospital in Kilifi, Kenya**. *Clinical infectious diseases* 2019, **69**(5):751-759.

14. Diallo AH, Shahid ASMSB, Khan AF, Saleem AF, Singa BO, Gnoumou BS, Tigoi C, Otieno CA, Bourdon C, Oduol CO: **Childhood mortality during and after acute illness in Africa and south Asia: a prospective cohort study**. *The Lancet Global Health* 2022, **10**(5):e673-e684.
15. Tornberg-Belanger SN, Rwigi D, Mugo M, Kitheka L, Onamu N, Ounga D, Diakhate MM, Atlas HE, Wald A, McClelland RS: **Antimicrobial resistance including Extended Spectrum Beta Lactamases (ESBL) among E. coli isolated from kenyan children at hospital discharge**. *PLoS neglected tropical diseases* 2022, **16**(3):e0010283.
16. Mutuma CK, Maingi J, Maina AK, Njeru J, Musyoki AM: **Asymptomatic gastrointestinal carriage of multidrug-resistant carbapenemase-producing Enterobacteriaceae among children under five years in a Kenyan hospital**. *IJID regions* 2023, **9**:25-31.
17. Muleme J, Musoke D, Balugaba BE, Kisaka S, Makumbi FE, Buregyeya E, Isunju JB, Wambi R, Mugambe RK, Kankya C: **Epidemiology of extended-spectrum beta-lactamase-producing Escherichia coli at the human-animal-environment interface in a farming community of central Uganda**. *PLoS Global Public Health* 2023, **3**(6):e0001344.
18. Cocker D, Chidziwisano K, Mphasa M, Mwapasa T, Lewis JM, Rowlingson B, Sammarro M, Bakali W, Salifu C, Zuza A: **Investigating One Health risks for human colonisation with extended spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae in Malawian households: a longitudinal cohort study**. *The Lancet Microbe* 2023, **4**(7):e534-e543.
19. Akenten CW, Khan NA, Mbwana J, Krumkamp R, Fosu D, Paintsil EK, Boahen KG, Osei-Mensa J, Maiga-Ascofare O, May J: **Carriage of ESBL-producing Klebsiella pneumoniae and Escherichia coli among children in rural Ghana: a cross-sectional study**. *Antimicrobial Resistance & Infection Control* 2023, **12**(1):60.
20. Caudell MA, Ayodo C, Ita T, Smith RM, Luvsansharav U-O, Styczynski AR, Ramay BM, Kariuki S, Palmer GH, Call DR: **Risk factors for colonization with multidrug-resistant bacteria in urban and rural communities in Kenya: an antimicrobial resistance in communities and hospitals (ARCH) study**. *Clinical Infectious Diseases* 2023, **77**(Supplement_1):S104-S110.
21. Aiken AM, Rehman AM, de Kraker ME, Madrid L, Kebede M, Labi A-K, Obeng-Nkrumah N, Nyamwaya B, Kagucia E, Cocker D: **Mortality associated with third-generation cephalosporin resistance in Enterobacterales bloodstream infections at eight sub-Saharan African hospitals (MBIRA): a prospective cohort study**. *The Lancet Infectious Diseases* 2023, **23**(11):1280-1290.
22. Dramowski A, Ong'ayo G, Rehman AM, Whitelaw A, Labi A-K, Obeng-Nkrumah N, Ndir A, Magwenzi MT, Onyedibe K, Wolkewitz M: **Mortality attributable to third-generation cephalosporin resistance in Gram-negative bloodstream infections in African hospitals: a multi-site retrospective study**. *JAC-antimicrobial Resistance* 2021, **3**(1):dlaa130.
23. Lewis JM, Mphasa M, Banda R, Beale MA, Mallewa J, Anscome C, Zuza A, Roberts AP, Heinz E, Thomson NR: **Genomic analysis of extended-spectrum beta-lactamase (ESBL) producing Escherichia coli colonising adults in Blantyre, Malawi reveals previously undescribed diversity**. *Microbial Genomics* 2023, **9**(6):001035.
24. Allel K, Stone J, Undurraga EA, Day L, Moore CE, Lin L, Furuya-Kanamori L, Yakob L: **The impact of inpatient bloodstream infections caused by antibiotic-**

resistant bacteria in low-and middle-income countries: A systematic review and meta-analysis. *PLoS medicine* 2023, **20**(6):e1004199.
25. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H: **Antibiotic resistance—the need for global solutions**. *The Lancet infectious diseases* 2013, **13**(12):1057-1098.
26. Gerber JS, Kronman MP, Ross RK, Hersh AL, Newland JG, Metjian TA, Zaoutis TE: **Identifying targets for antimicrobial stewardship in children's hospitals**. *Infection Control & Hospital Epidemiology* 2013, **34**(12):1252-1258.
27. Paterson DL: **Resistance in gram-negative bacteria: Enterobacteriaceae**. *American journal of infection control* 2006, **34**(5):S20-S28.
28. Shrivastava SR, Shrivastava PS, Ramasamy J: **World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics**. *Journal of Medical Society* 2018, **32**(1):76.
29. Partridge SR, Kwong SM, Firth N, Jensen SO: **Mobile genetic elements associated with antimicrobial resistance**. *Clinical microbiology reviews* 2018, **31**(4):e00088-00017.
30. Moxon CA, Paulus S: **Beta-lactamases in Enterobacteriaceae infections in children**. *Journal of Infection* 2016, **72**:S41-S49.
31. Ramasethu J: **Prevention and treatment of neonatal nosocomial infections**. *Maternal health, neonatology and perinatology* 2017, **3**:1-11.
32. Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, Knight GM, Robotham JV: **Estimating the burden of antimicrobial resistance: a systematic literature review**. *Antimicrobial Resistance & Infection Control* 2018, **7**(1):58.
33. Chemaly RF, Simmons S, Dale Jr C, Ghantoji SS, Rodriguez M, Gubb J, Stachowiak J, Stibich M: **The role of the healthcare environment in the spread of multidrug-resistant organisms: update on current best practices for containment**. *Therapeutic advances in infectious disease* 2014, **2**(3-4):79-90.
34. Kramer A, Schwebke I, Kampf G: **How long do nosocomial pathogens persist on inanimate surfaces? A systematic review**. *BMC infectious diseases* 2006, **6**(1):130.
35. Jadhav S, Sahasrabudhe T, Kalley V, Gandham N: **The microbial colonization profile of respiratory devices and the significance of the role of disinfection: a blinded study**. *Journal of clinical and diagnostic research: JCDR* 2013, **7**(6):1021.
36. Burgaya J, Marin J, Royer G, Condamine B, Gachet B, Clermont O, Jaureguy F, Burdet C, Lefort A, de Lastours V: **The bacterial genetic determinants of Escherichia coli capacity to cause bloodstream infections in humans**. *PLoS Genetics* 2023, **19**(8):e1010842.
37. Baker S, Thomson N, Weill F-X, Holt KE: **Genomic insights into the emergence and spread of antimicrobial-resistant bacterial pathogens**. *Science* 2018, **360**(6390):733-738.
38. Khezri A, Avershina E, Ahmad R: **Hybrid assembly provides improved resolution of plasmids, antimicrobial resistance genes, and virulence factors in Escherichia coli and Klebsiella pneumoniae clinical isolates**. *Microorganisms* 2021, **9**(12):2560.
39. Juraschek K, Borowiak M, Tausch SH, Malorny B, Käsbohrer A, Otani S, Schwarz S, Meemken D, Deneke C, Hammerl JA: **Outcome of different sequencing and assembly approaches on the detection of plasmids and localization of antimicrobial resistance genes in commensal Escherichia coli**. *Microorganisms* 2021, **9**(3):598.

40. Branger C, Zamfir O, Geoffroy S, Laurans G, Arlet G, Thien HV, Gouriou S, Picard B, Denamur E: **Genetic background of Escherichia coli and extended-spectrum β-lactamase type**. *Emerging infectious diseases* 2005, **11**(1):54.
41. Jean S-S, Harnod D, Hsueh P-R: **Global threat of carbapenem-resistant gram-negative bacteria**. *Frontiers in cellular and infection microbiology* 2022, **12**:823684.
42. Organization WH: **The evolving threat of antimicrobial resistance: options for action: executive summary**. In.: World Health Organization; 2012.
43. Njunge JM, Tickell K, Diallo AH, Shahid ASMSB, Gazi MA, Saleem A, Kazi Z, Ali S, Tigoi C, Mupere E: **The Childhood Acute Illness and Nutrition (CHAIN) network nested case-cohort study protocol: a multi-omics approach to understanding mortality among children in sub-Saharan Africa and South Asia**. *Gates open research* 2022, **6**.
44. Andrews S, Krueger F, Segonds-Pichon A, Biggins L, Krueger C, Wingett S: **FastQC**. *A quality control tool for high throughput sequence data* 2010, **370**.
45. Ewels P, Magnusson M, Lundin S, Käller M: **MultiQC: summarize analysis results for multiple tools and samples in a single report**. *Bioinformatics* 2016, **32**(19):3047-3048.
46. Gurevich A, Saveliev V, Vyahhi N, Tesler G: **QUAST: quality assessment tool for genome assemblies**. *Bioinformatics* 2013, **29**(8):1072-1075.
47. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW: **CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes**. *Genome research* 2015, **25**(7):1043-1055.
48. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH: **GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database**. In.: Oxford University Press; 2020.
49. Shen W, Le S, Li Y, Hu F: **SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation**. *PloS one* 2016, **11**(10):e0163962.
50. Abram K, Udaondo Z, Bleker C, Wanchai V, Wassenaar TM, Robeson MS, Ussery DW: **Mash-based analyses of Escherichia coli genomes reveal 14 distinct phylogroups**. *Communications Biology* 2021, **4**(1):117.
51. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, Edalatmand A, Petkau A, Syed SA, Tsang KK: **CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database**. *Nucleic acids research* 2023, **51**(D1):D690-D699.
52. Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, Jin Q: **VFDB: a reference database for bacterial virulence factors**. *Nucleic acids research* 2005, **33**(suppl_1):D325-D328.
53. Seemann T: **Prokka: rapid prokaryotic genome annotation**. *Bioinformatics* 2014, **30**(14):2068-2069.
54. Tonkin-Hill G, MacAlasdair N, Ruis C, Weimann A, Horesh G, Lees JA, Gladstone RA, Lo S, Beaudoin C, Floto RA: **Producing polished prokaryotic pangenomes with the Panaroo pipeline**. *Genome biology* 2020, **21**:1-21.
55. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, Harris SR: **SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments**. *Microbial genomics* 2016, **2**(4):e000056.
56. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R: **IQ-TREE 2: new models and efficient methods for phylogenetic**

inference in the genomic era. *Molecular biology and evolution* 2020, **37**(5):1530-1534.
57. Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS: **ModelFinder: fast model selection for accurate phylogenetic estimates**. *Nature methods* 2017, **14**(6):587-589.
58. Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS: **UFBoot2: improving the ultrafast bootstrap approximation**. *Molecular biology and evolution* 2018, **35**(2):518-522.
59. Letunic I, Bork P: **Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation**. *Nucleic acids research* 2021, **49**(W1):W293-W296.
60. Tartof SY, Solberg OD, Manges AR, Riley LW: **Analysis of a uropathogenic Escherichia coli clonal group by multilocus sequence typing**. *Journal of clinical microbiology* 2005, **43**(12):5860-5864.
61. Robertson J, Nash JH: **MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies**. *Microbial genomics* 2018, **4**(8):e000206.
62. Giufrè M, Errico G, Accogli M, Monaco M, Villa L, Distasi MA, Del Gaudio T, Pantosti A, Carattoli A, Cerquetti M: **Emergence of NDM-5-producing Escherichia coli sequence type 167 clone in Italy**. *International journal of antimicrobial agents* 2018, **52**(1):76-81.
63. Garcia-Fernandez A, Villa L, Bibbolino G, Bressan A, Trancassini M, Pietropaolo V, Venditti M, Antonelli G, Carattoli A: **Novel insights and features of the NDM-5-producing Escherichia coli sequence type 167 high-risk clone**. *MSphere* 2020, **5**(2):10.1128/msphere. 00269-00220.
64. Peirano G, Richardson D, Nigrin J, McGeer A, Loo V, Toye B, Alfa M, Pienaar C, Kibsey P, Pitout JD: **High prevalence of ST131 isolates producing CTX-M-15 and CTX-M-14 among extended-spectrum-β-lactamase-producing Escherichia coli isolates from Canada**. *Antimicrobial agents and chemotherapy* 2010, **54**(3):1327-1330.
65. Yu K, Huang Z, Xiao Y, Bai X, Gao H, Wang D: **Epidemiology and molecular characterization of CTX-M-type ESBLs producing Escherichia coli isolated from clinical settings**. *Journal of Global Antimicrobial Resistance* 2024, **36**:181-187.
66. Horesh G, Blackwell GA, Tonkin-Hill G, Corander J, Heinz E, Thomson NR: **A comprehensive and high-quality collection of Escherichia coli genomes and their genes**. *Microbial genomics* 2021, **7**(2):000499.
67. Kasanga M, Gajdács M, Muleya W, Ikhimiukor OO, Mudenda S, Kasanga M, Chizimu J, Shempela DM, Solochi BB, Mwikisa MJ: **Genotypic Characterisation and Antimicrobial Resistance of Extended-Spectrum β-Lactamase-Producing Escherichia coli in Humans, Animals, and the Environment from Lusaka, Zambia: public Health Implications and One Health Surveillance**. *Antibiotics* 2024, **13**(10):951.
68. Cuénod A, Agnetti J, Seth-Smith HM, Roloff T, Wälchli D, Shcherbakov D, Akbergenov R, Tschudin-Sutter S, Bassetti S, Siegemund M: **Bacterial genome-wide association study substantiates papGII of Escherichia coli as a major risk factor for urosepsis**. *Genome Medicine* 2023, **15**(1):89.
69. Mora Gutiérrez A, López Capón C, Dahbi G, Blanco Álvarez M, Blanco Álvarez JE, Alonso MP, Herrera Estévez A, Mamani Huarani RS, Bonacorsi S, Moulin Schouleur M: **Extraintestinal pathogenic Escherichia coli O1: K1: H7/NM from**

human and avian origin: detection of clonal groups B2 ST95 and D ST59 with different host distribution. In*: 2009*: BMC; 2009.

70. Ling W, Paterson DL, Harris PN, Furuya-Kanamori L, Edwards F, Laupland KB: **Mortality, hospital length of stay, and recurrent bloodstream infections associated with extended-spectrum beta-lactamase-producing Escherichia coli in a low prevalence region: A 20-year population-based large cohort study**. *International Journal of Infectious Diseases* 2024, **138**:84-90.
71. Pitout JD, Peirano G, Chen L, DeVinney R, Matsumura Y: **Escherichia coli ST1193: following in the footsteps of E. coli ST131**. *Antimicrobial Agents and Chemotherapy* 2022, **66**(7):e00511-00522.
72. Kocsis B, Gulyás D, Szabó D: **Emergence and dissemination of extraintestinal pathogenic high-risk international clones of Escherichia coli**. *Life* 2022, **12**(12):2077.
73. Pitout JD, Peirano G, Matsumura Y, DeVinney R, Chen L: **Escherichia coli sequence type 410 with carbapenemases: a paradigm shift within E. coli toward multidrug resistance**. *Antimicrobial Agents and Chemotherapy* 2024, **68**(2):e01339-01323.
74. Byarugaba DK, Osman TS, Sayyouh OM, Wokorach G, Kigen CK, Muturi JW, Onyonyi VN, Said MM, Nasrat SA, Gazo M: **Genomic epidemiology of multidrug-resistant Escherichia coli and Klebsiella pneumoniae in Kenya, Uganda, and Jordan**. *Emerging Infectious Diseases* 2024, **30**(Suppl 2):S33.
75. Fonseca EL, Morgado SM, Caldart RV, Vicente AC: **Global genomic epidemiology of Escherichia coli (ExPEC) ST38 lineage revealed a virulome associated with human infections**. *Microorganisms* 2022, **10**(12):2482.
76. Hounmanou YMG, Wanyana A, Alafi S, Wabwire-Mangen F, Christensen H, Olsen JE, Byarugaba DK: **Whole strains vs MGEs in short and longterm transmission of ESBL genes between healthcare and community settings in Uganda**. *Scientific Reports* 2023, **13**(1):10229.
77. Kagia N KP, Ooko M, Wafula L, Mturi N, Anampiu K, Mwarumba S, Njuguna P, Seale AC, Berkley JA, Bottomley C, Scott JAG, Morpeth SC.: **Carriage and Acquisition of Extended-spectrum β-Lactamase-producing Enterobacterales Among Neonates Admitted to Hospital in Kilifi, Kenya**.
78. El Chaar M, Khoury Y, Douglas GM, El Kazzi S, Jisr T, Soussi S, Merhi G, Moghnieh RA, Shapiro BJ: **Longitudinal genomic surveillance of multidrug-resistant Escherichia coli carriage in critical care patients**. *Microbiology Spectrum* 2024, **12**(2):e03128-03123.
79. Vrancianu CO, Popa LI, Bleotu C, Chifiriuc MC: **Targeting plasmids to limit acquisition and transmission of antimicrobial resistance**. *Frontiers in microbiology* 2020, **11**:761.

6 GENOMIC CHARACTERISTICS OF EXTENDED SPECTRUM BETA-LACATAMASE AND CARBEPENEM RESISTANT *ESCHERICHIA COLI* CARRIAGE, HOSPITAL ENVIRONMENT AND INVASIVE DISEASE

6.1 Introduction

The rise of antibiotic-resistant Enterobacterales, particularly extended-spectrum beta-lactamase and carbapenemase-producing strains, represents a critical global health challenge, with low- and middle-income countries (LMICs) facing disproportionate impacts[25, 26]. Globally, *Escherichia coli* and *Klebsiella pneumoniae* are major pathogens within Enterobacterales, causing healthcare- and community-associated infections, exacerbated by the acquisition of mobile genetic elements (MGEs) such as plasmids, transposons, and integrons encoding resistance genes, like $bla_{\text{CTX-M}}$, bla_{NDM}, and $bla_{\text{OXA-48}}$[27, 28]. MGEs facilitate horizontal gene transfer, enabling organisms like *E. coli* to acquire resistance to antibiotics critical for clinical care, exemplified by ESBL-producing and carbapenem-resistant Enterobacterales (ESBL-E and CRE respectively)[29]. Asymptomatic carriage of ESBL-E may be present in healthy individuals in the community, including gastrointestinal colonisation established in early life[30]. Colonisation can be a precursor to sepsis in the individual or be the source of invasive infection to others in hospital and in the community[31]. Asymptomatic carriage of ESBL-E in healthy individuals as well as community acquired infections are increasingly reported[30]. The emergence and spread of resistance in Enterobacterales may lead to serious disease due to strains that are resistant to all available agents[32].

Hospitalisation, especially in resource-limited settings, such as urban hospitals in Kenya, and in high-intensity treatment contexts involving selective pressure from antimicrobial

use, patient crowding and limited IPC creates a high-risk environment for colonisation with ESBL-E and CRE[27, 30, 33]. Surfaces, liquids including IV fluids, drug diluents and oxygen humidification fluids, sinks, drains and patient colonisation can provide a prolonged reservoir of resistant bacteria and a continuous source of transmission[34, 35]. Hospital-associated *E. coli* also commonly acquires additional resistance and virulence determinants, potentially contributing to the severity of infections[35, 36].

Genomics has changed the practice of bacterial surveillance and outbreak investigation by providing a high-resolution methodology to understand the spread of multidrug-resistant pathogens.[37] Complete reconstruction of genomes using hybrid sequencing approaches combining long- (e.g., Oxford-Nanopore) and short-read (e.g. Illumina) sequencing data enables the complete and accurate reconstruction of bacterial genomes, including plasmids[38, 39]. Decreasing sequencing costs have enabled these methods to be increasingly used on large datasets, enabling accurate reconstruction of transmission pathways to appropriately reflect genetically diverse and highly dynamic AMR gene transmission networks. Longitudinal genomic studies have also highlighted dynamic shifts in resistance mechanisms during hospitalisation, including the acquisition or loss of plasmids and changes in clonal structure influenced by nosocomial transmission dynamics[40, 41]. An evolving resistance profile can complicate treatment strategies, especially in resource-constrained settings where therapeutic options are limited[42].

I aimed to evaluate genomic characteristics of ESBL-E/CRE carriage in the community and changes over time during admission, discharge and post-discharge using whole genome sequencing and phylogenetic analysis of *E. coli* strains in Kenya.

6.2 Research Questions

1. What is the genetic relatedness of *E. coli* paediatric carriage and invasive isolates at Kenyan hospitals?
2. Which sequence types (STs), phylogroups, antibiotic resistance associated genes (ARGs) and virulence genes are carried at admission to hospital, acquired in hospital, carried in the community and are associated with persistent carriage at 180 days post discharge?
3. What are the distinct plasmid types present in *E. coli*, their mobility status, and what proportion of antimicrobial resistance genes are located on plasmids versus on chromosomal DNA?

6.3 Study design

This is primarily an analysis of a stratified prospective cohort study of acutely ill children enrolled into the Childhood Acute Illness & Nutrition (CHAIN) Network cohort study[8, 43]. The study included children aged 2 to 23 months who were observed during their index hospital admission, followed for 180 days post-discharge or readmitted to hospital. Three CHAIN Network sites in Kenya included in this genomic analysis were Kilifi County Hospital, Mbagathi Sub-County Hospital (Nairobi), and Migori County Hospital (Figure 6.1). To establish community norms, well children in the same age range and living in the same communities as the hospitalised CHAIN Network cohort were sampled at a single time point. In addition, invasive isolates were obtained from two additional hospital sites from parallel ongoing surveillance studies in Kenya: Coast General Hospital Mombasa, and Kiambu Hospital, Nairobi. Hospital environmental isolates collected on a quarterly basis as part of IPC in the two hospitals were also included.

6.4 Sample Selection

Longitudinal rectal isolates: *E. coli* isolates (ESBL-E and CRE positive/negative) from rectal swabs collected at admission, discharge, day 45, day 90, day 180 and readmission after discharge, were included (figure 6.2).

Invasive isolates: Blood culture *E. coli* isolates from hospitalised children and neonates at CHAIN Network sites and two other hospitals under ongoing surveillance (figure 6.2).

Environmental isolates: Hospital environmental samples collected quarterly as part of infection control programs in the sites included. These swabs identified resistant bacteria from surfaces such as equipment, sinks, taps, and staff hands (figure 6.2).

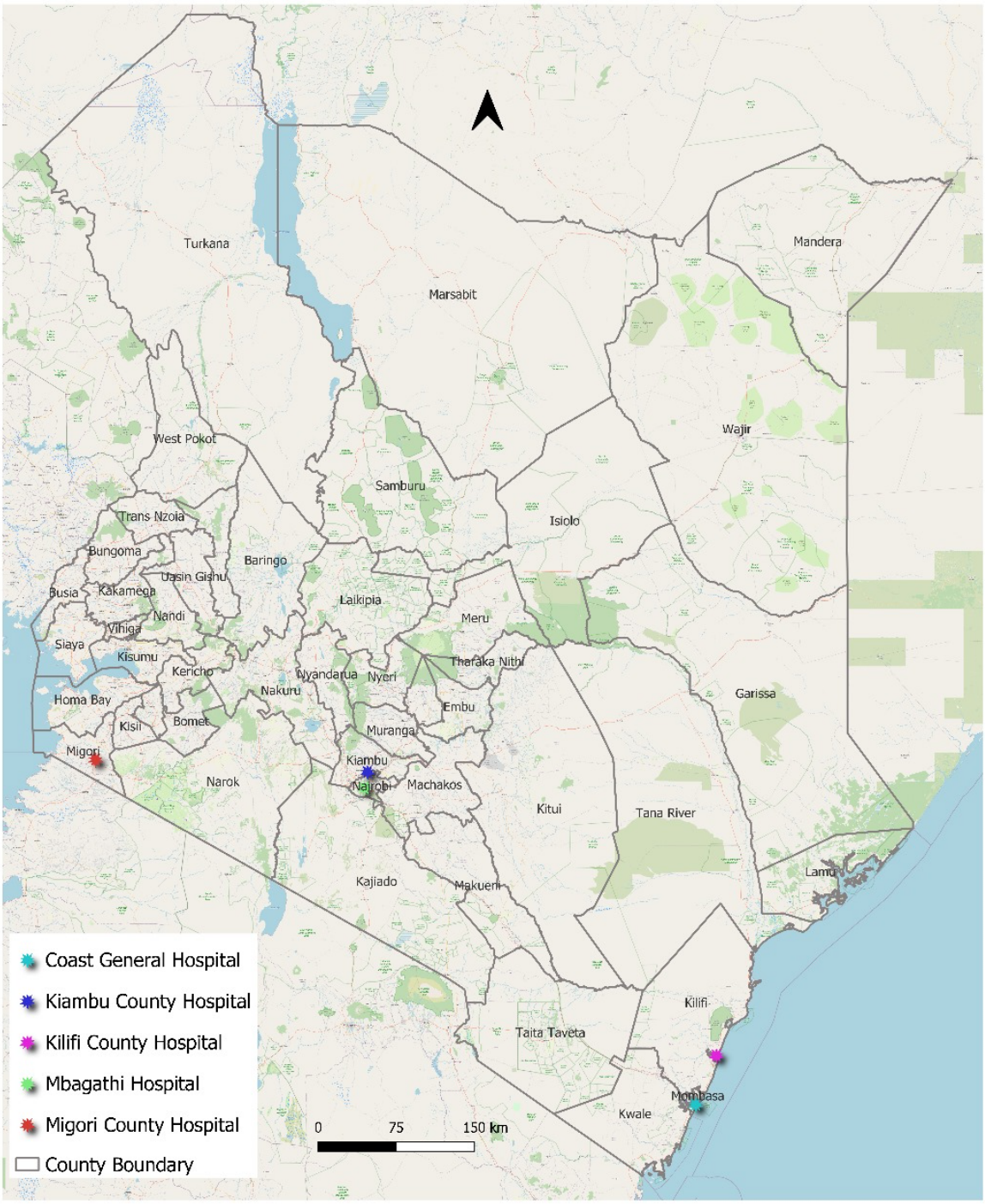


***Figure 6.1**: Kenyan study sites included in the analysis, Kilifi and Migori are rural sites while Mbagathi, Kiambu and Coast General hospitals are urban sites.*

6.5 Study setting and sample collection

To determine the *E. coli* phylogeny and the acquisition or loss of ESBL-E, CRE, and virulence genes at admission, discharge, post-discharge, readmission, in the community, in

the hospital environment, and in paediatric invasive disease, I analysed 538 stored *E. coli* isolates (Figure 6.2).

6.5.1 Selection of Isolates

Faecal isolates (440): To characterise ESBL-E or CRE carriage, children with one or more phenotypically positive rectal swab (ESBL-E or CRE) taken at admission, discharge, day 45, day 90, day 180 or readmission post-discharge were included and all available *E. coli* isolates from their additional time points comprising both ESBL-E positive and negative strains were analysed. There were 242 ESBL-E positive isolates and 38 CRE positive carriage isolates from Kilifi (n=178), Mbagathi (n=154) and Migori (n=108).

Environmental isolates (18): Hospital environmental *E. coli* isolates were included irrespective of resistance (ESBL-E or CRE) from Kilifi (n=7), Mbagathi (n=7), Mombasa (n=1), Migori (n=2) and Kiambu (n=1).

Invasive isolates (80): Paediatric invasive *E. coli* isolates from blood cultures were similarly selected without conditioning on resistance from Kilifi (n=49), Mbagathi (n=19), Mombasa (n=7) and Kiambu (n=5).

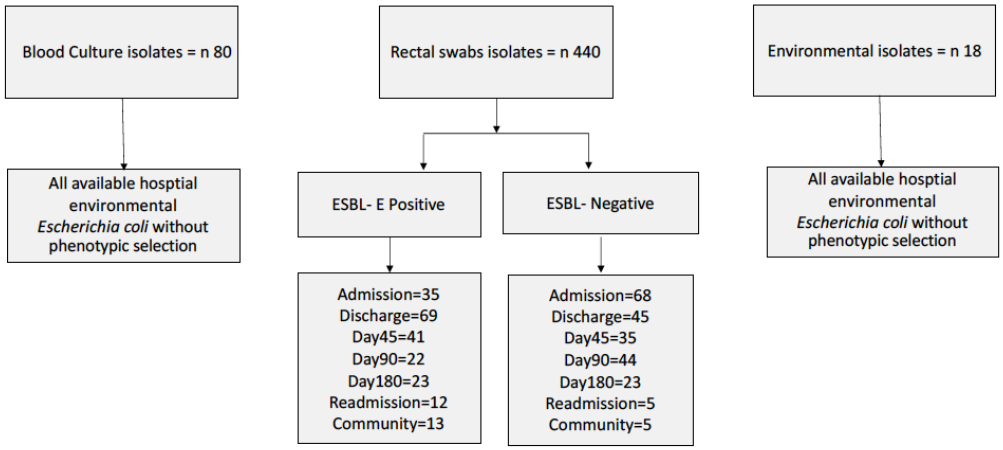


Figure 6.2: *Sample/isolate selection flow diagram*

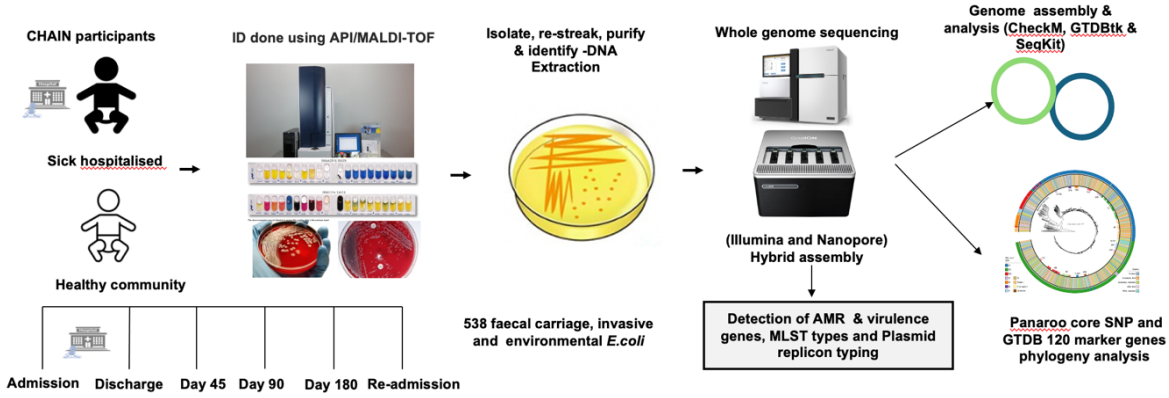


Figure 6.3: *Sample-processing flow diagram*

This work was conducted in collaboration with the Wellcome Sanger Institute, where I received training and mentorship. All the 538 *E. coli* isolates were sequenced using Illumina (short-read) and Nanopore (long-read) platforms. DNA extraction was performed using the MasterPure Complete DNA and RNA Purification Kit. Library preparation was followed by whole-genome sequencing (WGS) using both the Illumina NovaSeq 6000 platform for short reads (150 bp read length) and the Oxford Nanopore platform for long reads (Figure 6.3 and 6.4).

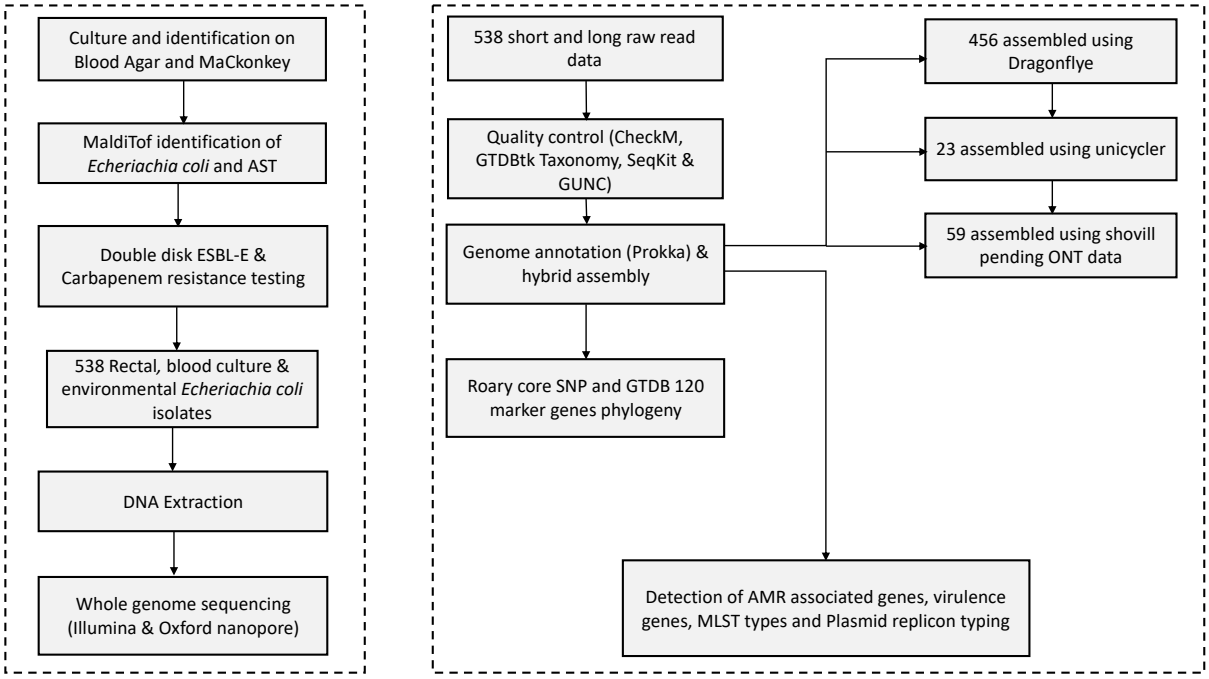


Figure 6.4: The study sample-processing and sequence data analysis flow diagram

6.5.2 Quality control and genome assembly

I performed initial quality control of sequencing reads using FastQC (v0.11.4.)[44] and MultiQC (v1.17)[45], removing read sets with bimodal GC content peaks or substantially abnormal quality metrics. Based on the quality control outcomes and the specific requirements of the dataset, I utilized different assemblers for de novo genome assembly. Specifically, Dragonflye was used for 456 genomes, Unicycler for 23 genomes, and Shovill for 59 genomes. Additional QC was performed on the de novo assemblies using Quast (v5.0.2)[46] and CheckM (v1.1.2--py_1)[47] to calculate the genome contamination and completeness. Assemblies were filtered to retain high quality assemblies based on genome length (4.5-5.5 MB), genome contamination (<5%) and genome completeness (>95%). A total of three were contaminated which were resolved through purity plating and repeat sequencing and three incomplete short reads, resolved through hybrid assembly using Dragonflye (short reads used to polish long reads).

6.5.3 Species identification and distinction

I initially used gtdbtk (v2.2.3--pyhdfd78af_1) [48] to assign taxonomic labels, followed by Seqkit (v2.3.1--h9ee0642_0)[49] assess the species abundance and genome statistics, respectively. This was followed by an assessment of *E. coli* phylogroups using Mash-based analyses, a program that approximates similarity between two genomes in nucleotide content, and an in-house Sanger Python script that integrates additional annotations and species-specific features to determine phylogroup assignments. This matrix was used to generate the population structure of these genomes to differentiate 14 different phylogroups, consisting of 14 *E. coli* groups: G, B2-1, B2-2, F, D1, D2, D3, E2(O157), E1, A, C, and B1, and 2 *Shigella* groups: Shig1 and Shig2[50] .

6.5.4 Prediction of antimicrobial resistance and virulence genes

Antimicrobial resistance genes were identified from the assembled contigs using ABRicate v1.0.1 (available at https://github.com/tseemann/abricate) to query the Comprehensive Antimicrobial Resistance Database (CARD)[51] where the CARD identification threshold was set at 80%. Identification of virulence genes was done using ABRicate with the Virulence Finder Database[52].

6.5.5 Genome annotation and phylogenetic analysis

Draft *E. coli* genomes were annotated using PROKKA (v1.4.5-c1)[53]. Panaroo v1.3.0-c4[54] was used to infer a pangenome and I used the resulting core gene alignment (comprised of 2,417 core genes) for phylogenetic analysis. I extracted variable sites using SNP-sites v2.51[55] and the resulting multiple sequence alignment file was used to infer a phylogeny with IQ-TREE (v2.1)[56], using a general time reversible model of nucleotide substitution with a FreeRate model of heterogeneity[57], incorporating invariant sites using

the '-fconst' parameter, and with 1,000 UltraFast Bootstraps[58]. Phylogenetic trees and associated metadata were visualized in iTOL[59].

6.5.6 Multi-locus Sequence Typing (MLST) and Subtyping of *E. coli* isolates

I used the *mlst* tool v2.22 MLST based on the seven-gene Achtman scheme described at the website https://pubmlst.org/[60].This MLST scheme uses seven housekeeping genes, namely, *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA* to subtype *E. coli* further into Sequence Types (STs). Novel alleles and STs were submitted to the *E. coli* MLST database via pubmlst.org for naming. All sequence types were further integrated with genomic and phenotypic data using phylogenetic clustering to provide a comprehensive view of its architecture within the studied *E. coli* populations in relation to site, phylogroups and sample type.

6.5.7 Mobsuite Plasmid Analysis of *E. coli* isolates

Plasmid analysis was conducted using the Mobsuite toolkit (available at https://github.com/phac-nml/mob-suite), a robust computational pipeline designed for comprehensive plasmid identification and characterization in bacterial genomes[61]. Mobsuite was employed to analyse the plasmid content of *E. coli* genomes, focusing on replicon typing, plasmid clustering, and mobility profiling. The pipeline includes tools for detecting plasmid-associated sequences, assigning plasmids to known incompatibility groups, and identifying the presence of mobility elements such as relaxases and conjugative transfer systems.

The plasmid analysis began with the identification of plasmid replicons, using a curated database of known replicon sequences to classify plasmids into incompatibility groups. This step provided insights into the distribution of plasmid types across the *E. coli* isolates

and their potential roles in mediating resistance gene transfer. Plasmid clustering was performed to group related plasmid sequences based on genetic similarity, enabling the detection of conserved plasmid backbones and variants contributing to the dissemination of antimicrobial resistance genes.

Additionally, Mobsuite's mobility prediction capabilities were utilized to distinguish between conjugative, mobilizable, and non-mobilizable plasmids. This classification was critical for understanding the mechanisms driving horizontal gene transfer among the isolates. MOB-typer (as part of Mobsuite) annotates relaxases (*mob*), mating pair formation (MPF) complexes, and *oriT* genes. Plasmids were labelled as conjugative if they had both a relaxase and MPF, mobilizable if they had either a relaxase or *oriT* but no MPF, and non-mobilizable if they had no relaxase and no *oriT*.

6.5.8 Ethical considerations

Ethical approval for this work was obtained from the Oxford University Tropical Research Ethics Committee and the Kenya Medical Research Institute (KEMRI) Scientific Ethical and Research Unit (SERU no. 3318). Written informed consent was obtained from parents or guardians for all enrolled children.

6.6 Results

6.6.1 Population structure of *E. coli* isolates

A total of 538 *E. coli* genomes from bloodstream, environmental, and rectal swab isolates were sequenced using both Illumina (short-read) and Nanopore (long-read) platforms. Hybrid assembly was performed, with 456 genomes assembled using Dragonflye and 23 using Unicycler, yielding high-quality assemblies. Additionally, 59 short-read sequences were assembled using Shovill, as their corresponding long-read sequences failed QC, and

these were also included in the analysis. To understand the genomics of carriage, hospital environment versus invasive *E. coli* isolates I evaluated the phylogeny reconstructed using these 538 assembled genomes. I found that *E. coli* isolates represent diverse phylogroups, and there was no clear structuring by site. I identified 12 phylogroups in Kenya against 14 that have been identified globally[50]. The most predominant phylogroup being A (152/538 [28%]), followed by D3 (85/538 [16%]), B1 (81/538 [15%]), B2-1 (58/538 [11%]), B2-2 (54/538 [10%]) and D1 (49/538 [9%]). Some of these phylogroups have been associated with invasive disease including B1, B2, D1 and D3 (Figure 6.5). Invasive and carriage isolates were intermixed in the phylogeny consistent with ST69, ST38 and ST95 clustering together (Figure 6.5). Interestingly, environmental isolates were restricted to a cluster that encompassed an unknown sequence type and included invasive isolates in 5 hospitals (Kilifi, Migori, Mbagathi, Kilifi and Mombasa) (Figure 6.5).

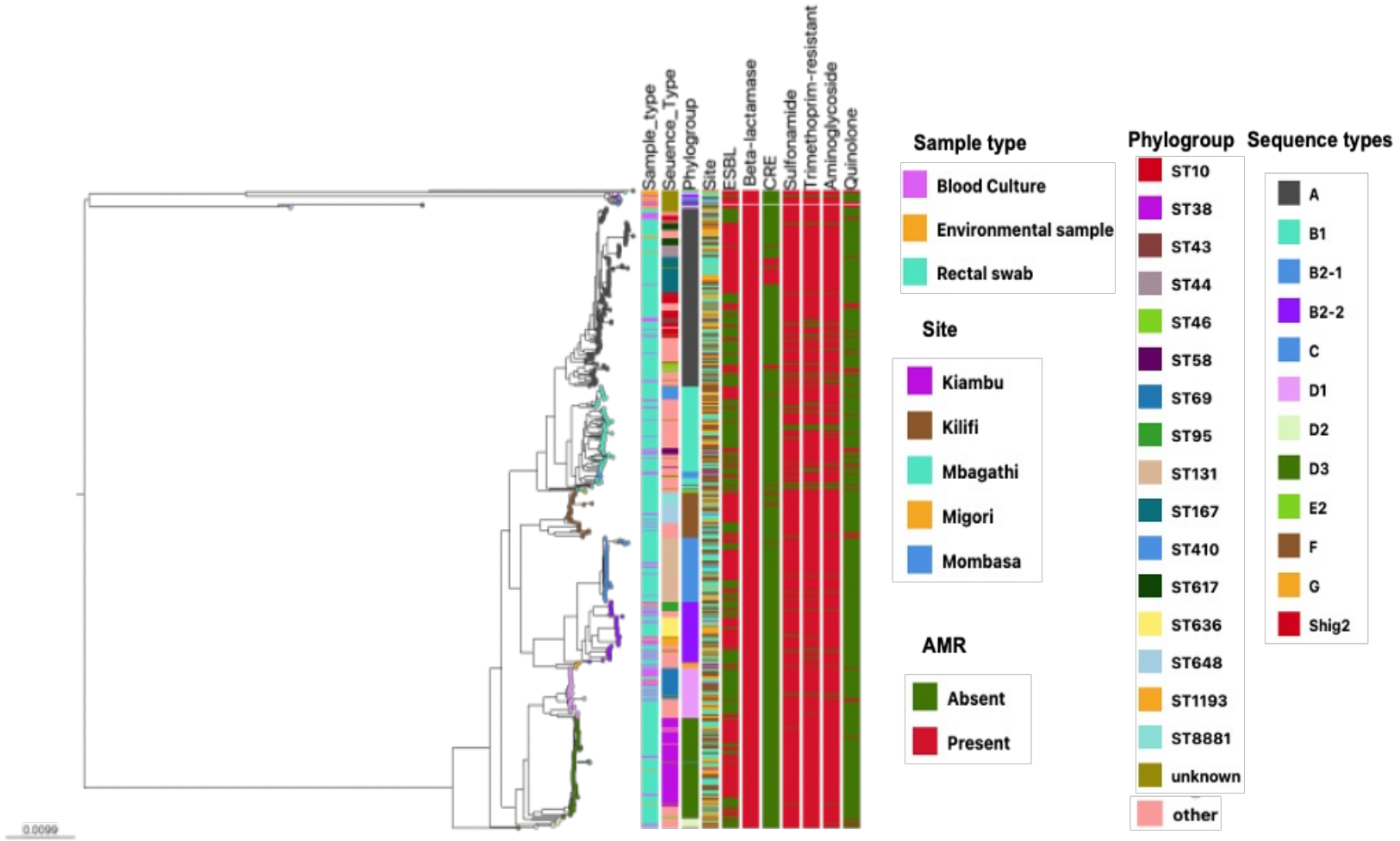


Figure 6.5: *A midpoint rooted maximum likelihood phylogenetic tree of E. coli genomes: n = 538 blood stream, environmental and rectal swab isolate genomes showing the distribution of phylogroups and their association with Sequence types. The colour strip shows the sample type, the second colour strip shows the sequence type, then phylogroup, site and AMR genes. The sample types included were: Rectal swab, hospital environment samples and blood culture samples. 12 phylogroups were identified in this dataset. It is evident that the most predominant phylogroup is A (152/538 [28%]), followed by D3 (85/538 [16%]), B1 (81/538 [15%]), B2-1 (58/538 [11%]), B2-2 (54/538 [10%]) and D1 (49/538 [9%]).*

6.6.2 Prevalence of AMR genes

A total of 5573 unique AMR gene variants were identified in all faecal carriage, environmental and invasive isolates, conferring resistance to 12 antibiotic classes (Figure 6.6). The most predominant AMR genes conferred resistance to aminoglycosides (1421), followed by beta-lactamases (1183), then sulphonamides (768) and ESBL-E/AMPCs (322) while the least were carbapenems (34) quinolones (47) and fosfomycin (14). Among these, 322/538 (60%) ESBL-E isolates were detected, with the most prevalent variant being *bla*$_{\text{CTX-M-15}}$ (235 isolates), followed by *bla*$_{\text{CTX-M-9}}$ (19 isolates) and *bla*$_{\text{CTX-M-27}}$ (15 isolates). Additionally, four carbapenemase variants were identified: *bla*$_{\text{NDM-5}}$ (n=32) and *bla*$_{\text{OXA-181}}$ (n=2) (Figure 6.7a and 6.7b).

To better interpret these data, ESBL-E and CRE gene proportions were calculated relative to the total number of isolates collected at each time point for faecal samples only. This analysis included a total of 440 rectal swabs only with ESBL-E and CRE genes results. Overall ESBL-E carriage proportion was 68% (299/440) and for CRE was 7% (30/440). Distribution patterns of AMR genes increased during hospitalisation/discharge, particularly for *bla*$_{\text{CTX-M-15,}}$ *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{OXA-181}}$, which were carried for over 6 months post-discharge from hospital. ESBL-E genes like. Very little circulation of CRE genes was detected in the community with one to 4 genes maximum detected in the community participants and during the follow-up period. Two *bla*$_{\text{NDM-5}}$ genes were rarely detected at discharge and day 180.

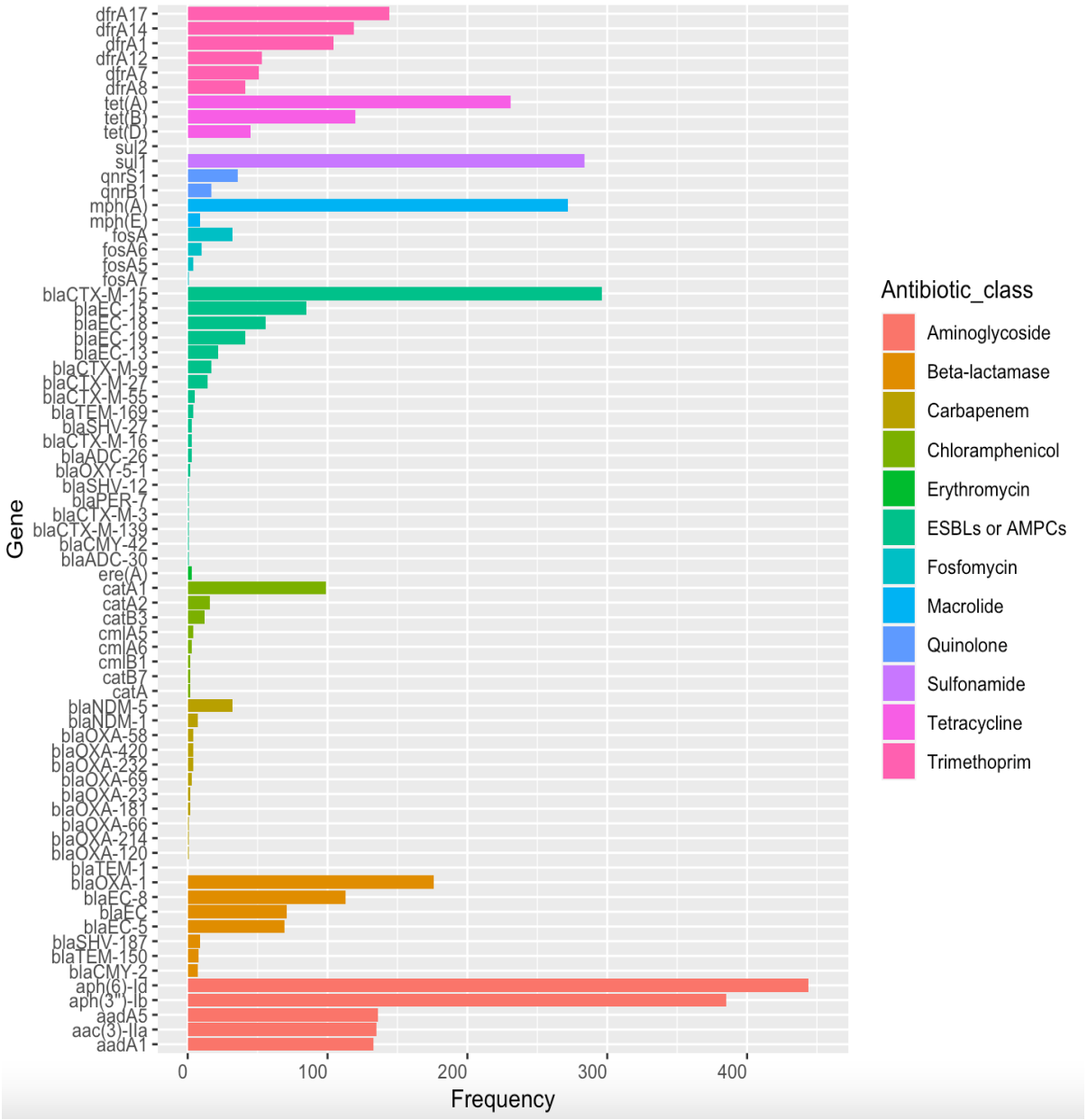


Figure 6.6: *Overall AMR genes detected in E. coli genomes*

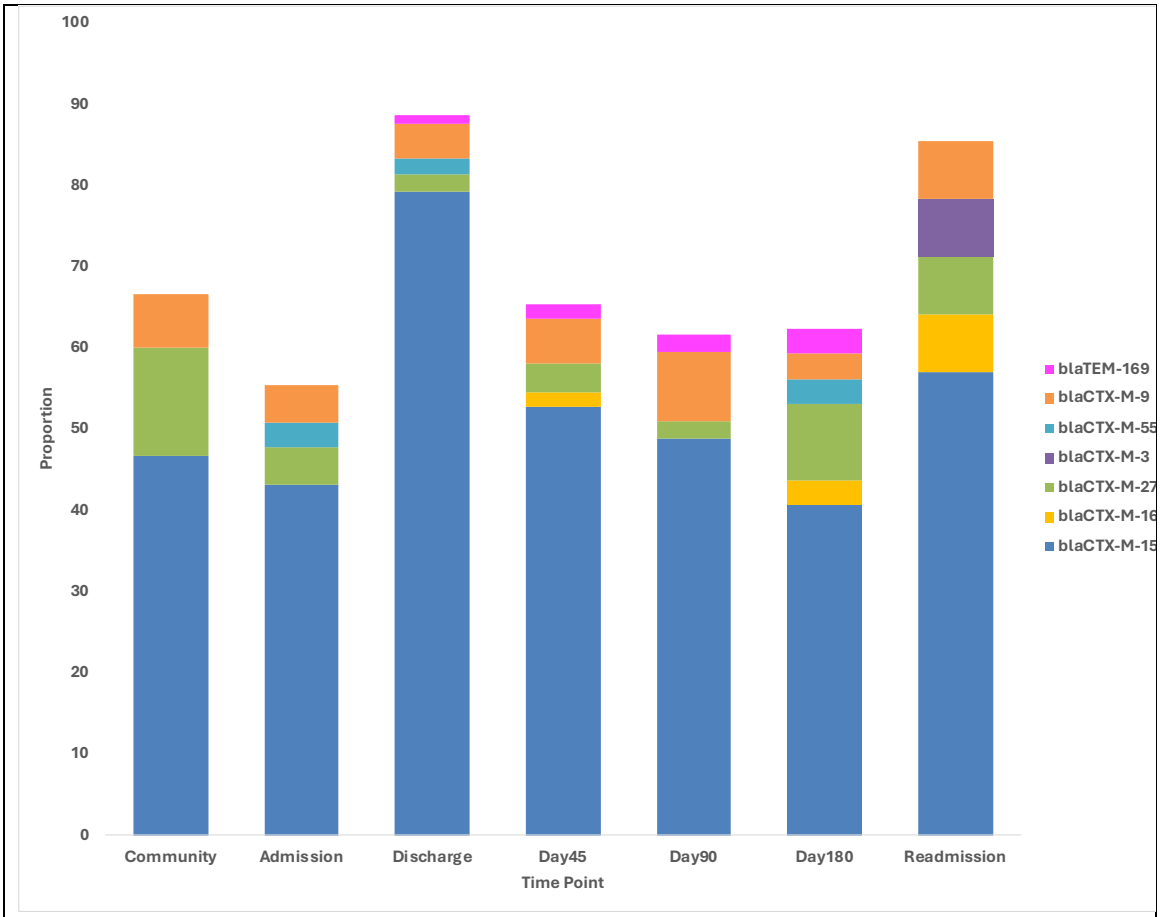


***Figure 6.7a**: ESBL-E genes across time points*

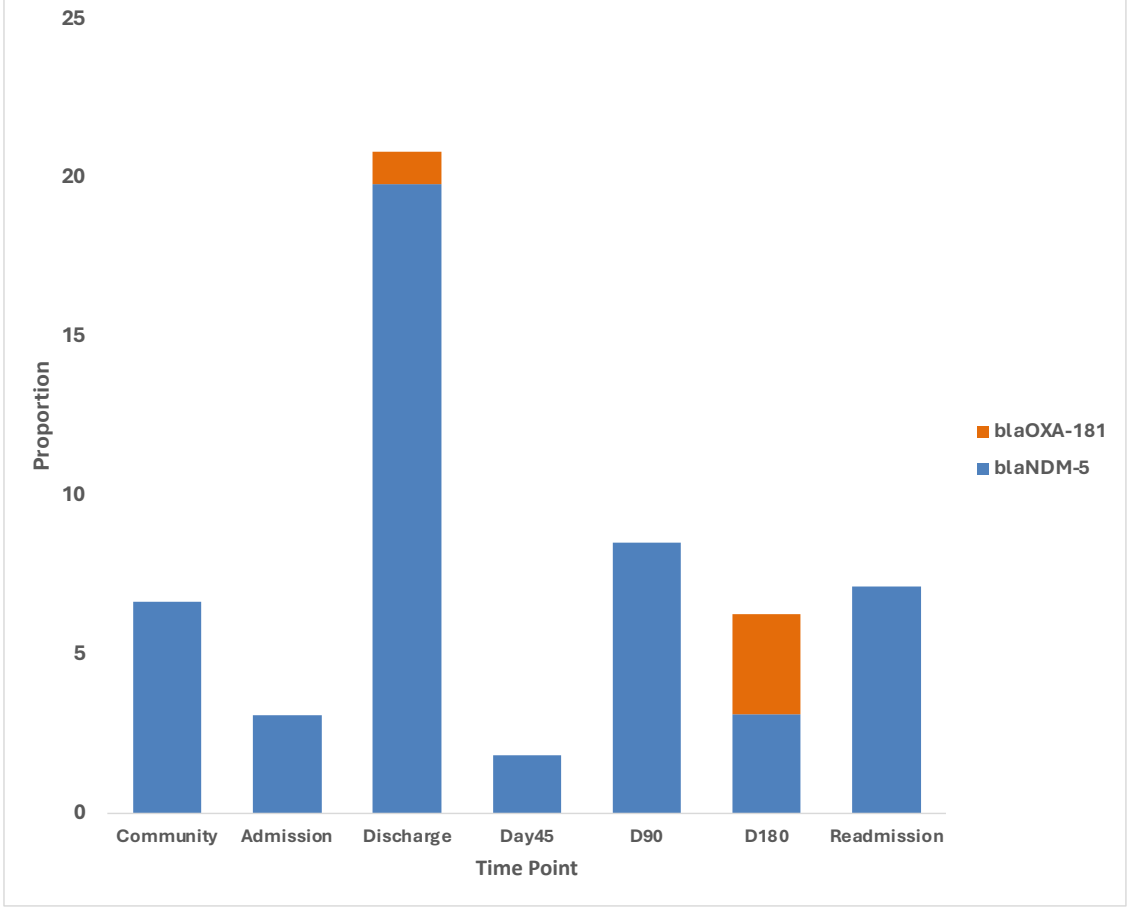


Figure 6.7b: *CRE genes across time point*

Figure 6.7a and 6.7b: *Proportion of ESBL-E and CRE genes across time point*

6.6.3 Virulence genes

Genes thought to be linked to intestinal or extraintestinal disease in humans, included adhesins, invasins, toxins and iron-acquisition genes such as *fyuA*, several *fim* and *pap genes* and *aslA* were identified. I did not detect any of the well-known markers of Enteropathogenic *E. coli* (EPEC) (*eae, bfpA, stx1*, or *stx2*). The most predominant functions of genes detected were those of iron uptake, immune evasion and system secretion.

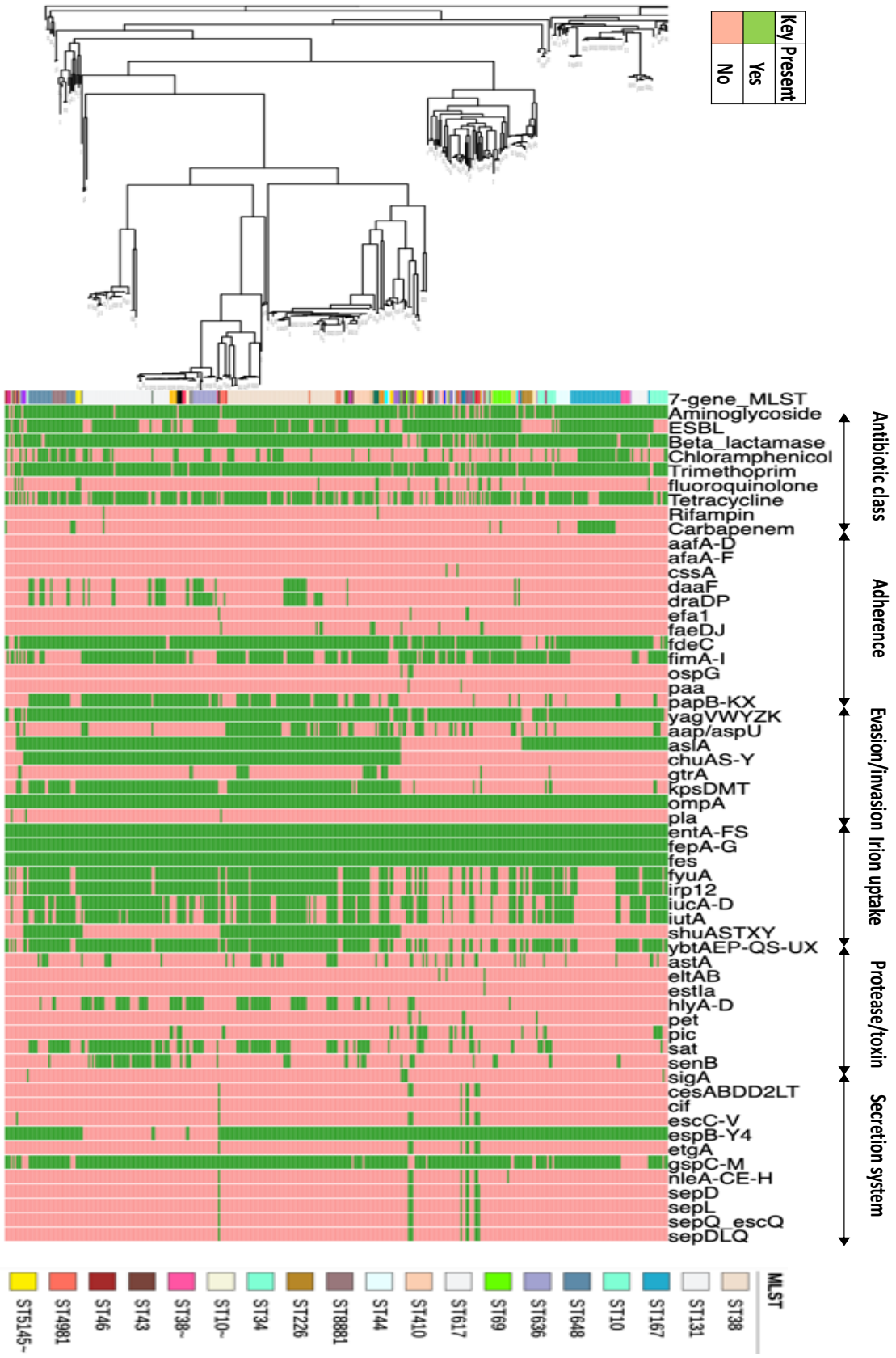


Figure 6.8: *Heatmap showing the distribution of AMR, virulence genes and ST types identified in 538 E. coli genomes from faecal (440), environmental (18) and invasive (80) isolates.*

6.6.4 Sequence Types detected in *E. coli* genomes

Out of the 538 *E. coli* isolates with sequences analysed, 126 unique sequence types were identified. The commonest sequence types identified were ST38, 13% (n=69 isolates), ST131, 10% (n=52) and ST167, 5%(n=29) (Figure 6.9a). The patterns reveal that hospital-associated sequence types, such as ST167, ST410, and ST44, were more prevalent within healthcare settings, whereas while ST10, ST648, ST8881 and ST636 predominated in the community (Figure 6.9b). ST167 has been associated with carbapenemases in other studies[62, 63] while ST131 has been associated CTX-M genes[64, 65]. The heatmap (Figure 6.8) also shows that, some of the ST131 were MDR due to presence of ESBL-producing Enterobacterales (ESBL-E), as well as aminoglycoside, tetracycline and chloramphenicol resistance genes. These were also hypervirulent due to presence of adhesion (fimH and papG), toxins (hlyA,) and iron acquisition (yersiniabactin) (Figure 6.8). On the other hand, ST167 was an NDM producing strain seen to be clustering with carbapenem resistance genes (Figure 6.8) which were predominantly NDM5. ST167 (17/29 cases) and ST410 (9/11) appeared to be acquired in hospital after hospital admission/discharge and with ST167 being seen in readmissions as well. ST38 and ST131was predominant across all time point circulating with an expansion within hospital readmissions seen in 19 cases (Figure 6.9b). Common sequence types between community participants and hospitalised cohort were ST8881, ST648 and ST636. The distribution patterns highlighted that hospital-associated sequence types, including ST167, ST410, ST10, ST131, ST38 and ST648, (Figure 6.9b). Carriage at readmission was dominated by 4 sequence types including ST38, ST131, ST44 and ST167 which was amplified at discharge was found in hospital environment. Sequence types found in hospital environment include ST38, ST69, ST44, ST95 and unknown STs which was the highest seen (Figure 6.9b).

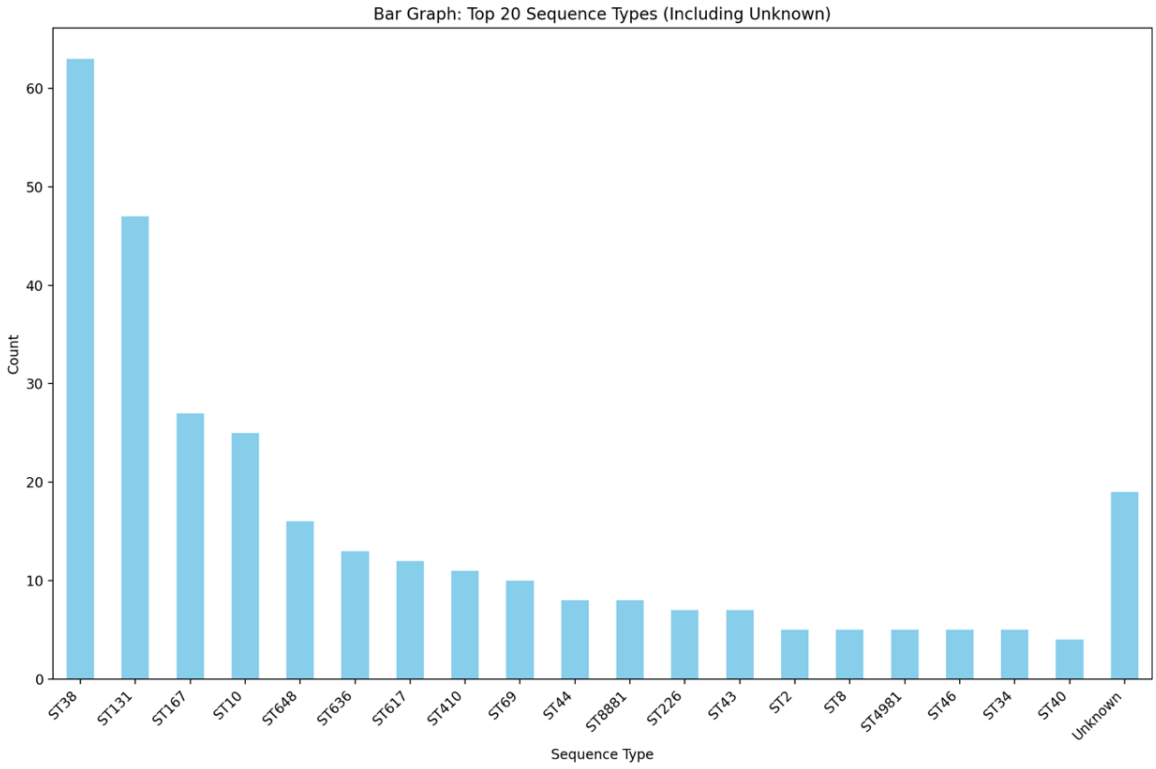


***Figure 6.9a**: Overall count of top 20 sequence types found in E. coli genomes*

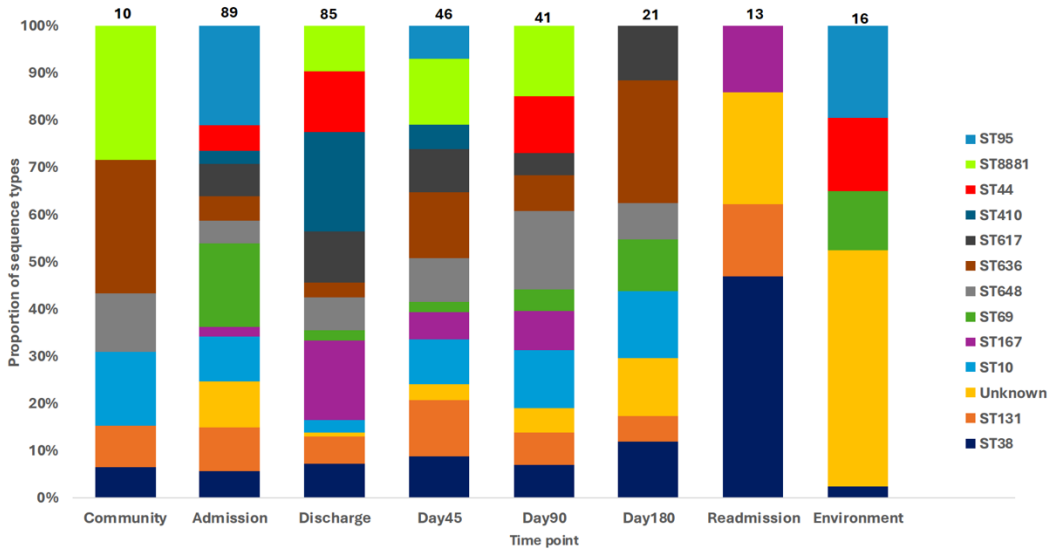


***Figure 6.9b:** This plot illustrates the distribution of 12 sequence types (STs) across different time points and in hospital environment. The numbers above each bar graph indicates the number of isolates with the sequence types.*

***Figure 6. 9a and 6.9b:** Sequence type distribution overall and by time point*

6.6.5 Plasmid analysis

6.6.5.1 Plasmid size and replicon types

Overall, 1881 fully assembled (i.e. circularised) plasmids were reconstructed from 369,003 plasmid contigs/genomes. Of the 1881 circularised plasmids, majority (n=1362) were classified as large plasmids (≥50 kb), 298 as medium-sized (10–50 kb), 148 as small (5–10 kb), and 73 as very small (<5 kb) (Figure 6.10a and 6.10b). Most plasmids (86.9%) carried between 1-4 replicon types, with a median of 1 replicon type (n = 1636/1881)) and 52 plasmids carrying no replicon types. Only circularised plasmids were included after quality control, and a few (n = 193/1881) carried 5 or more replication types (Figure 6.10c). The analysis identified *IncFIA, IncFIB*, and *IncFII* as the most predominant replicon types, with 1252/1881 of plasmids carrying at least one of the 3 types, with *IncFIA* being the most prevalent at 65.4% (1231/1881) (Figure 6.10c and 6.10d).

6.6.5.2 Association of replicon types with ESBL and carbapenemase genes

Among the total of 1881 unique plasmids analysed, 104 (5.5%) plasmids were associated with CRE genes, while 745(39.6%) plasmids were associated with ESBL-E resistance genes (Figure 6.10e and 6.10f). The leading replicon types encoding these ESBL-E resistance genes were *IncFIA* (n=461/745, 17.1%), *IncFIB* (n=417/745, 15.5%) and *IncFII* (n=278/745, 10.3%). For CRE-associated plasmids, the most common replicon types were *IncFIA, IncFIA, IncFIC* (n=50/104,48.1%), followed by ColRNAI_rep_cluster_1987 (n=34/104, 32.7%), IncI-gamma/K1 (n = 19/104, 18.3%), rep_cluster_2350 (n = 19/104, 18.3%), and rep_cluster_1778 (n = 14/104, 13.5%). Notably, 7 replicon types were found to harbour both CRE and ESBL-E resistance genes, representing significant overlap in resistance determinants. These replicon types included *IncFIA*, *IncFIB*, *IncFII*, *IncFIC,* rep_cluster_2131, rep_cluster_1226 and

rep_cluster_2401, with *IncF*-types prominently contributing to the co-carriage of resistance genes, reinforcing their critical role in plasmid-mediated AMR dissemination. (Figure 6.10e and 6.10f).

Overall, replicon types such as *IncFIA, IncFIB* and *IncFII* encoded 13 antibiotic classes each, while *IncFIC, IncQ1* and rep_cluster_2131 encoded 12 antibiotic classes, indicating a specialized role in carrying diverse but specific resistance genes (Figure 6.10e and 6.10f). The most predominant ESBL-E genes identified were $bla_{\text{CTX-M-107}}$ and $bla_{\text{CTX-M-15,}}$ while for CRE genes were $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-21}}$ (Figure 6.10g).

6.6.5.3 Association of plasmid size, other AMR genes and predicted plasmid mobility

Large plasmids (>100 kb) carried the highest number of AMR genes, with 1362 plasmids encoding resistance to an average of 5.57 antibiotic classes each. Medium-sized plasmids (50–100 kb) carried an average of 5.33 antibiotic classes per plasmid. Small plasmids (10–50 kb) carried an average of 4.73 antibiotic classes, while very small plasmids (<10 kb) had the lowest average, at 4.49 antibiotic classes per plasmid (Figure 6.10h).

In terms of predicted mobility, 46.4% (873/1881) of plasmids were classified as mobile, with a majority being large and medium plasmids. These mobile plasmids carried most of the resistance genes (Figure 6.10i and 6.10j). In contrast, non-mobile plasmids accounted for 53.6% (1008/1881). Notably, carbapenem resistance genes were detected in both small and large plasmids, while ESBL genes were present across all plasmid size categories (Figure 6.10h).

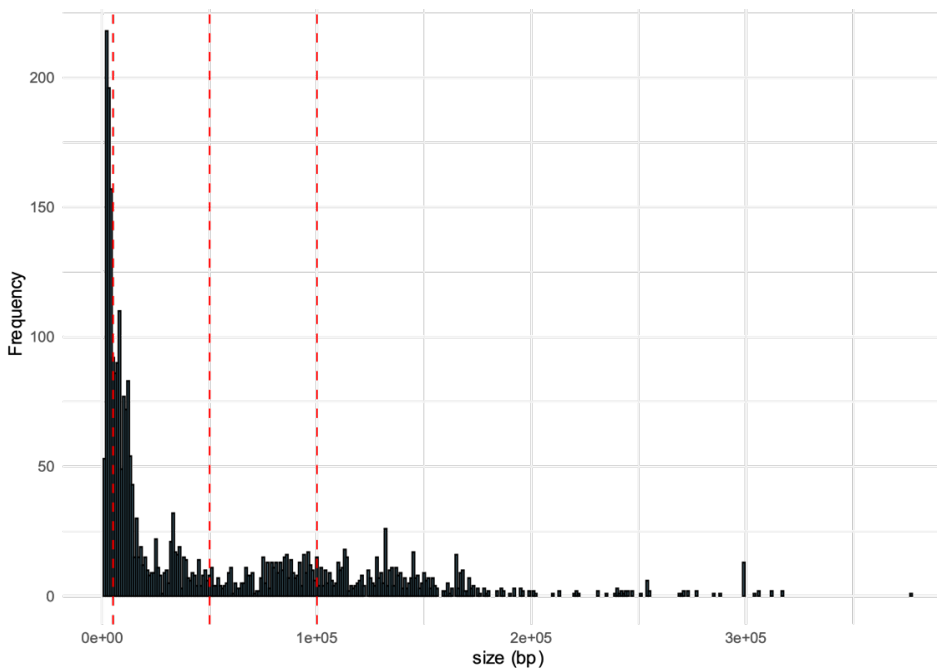


Figure 6.10a: *Distribution of plasmids by size*

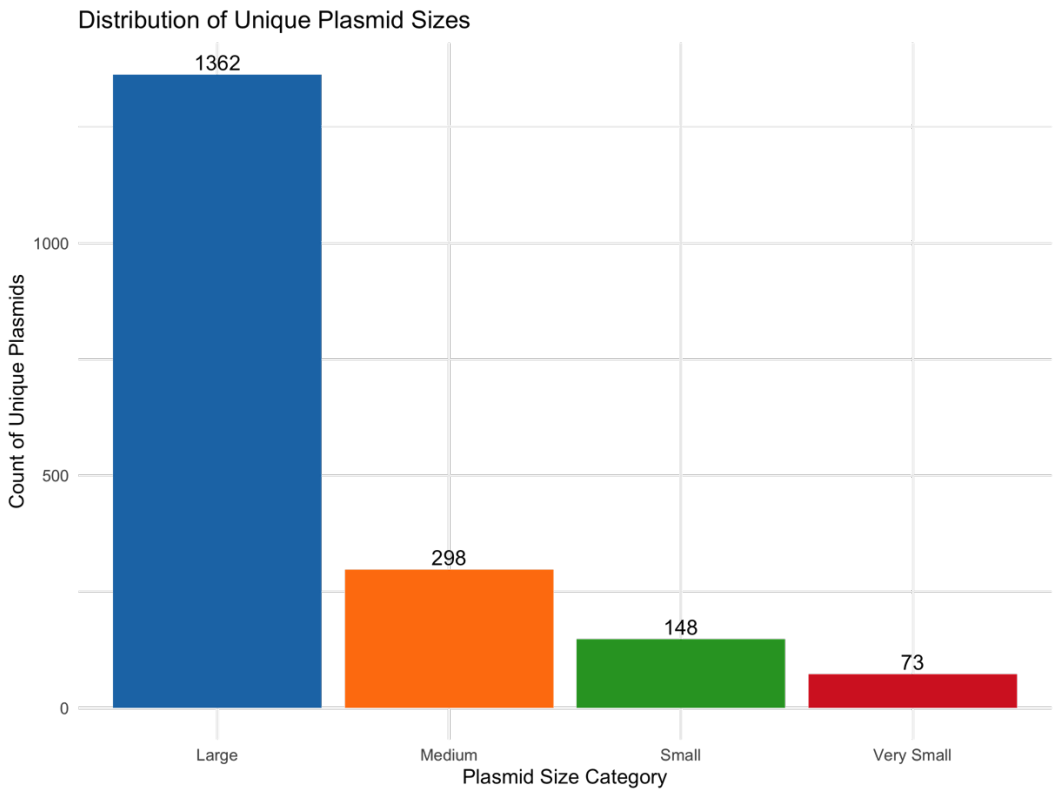


Figure 6.10b: *This bar plot illustrates the distribution of plasmid sizes across four categories. Of the 1881 plasmids analysed, the majority (n = 1362) are classified as large plasmids (≥50 kb). Additionally, 298 are medium-sized (10–50 kb), 148 are small (5–10 kb), and 73 are very small (<5 kb). This distribution highlights the dominance of large plasmids.*

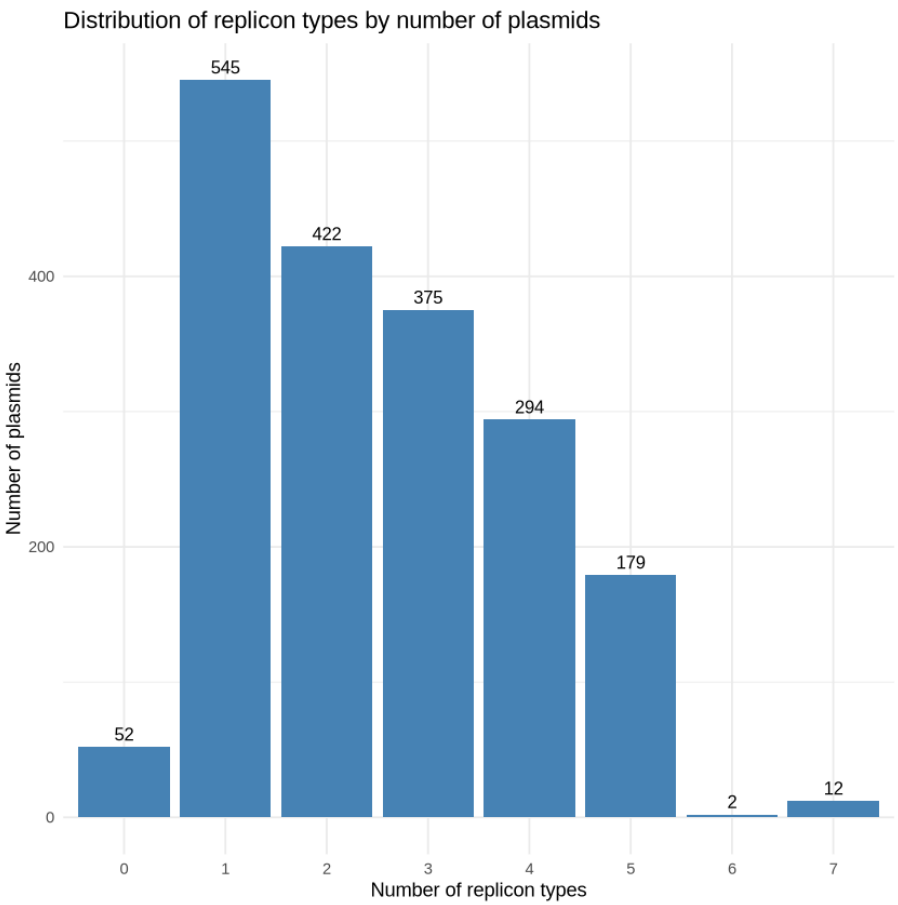


Figure 6.10c: *Distribution of replicon types by plasmids*

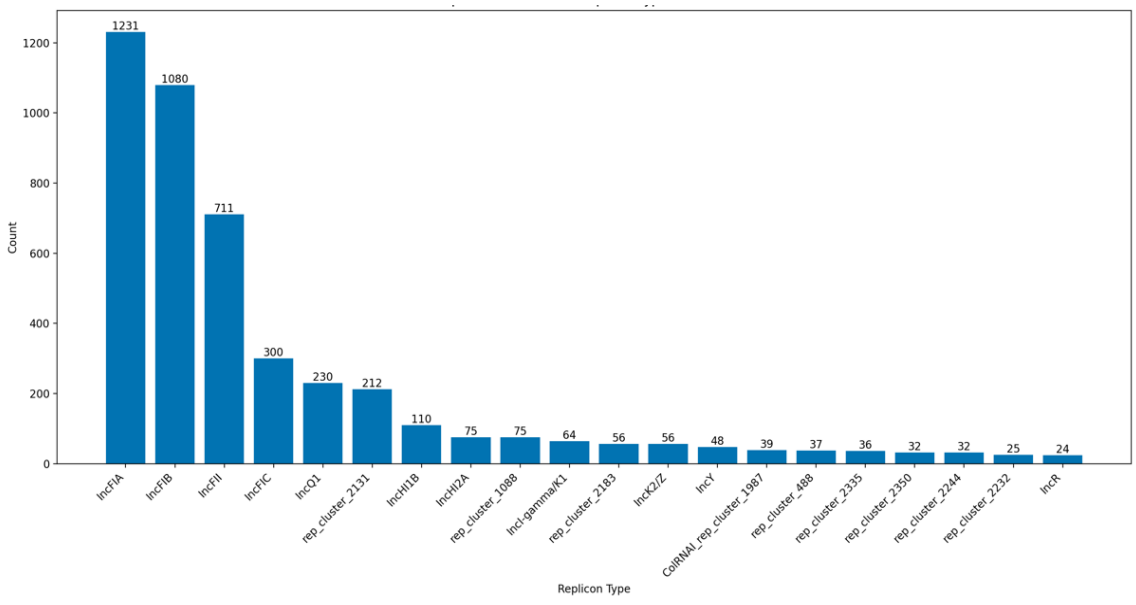


Figure 6.10d: *Count of the most common replicon types in plasmids*

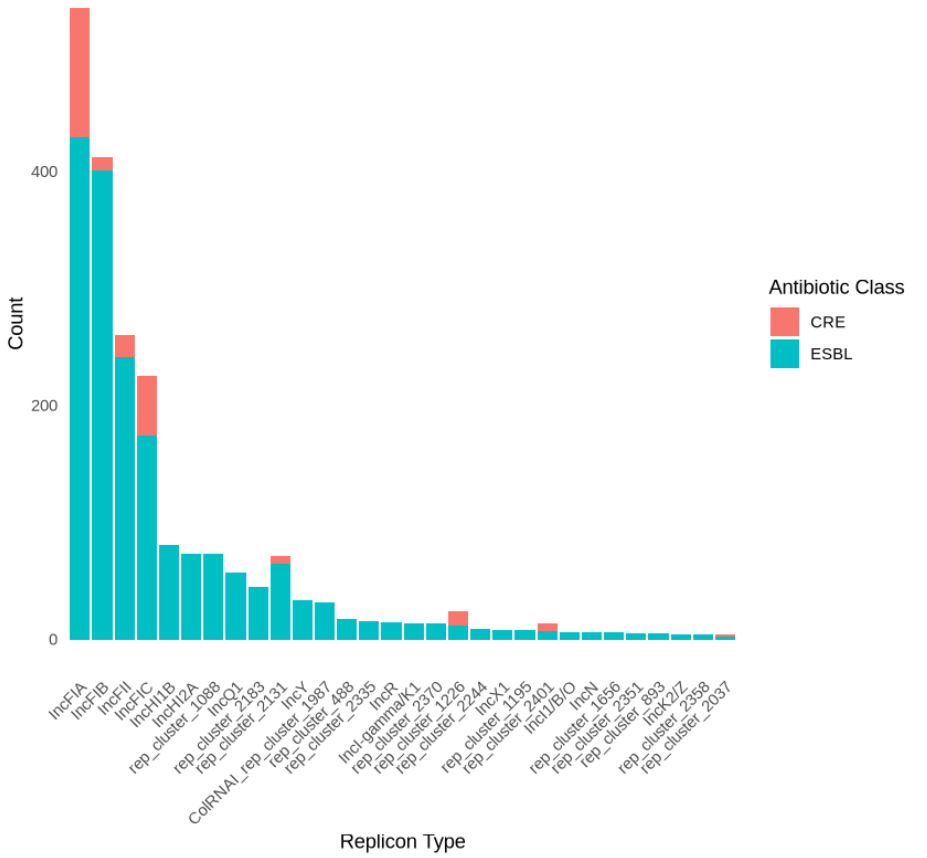


Figure 6.10e: *Count of replicon types with ESBL-E and CRE genes*

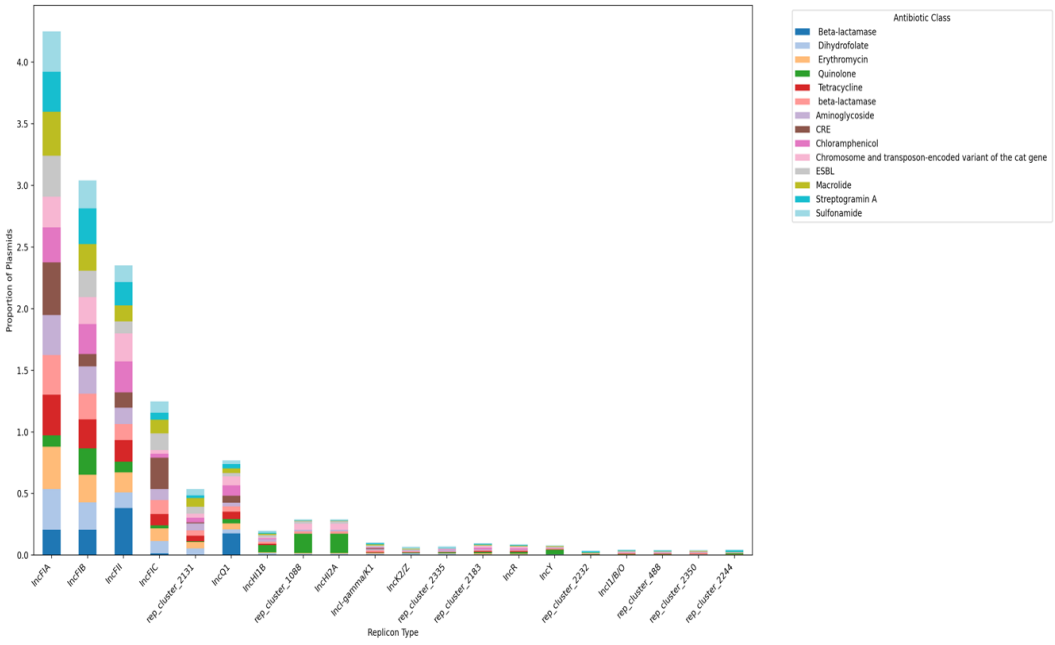


Figure 6.10f: *Proportion of plasmids replicon types by antibiotic class*

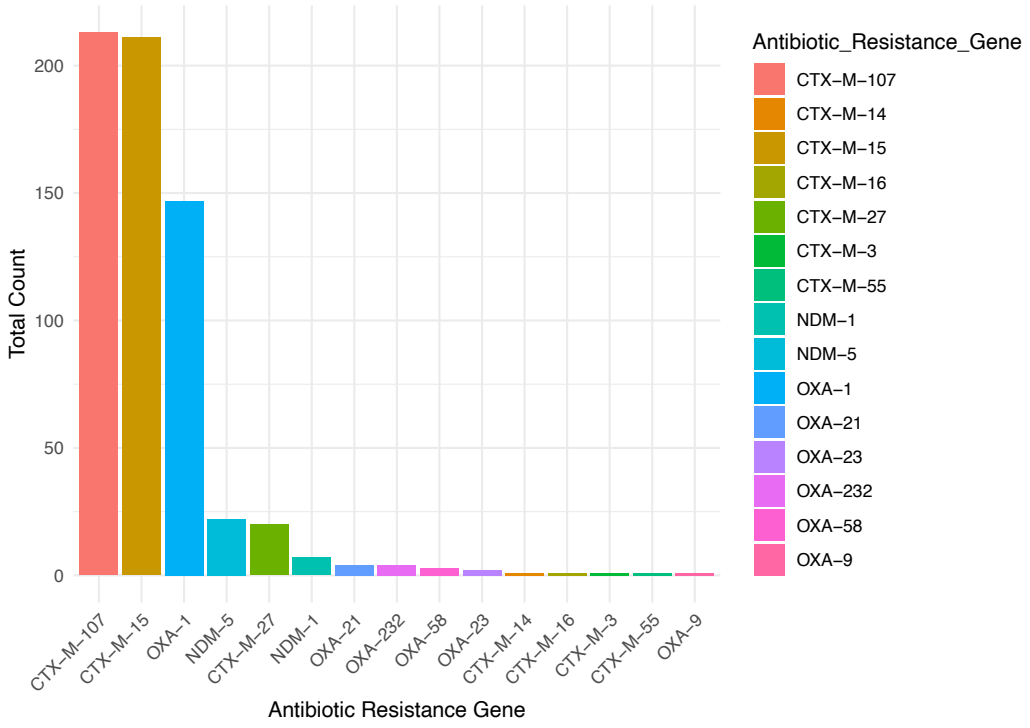


Figure 6.10g: *Total count of ESBL-E and CRE genes located within the plasmids*

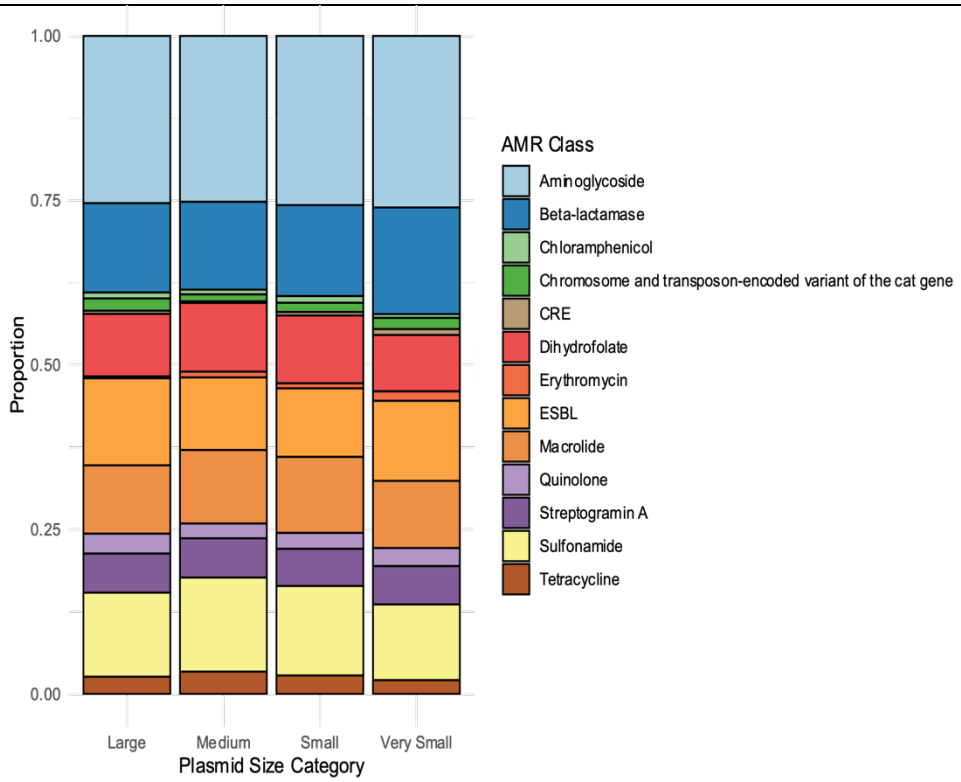
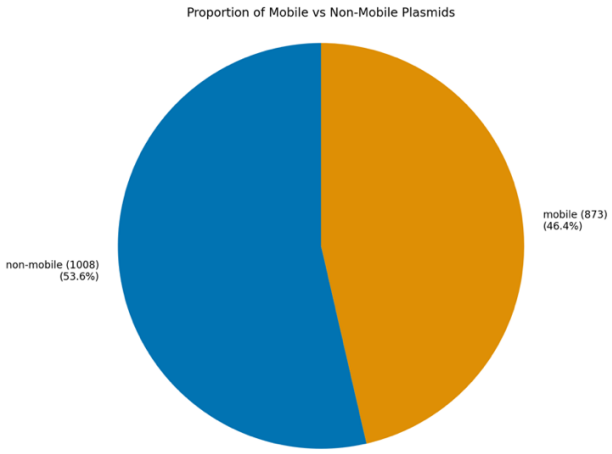


Figure 6.10h: *Distribution of AMR classes by plasmid size*



6.10i: *Proportion of mobile and non-mobile plasmids*

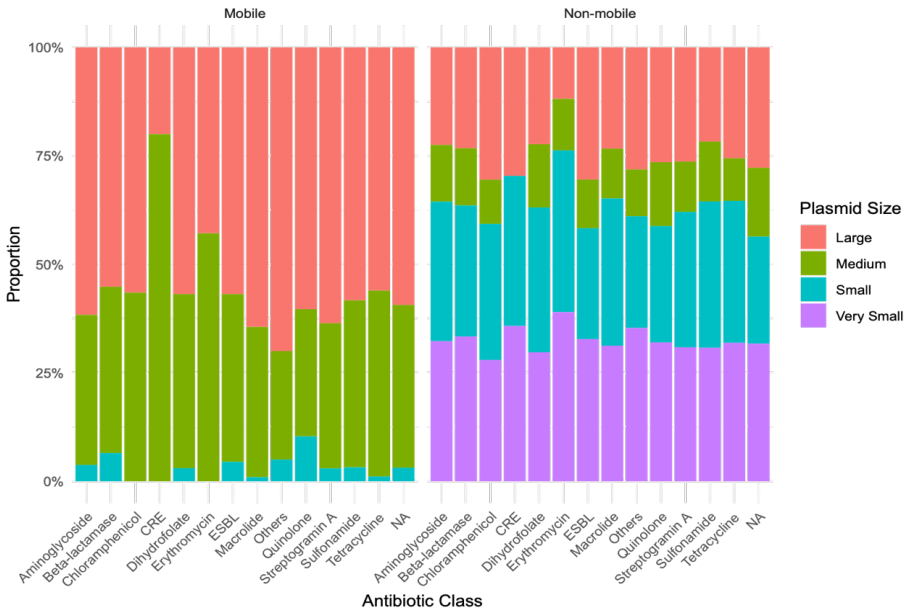


Figure 6.10j: *Proportion of plasmid size by antibiotic class and mobility status showing a large proportion of medium and large plasmids to be mobile*

Figure 6.10a-j: *Figures showing the plasmid types, size, predicted mobility, replicon types and AMR gene's location in the plasmids.*

6.7 Discussion

I found substantial diversity in *E. coli* phylogroups and sequence types (STs) across sites, aligning with other African studies that demonstrate both regional and global patterns of

AMR dissemination[66]. By providing data from under-sampled locations where most of the existing genomic data were from high income countries due to limited laboratory testing and sequencing capacities, this work analysing both short and long read sequence data fills critical gaps on genomic diversity and resistance patterns of *E. coli*, as well as location of AMR genes within the plasmids in diverse settings (healthcare, environmental and carriage in the gut). This study in Kenya, identified 12 distinct phylogroups, compared to the 14 phylogroups documented globally among *E. coli* isolates. The predominant phylogroups were A, D3, B1, B2-1, B2-2 and D1, similar to the results found by Kasanga *et al*., 2024 in Zambia and Lewis *et al.,* 2023 in Malawi, where Phylogroups A, B1, B2 and D were the commonest[50, 66, 67]. Notably, phylogroups B1, B2, D1, and D3, which have been previously linked to invasive disease and linked to ExPEC strains by Horesh *et al*., 2021 and urinary tract infections by Cuénod *et al*., 2023, were well-represented in the population, clustering together with invasive ST types like ST69 with D1, ST131 with B2-1 and B2-2 with ST95[66, 68]. ST95 is a genetic lineage within the larger B2 group, often associated with extraintestinal pathogenic E. coli (ExPEC) strains that can cause infections outside the gut in humans and animals, indicating potential for zoonotic transmission[69].

Among the 126 unique STs identified, several globally recognized high-risk clones associated with invasive infections such as ST38, ST131, ST44, ST69, ST410, and ST648 were detected. These sequence types associated with extended-spectrum beta-lactamase-producing *E. coli* have been linked to severe disease outcomes, including urinary tract infections, recurrent *E. coli* bloodstream infections, mortality and neonatal sepsis, and their presence poses significant treatment challenges due to extensive multidrug resistance[70]. ST131, for instance, is a predominant clone associated with extraintestinal pathogenic *E. coli* (ExPEC) infections globally and is notorious for disseminating extended-spectrum

beta-lactamase (ESBL) genes, including *bla*$_{CTX\text{-}M\text{-}15}$ and *bla*$_{CTX\text{-}M\text{-}14}$. This lineage has been implicated in urinary tract and bloodstream infections and is resistant to fluoroquinolones and other critical antibiotics, compounding treatment difficulties[71]. Similarly, ST69, ST38, and ST648 clones are frequently reported lineages in both hospital- and community-associated infections, considered high-risk due to their widespread distribution and association with diverse antimicrobial resistance determinants[72]. ST410, an emerging clone linked to carbapenemase production and multidrug resistance, represents a particularly significant global threat, with the potential to further drive carbapenem resistance in *E. coli* populations[73]. East Africa have also documented the presence of multidrug-resistant *E. coli* clones, including ST131, ST69, ST410, and ST648[74].

Similarly, ST167, a known producer of New Delhi metallo-beta-lactamase (NDM), was identified in this study, consistent with findings by Aurora *et al*. (2020), which highlight its role as a high-risk clone implicated in outbreaks of carbapenem-resistant infection in hospital-acquired settings[63]. While ST167, ST410, and ST617 were predominantly acquired in hospitals, community-dominant STs such as ST10, ST1881, and ST636 coexisted with ST38 and ST131 across time points, demonstrating shared reservoirs of sequence type determinants between healthcare and community settings, and also seen by Lewis *et al* (2023) as the most predominant sequence types in Malawi[23]. The expansion of ST38 during admission and readmissions is particularly significant. As a high-risk, globally disseminated ExPEC sequence type, ST38 is associated with carbapenem resistance and zoonotic reservoirs, raising concerns about interspecies transmission and its persistence across human and animal populations[75].Its emergence in this cohort aligns with previous reports of its involvement in invasive infections and hospital outbreaks, further underscoring its critical role in the AMR landscape[75]. In Malawi, studies by

Lewis *et al.* (2023) have reported dominant high-risk ESBL-producing sequence types including T131, ST410 and ST167, in *E. coli* colonising adults, implicated by other studies to have a role in carbapenem-resistance, limiting therapeutic options in settings with limited treatment options[23]. Similarly, the coexistence of hospital-acquired and community-dominant STs, with ST38, ST131, ST167, and ST410 frequently identified have been documented in Kenya and Uganda[74].

This study is among the first in Africa to track ESBL-E carriage longitudinally post-discharge, revealing the prolonged dominance of AMR genes like $bla_{\text{CTX-M-15}}$ and $bla_{\text{NDM-5}}$ and similarity in clones being acquired in hospital with those brought into the hospital at admission like $bla_{\text{CTX-M-15}}$ AMR genes and sequence type ST38 being expanded in hospital and spread on plasmids with interchange between hospital and the community, were also seen in Uganda[76]. These two most dominant ESBL family $bla_{\text{CTX-M-15}}$ and carbapenemase encoding gene $bla_{\text{NDM-5}}$ have been seen globally and in Africa too encoded by *IncF* plasmids [23]. The observed genetic intermixing of carriage and invasive strains supports the hypothesis that colonization often precedes invasive disease. Examples such as ST69, ST38, and ST95 validate this hypothesis, highlighting the interconnectedness of colonization and invasive infections[4]. Hospital admissions and readmissions were associated with increased ESBL-E and carbapenem-resistant Enterobacterales (CRE) carriage, suggesting transmission within healthcare settings or selection under antibiotic pressure[77]. This finding aligns with previous African studies emphasizing the role of carriage strains as precursors to infection and underscoring the need for early detection and monitoring as part of infection prevention strategies[4].

The genetic diversity of AMR determinants was considerable, with 5,573 AMR gene variants identified across all major antibiotic classes. Among the 322 ESBL-E variants detected, *bla*$_{\text{CTX-M-15}}$ was the most prevalent, followed by *bla*$_{\text{CTX-M-9}}$ and *bla*$_{\text{CTX-M-27}}$. Carbapenemase production, though less common, was primarily driven by *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{OXA-181}}$. These align with results found in a study conducted in Lusaka, Zambia where *E. coli* in humans, animals and environments had the highest prevalence of *bla*$_{\text{CTX-M-15}}$ in ST131 and *bla*$_{\text{NDM-5}}$, in both environment and clinical settings, illustrating its ease of dissemination across diverse environments[67]. Longitudinal analysis revealed that *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{NDM-5}}$ AMR genes were carried for extended durations post-discharge, indicating potential for community dissemination[76, 78]. The comparatively low circulation of carbapenemase genes in the community versus healthcare settings emphasizes the critical role of hospitals as reservoirs and amplifiers of resistance determinants[67, 76].

Plasmid analysis provided key insights into the mechanisms driving AMR dissemination. The predominant plasmid types, Col and *IncF*, carried diverse resistance genes. *IncF* plasmids, particularly the *IncFII* subtype, were frequent vectors for *bla*$_{\text{CTX-M}}$ genes and were closely associated with ST131 clones, consistent with results reported in a multi-site project conducted in Malawi, Uganda, Kenya and Jordan where *IncF* plasmids had a role in spreading bla$_{\text{CTX-M-15}}$ resistance gene[23]. While these plasmids displayed genetic diversity, they were predominantly restricted to *E. coli* and related species from human and animal sources, reinforcing the interconnectedness of human, animal, and environmental reservoirs in AMR transmission[74]. The dominance of *IncF* plasmids in the population, due to their large size, adaptability, and ability to carry multiple resistance determinants, underscores their critical role in AMR spread and highlights them as potential targets for intervention strategies[79].

There remains significant untapped potential in the data for deeper genomic analyses. More granular approaches such as mapping single nucleotide variant (SNV) distances could refine phylogenetic reconstructions and elucidate genetic relationships within and between STs. Comparative genomic analyses of plasmids could further clarify patterns of resistance gene acquisition and dissemination. Additionally, distinguishing between plasmid- and mobile genetic element-mediated resistance spread could shed light on underlying mechanisms of AMR persistence.

Longitudinal plasmid data analyses could offer critical insights into the dynamics of plasmid persistence and transmission in both hospital and community settings. Identifying specific plasmid types or resistance genes associated with prolonged carriage or readmission could inform targeted interventions. This study provides a foundation for future research into the interconnectedness of AMR reservoirs and offers actionable insights to mitigate the AMR burden given the alarming diversity of resistance mechanisms in the region. Enhanced infection prevention and control (IPC) measures, coupled with genomic surveillance and innovative decolonization strategies, are essential to combat AMR in Africa and globally.

6.8 Limitations

One limitation of this study is the potential bias in isolate selection. Carriage samples were included if they were phenotypically positive at admission, discharge, or any other time point, aiming to represent both positive and negative isolates. This approach, however, may have led to oversampling at certain time points and could have led to unrepresentative ST and gene profiles. Additionally, to identify Enterobacterales in blood cultures, I cultured

them on 5% horse blood agar. Rectal and environmental swabs were also inoculated onto 5% horse blood agar, MacConkey agar and MacConkey agar supplemented with 8% gentamicin. Cefotaxime (30 μg) and ceftazidime (30 μg) antibiotic disks (Oxoid, United Kingdom) were added on the second and fourth streaking zones on the blood agar plate to detect bacteria resistant to third-generation cephalosporins. The use of selective media could have skewed the results by emphasizing antibiotic-resistant strains, particularly ESBL-producing isolates, while potentially underrepresenting other phenotypes. Nevertheless, MacConkey agar likely captured isolates that may have been missed on other media.

Prior antibiotic and healthcare exposure, which is common in hospitalised populations, could have further influenced the resistance profiles and bacterial population structure observed in this study. This confounding factor might partially explain the high prevalence of hospital-associated antimicrobial resistance determinants.

6.9 Conclusions

This chapter contributes to a growing body of evidence emphasizing the interconnectedness of hospital and community reservoirs in AMR dissemination. By linking whole-genome sequencing data to phylogenetic clustering, this study identifying key high-risk clones such as ST38, ST131, and ST167, and plasmid types like *IncF*, these findings align with regional and global trends while providing new insights into the Kenyan context. The observed genetic intermixing between invasive and carriage strains underscores the interconnectedness of reservoirs and the importance of integrated approaches to AMR control.

Children acquired hospital-associated sequence types and AMR genes similar to those carried at admission. The expansion of ST38 during admission and readmission highlights the importance of enhanced infection prevention and control (IPC) measures, such as rigorous hand hygiene practices, environmental decontamination, and antimicrobial stewardship programs, coupled with targeted genomic surveillance strategies like monitoring high-risk clones and plasmid-mediated resistance genes, are essential to curbing the spread of high-risk clones and resistance determinants. Future studies should prioritize exploring the genomic epidemiology of AMR at a finer resolution, particularly focusing on plasmid dynamics, resistance gene contexts, and their role in long-term persistence and transmission.

Integrating genomic surveillance with public health strategies, including vaccination and IPC measures may help reduce invasive *E. coli* infections and mitigate the growing threat of AMR.

REFERENCES

1. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H: **Antibiotic resistance—the need for global solutions**. *The Lancet infectious diseases* 2013, **13**(12):1057-1098.
2. Gerber JS, Kronman MP, Ross RK, Hersh AL, Newland JG, Metjian TA, Zaoutis TE: **Identifying targets for antimicrobial stewardship in children's hospitals**. *Infection Control & Hospital Epidemiology* 2013, **34**(12):1252-1258.
3. Paterson DL: **Resistance in gram-negative bacteria: Enterobacteriaceae**. *American journal of infection control* 2006, **34**(5):S20-S28.
4. Shrivastava SR, Shrivastava PS, Ramasamy J: **World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics**. *Journal of Medical Society* 2018, **32**(1):76.
5. Partridge SR, Kwong SM, Firth N, Jensen SO: **Mobile genetic elements associated with antimicrobial resistance**. *Clinical microbiology reviews* 2018, **31**(4):e00088-00017.
6. Moxon CA, Paulus S: **Beta-lactamases in Enterobacteriaceae infections in children**. *Journal of Infection* 2016, **72**:S41-S49.
7. Ramasethu J: **Prevention and treatment of neonatal nosocomial infections**. *Maternal health, neonatology and perinatology* 2017, **3**:1-11.
8. Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, Knight GM, Robotham JV: **Estimating the burden of antimicrobial resistance: a systematic literature review**. *Antimicrobial Resistance & Infection Control* 2018, **7**(1):58.
9. Chemaly RF, Simmons S, Dale Jr C, Ghantoji SS, Rodriguez M, Gubb J, Stachowiak J, Stibich M: **The role of the healthcare environment in the spread of multidrug-resistant organisms: update on current best practices for containment**. *Therapeutic advances in infectious disease* 2014, **2**(3-4):79-90.
10. Kramer A, Schwebke I, Kampf G: **How long do nosocomial pathogens persist on inanimate surfaces? A systematic review**. *BMC infectious diseases* 2006, **6**(1):130.
11. Jadhav S, Sahasrabudhe T, Kalley V, Gandham N: **The microbial colonization profile of respiratory devices and the significance of the role of disinfection: a blinded study**. *Journal of clinical and diagnostic research: JCDR* 2013, **7**(6):1021.
12. Burgaya J, Marin J, Royer G, Condamine B, Gachet B, Clermont O, Jaureguy F, Burdet C, Lefort A, de Lastours V: **The bacterial genetic determinants of Escherichia coli capacity to cause bloodstream infections in humans**. *PLoS Genetics* 2023, **19**(8):e1010842.
13. Baker S, Thomson N, Weill F-X, Holt KE: **Genomic insights into the emergence and spread of antimicrobial-resistant bacterial pathogens**. *Science* 2018, **360**(6390):733-738.
14. Khezri A, Avershina E, Ahmad R: **Hybrid assembly provides improved resolution of plasmids, antimicrobial resistance genes, and virulence factors in Escherichia coli and Klebsiella pneumoniae clinical isolates**. *Microorganisms* 2021, **9**(12):2560.
15. Juraschek K, Borowiak M, Tausch SH, Malorny B, Käsbohrer A, Otani S, Schwarz S, Meemken D, Deneke C, Hammerl JA: **Outcome of different sequencing and assembly approaches on the detection of plasmids and localization of antimicrobial resistance genes in commensal Escherichia coli**. *Microorganisms* 2021, **9**(3):598.

16. Branger C, Zamfir O, Geoffroy S, Laurans G, Arlet G, Thien HV, Gouriou S, Picard B, Denamur E: **Genetic background of Escherichia coli and extended-spectrum β-lactamase type**. *Emerging infectious diseases* 2005, **11**(1):54.
17. Jean S-S, Harnod D, Hsueh P-R: **Global threat of carbapenem-resistant gram-negative bacteria**. *Frontiers in cellular and infection microbiology* 2022, **12**:823684.
18. Organization WH: **The evolving threat of antimicrobial resistance: options for action: executive summary**. In.: World Health Organization; 2012.
19. Njunge JM, Tickell K, Diallo AH, Shahid ASMSB, Gazi MA, Saleem A, Kazi Z, Ali S, Tigoi C, Mupere E: **The Childhood Acute Illness and Nutrition (CHAIN) network nested case-cohort study protocol: a multi-omics approach to understanding mortality among children in sub-Saharan Africa and South Asia**. *Gates open research* 2022, **6**.
20. Childhood Acute Illness Nutrition Network: **Childhood Acute Illness and Nutrition (CHAIN) Network: a protocol for a multi-site prospective cohort study to identify modifiable risk factors for mortality among acutely ill children in Africa and Asia**. *BMJ Open* 2019, **9**(5):e028454.
21. Andrews S, Krueger F, Segonds-Pichon A, Biggins L, Krueger C, Wingett S: **FastQC**. *A quality control tool for high throughput sequence data* 2010, **370**.
22. Ewels P, Magnusson M, Lundin S, Käller M: **MultiQC: summarize analysis results for multiple tools and samples in a single report**. *Bioinformatics* 2016, **32**(19):3047-3048.
23. Gurevich A, Saveliev V, Vyahhi N, Tesler G: **QUAST: quality assessment tool for genome assemblies**. *Bioinformatics* 2013, **29**(8):1072-1075.
24. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW: **CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes**. *Genome research* 2015, **25**(7):1043-1055.
25. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH: **GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database**. In.: Oxford University Press; 2020.
26. Shen W, Le S, Li Y, Hu F: **SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation**. *PloS one* 2016, **11**(10):e0163962.
27. Abram K, Udaondo Z, Bleker C, Wanchai V, Wassenaar TM, Robeson MS, Ussery DW: **Mash-based analyses of Escherichia coli genomes reveal 14 distinct phylogroups**. *Communications Biology* 2021, **4**(1):117.
28. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, Edalatmand A, Petkau A, Syed SA, Tsang KK: **CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database**. *Nucleic acids research* 2023, **51**(D1):D690-D699.
29. Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, Jin Q: **VFDB: a reference database for bacterial virulence factors**. *Nucleic acids research* 2005, **33**(suppl_1):D325-D328.
30. Seemann T: **Prokka: rapid prokaryotic genome annotation**. *Bioinformatics* 2014, **30**(14):2068-2069.
31. Tonkin-Hill G, MacAlasdair N, Ruis C, Weimann A, Horesh G, Lees JA, Gladstone RA, Lo S, Beaudoin C, Floto RA: **Producing polished prokaryotic pangenomes with the Panaroo pipeline**. *Genome biology* 2020, **21**:1-21.

32. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, Harris SR: **SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments**. *Microbial genomics* 2016, **2**(4):e000056.
33. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R: **IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era**. *Molecular biology and evolution* 2020, **37**(5):1530-1534.
34. Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS: **ModelFinder: fast model selection for accurate phylogenetic estimates**. *Nature methods* 2017, **14**(6):587-589.
35. Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS: **UFBoot2: improving the ultrafast bootstrap approximation**. *Molecular biology and evolution* 2018, **35**(2):518-522.
36. Letunic I, Bork P: **Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation**. *Nucleic acids research* 2021, **49**(W1):W293-W296.
37. Tartof SY, Solberg OD, Manges AR, Riley LW: **Analysis of a uropathogenic Escherichia coli clonal group by multilocus sequence typing**. *Journal of clinical microbiology* 2005, **43**(12):5860-5864.
38. Robertson J, Nash JH: **MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies**. *Microbial genomics* 2018, **4**(8):e000206.
39. Giufrè M, Errico G, Accogli M, Monaco M, Villa L, Distasi MA, Del Gaudio T, Pantosti A, Carattoli A, Cerquetti M: **Emergence of NDM-5-producing Escherichia coli sequence type 167 clone in Italy**. *International journal of antimicrobial agents* 2018, **52**(1):76-81.
40. Garcia-Fernandez A, Villa L, Bibbolino G, Bressan A, Trancassini M, Pietropaolo V, Venditti M, Antonelli G, Carattoli A: **Novel insights and features of the NDM-5-producing Escherichia coli sequence type 167 high-risk clone**. *MSphere* 2020, **5**(2):10.1128/msphere. 00269-00220.
41. Peirano G, Richardson D, Nigrin J, McGeer A, Loo V, Toye B, Alfa M, Pienaar C, Kibsey P, Pitout JD: **High prevalence of ST131 isolates producing CTX-M-15 and CTX-M-14 among extended-spectrum-β-lactamase-producing Escherichia coli isolates from Canada**. *Antimicrobial agents and chemotherapy* 2010, **54**(3):1327-1330.
42. Yu K, Huang Z, Xiao Y, Bai X, Gao H, Wang D: **Epidemiology and molecular characterization of CTX-M-type ESBLs producing Escherichia coli isolated from clinical settings**. *Journal of Global Antimicrobial Resistance* 2024, **36**:181-187.
43. Horesh G, Blackwell GA, Tonkin-Hill G, Corander J, Heinz E, Thomson NR: **A comprehensive and high-quality collection of Escherichia coli genomes and their genes**. *Microbial genomics* 2021, **7**(2):000499.
44. Kasanga M, Gajdács M, Muleya W, Ikhimiukor OO, Mudenda S, Kasanga M, Chizimu J, Shempela DM, Solochi BB, Mwikisa MJ: **Genotypic Characterisation and Antimicrobial Resistance of Extended-Spectrum β-Lactamase-Producing Escherichia coli in Humans, Animals, and the Environment from Lusaka, Zambia: public Health Implications and One Health Surveillance**. *Antibiotics* 2024, **13**(10):951.
45. Cuénod A, Agnetti J, Seth-Smith HM, Roloff T, Wälchli D, Shcherbakov D, Akbergenov R, Tschudin-Sutter S, Bassetti S, Siegemund M: **Bacterial genome-**

wide association study substantiates papGII of Escherichia coli as a major risk factor for urosepsis. *Genome Medicine* 2023, **15**(1):89.
46. Mora Gutiérrez A, López Capón C, Dahbi G, Blanco Álvarez M, Blanco Álvarez JE, Alonso MP, Herrera Estévez A, Mamani Huarani RS, Bonacorsi S, Moulin Schouleur M: **Extraintestinal pathogenic Escherichia coli O1: K1: H7/NM from human and avian origin: detection of clonal groups B2 ST95 and D ST59 with different host distribution**. In*: 2009*: BMC; 2009.
47. Ling W, Paterson DL, Harris PN, Furuya-Kanamori L, Edwards F, Laupland KB: **Mortality, hospital length of stay, and recurrent bloodstream infections associated with extended-spectrum beta-lactamase-producing Escherichia coli in a low prevalence region: A 20-year population-based large cohort study**. *International Journal of Infectious Diseases* 2024, **138**:84-90.
48. Pitout JD, Peirano G, Chen L, DeVinney R, Matsumura Y: **Escherichia coli ST1193: following in the footsteps of E. coli ST131**. *Antimicrobial Agents and Chemotherapy* 2022, **66**(7):e00511-00522.
49. Kocsis B, Gulyás D, Szabó D: **Emergence and dissemination of extraintestinal pathogenic high-risk international clones of Escherichia coli**. *Life* 2022, **12**(12):2077.
50. Pitout JD, Peirano G, Matsumura Y, DeVinney R, Chen L: **Escherichia coli sequence type 410 with carbapenemases: a paradigm shift within E. coli toward multidrug resistance**. *Antimicrobial Agents and Chemotherapy* 2024, **68**(2):e01339-01323.
51. Byarugaba DK, Osman TS, Sayyouh OM, Wokorach G, Kigen CK, Muturi JW, Onyonyi VN, Said MM, Nasrat SA, Gazo M: **Genomic epidemiology of multidrug-resistant Escherichia coli and Klebsiella pneumoniae in Kenya, Uganda, and Jordan**. *Emerging Infectious Diseases* 2024, **30**(Suppl 2):S33.
52. Lewis JM, Mphasa M, Banda R, Beale MA, Mallewa J, Anscome C, Zuza A, Roberts AP, Heinz E, Thomson NR: **Genomic analysis of extended-spectrum beta-lactamase (ESBL) producing Escherichia coli colonising adults in Blantyre, Malawi reveals previously undescribed diversity**. *Microbial Genomics* 2023, **9**(6):001035.
53. Fonseca EL, Morgado SM, Caldart RV, Vicente AC: **Global genomic epidemiology of Escherichia coli (ExPEC) ST38 lineage revealed a virulome associated with human infections**. *Microorganisms* 2022, **10**(12):2482.
54. Hounmanou YMG, Wanyana A, Alafi S, Wabwire-Mangen F, Christensen H, Olsen JE, Byarugaba DK: **Whole strains vs MGEs in short and longterm transmission of ESBL genes between healthcare and community settings in Uganda**. *Scientific Reports* 2023, **13**(1):10229.
55. Lewis JM, Mphasa M, Banda R, Beale MA, Heinz E, Mallewa J, Jewell C, Faragher B, Thomson NR, Feasey NA: **Colonization dynamics of extended-spectrum beta-lactamase-producing Enterobacterales in the gut of Malawian adults**. *Nature microbiology* 2022, **7**(10):1593-1604.
56. Kagia N KP, Ooko M, Wafula L, Mturi N, Anampiu K, Mwarumba S, Njuguna P, Seale AC, Berkley JA, Bottomley C, Scott JAG, Morpeth SC.: **Carriage and Acquisition of Extended-spectrum β-Lactamase-producing Enterobacterales Among Neonates Admitted to Hospital in Kilifi, Kenya**.
57. El Chaar M, Khoury Y, Douglas GM, El Kazzi S, Jisr T, Soussi S, Merhi G, Moghnieh RA, Shapiro BJ: **Longitudinal genomic surveillance of multidrug-resistant Escherichia coli carriage in critical care patients**. *Microbiology Spectrum* 2024, **12**(2):e03128-03123.

58. Vrancianu CO, Popa LI, Bleotu C, Chifiriuc MC: **Targeting plasmids to limit acquisition and transmission of antimicrobial resistance**. *Frontiers in microbiology* 2020, **11**:761.

7 OVERALL THESIS DISCUSSION AND FUTURE WORK

This thesis advances our understanding of AMR dynamics in children, particularly focusing on extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) and carbapenem-resistant Enterobacterales (CRE) in Kenyan hospital and community settings. Through detailed literature review of healthcare acquired Gram negative blood stream infections, an analysis of antimicrobial prescribing patterns in hospital, the phenotypic and genomic analyses of antimicrobial resistance focusing on both clinical and genomic determinants of AMR, this work highlights the substantial amplification of AMR within hospitals, the genetic overlap between community and hospital strains, and the complex factors driving acquisition and persistence of resistance. The initial literature review of the burden of AMR in African Healthcare Settings has expanded and added knowledge on existing data on ESBL-E and CRE that significantly contribute to healthcare-acquired Gram-negative bloodstream infections (HA-GNBSIs) among children and neonates, with *K. pneumoniae* and *E. coli* being the predominant pathogens also observed in a global neonatal sepsis observational cohort study (NeoOBS), the CHAMPS & BARNARDS networks and a systematic review by Zelellw *et al*., [1-4]. High ESBL-E prevalence at discharge (71%) with persistence post-discharge (27%) at six months illustrates the enduring impact of hospital-acquired colonization. Detailed analysis of daily hospital treatment data has highlighted the critical role of antibiotic prescribing practices and infection control measures in curbing the burden of AMR.

Chapter 3 synthesised findings from existing literature and meta-analyses on HA-GNBSIs caused by ESBL-E and CRE in children across Africa. These infections are characterized by high morbidity and mortality, with *K. pneumoniae, A. baumannii* and *E. coli* being the predominant ESBL and CRE pathogens in both children and neonates and have been

associated with high morbidity and mortality in these groups as identified in the recent study by CHAMPs where *E. coli*, *A. baumanii* and *K. pneumoniae* were in the causal pathway of 87% of deaths associated with multidrug resistant nosocomial infections[5]. Some of the major factors identified to be associated with HA-GNBSIs were lower age, antibiotic use in before and during hospitalisation, prolonged admission in hospital or ICU and malnutrition similar with what has been documented before[6, 7]. The findings emphasise the high prevalence of resistant infections, the limited availability of appropriate diagnostics, and suboptimal implementation of IPC measures that should target identified associated factors through vaccine development and improved/new therapeutics.

Chapter 4 is a detailed analysis of daily hospital data on antimicrobial treatment in acutely ill hospitalised children aged 2-23 months across nine hospitals in Africa and South Asia has provided critical insights into antimicrobial prescribing patterns. My findings highlight a high prevalence of antimicrobial use, with most children receiving antimicrobials shortly after admission. 35% of children received WHO Watch antimicrobials, while Reserve antimicrobials were underutilized despite elevated risks of resistant infections. While adherence to WHO guidelines was observed, there was some evidence of overuse, particularly in cases without clear indications, and underuse in children at elevated risk for mortality or resistant infections. The limited escalation and de-escalation of therapy reflect the challenges of applying guidelines in the absence of relevant antimicrobial sensitivity data hence the need for updated risk-based prescribing guidelines. Inappropriate prescribing, particularly in cases of uncomplicated illnesses, and the lack of targeted antimicrobial use in high-risk groups such as those with severe malnutrition, calls for more nuanced approaches. Additionally, limitations in microbiological capacity and the generalizability of pooled antibiograms further constrain appropriate prescribing[8]. These

challenges, compounded by the high early mortality rate and lack of paediatric formulations, highlight significant gaps in current care. Future work should prioritize clinical trials to evaluate risk-differentiated antimicrobial treatment pathways rather than focusing solely on individual drugs. Such trials could address key questions about the timing and choice of antimicrobials, particularly in severely ill children with prior antibiotic exposure or hospital-acquired infections[9]. Additionally, developing syndrome and context-specific antibiograms tailored to paediatric populations in LMICs could greatly enhance empiric prescribing and improve outcomes[10]. Investments in laboratory capacity for culture and sensitivity testing are essential, as are efforts to refine and expand antimicrobial stewardship training. Further research is needed to understand the impact of guidelines on prescribing practices and to explore strategies for improving the timely initiation of appropriate antimicrobials in high-risk groups. Finally, ongoing surveillance and detailed analysis of antimicrobial use should continue, integrating real-time clinical and microbiological data to inform adaptive and context-specific interventions. By addressing these gaps, we can move closer to achieving an optimal balance between reducing inappropriate antimicrobial use and ensuring adequate treatment for vulnerable paediatric populations, ultimately mitigating the global threat of antimicrobial resistance. Limited capacity for microbial diagnostics and empiric prescribing practices highlight the need for enhanced antimicrobial stewardship.

Chapter 5 reveals that nearly three-quarters of children discharged from hospitals carry ESBL-E, with more than a quarter still colonized six months post-discharge. This persistence highlights the long-term impact of hospital-acquired colonization. Risk factors such as prolonged/previous hospital stays, use of antibiotics before and after admission, underlying conditions and readmission to hospital remained significant contributors to the

risk of ESBL-E acquisition and carriage. Limited resources, poor hygiene practices, and overcrowding exacerbate these challenges, while inadequate access to microbiological diagnostics hinders effective treatment and surveillance addresses the critical challenges identified in this chapter, future work should focus on several key areas. Enhanced infection control measures are paramount, particularly through strengthening IPC programs in neonatal and intensive care units. These efforts should aim to improve hand hygiene compliance, optimize the use of invasive devices, and mitigate hospital overcrowding by expanding access to water, sanitation, and hygiene (WASH) and improved antimicrobial stewardship to reduce infection transmission in healthcare settings[11]. Antimicrobial stewardship programs should be developed and implemented to curb inappropriate antibiotic use in these settings. Clinical trials should be conducted to evaluate empiric regimens based on mortality risk and the likelihood of resistant infections. Additionally, they should assess the safety, efficacy, and cost-effectiveness of targeted escalation to Watch or Reserve antibiotics for high-risk groups. Strengthening research and healthcare infrastructure is crucial to address disparities in surveillance and healthcare resources across different regions[12]. Inclusive research efforts should be promoted to generate comprehensive data on HAIs in secondary and rural healthcare settings. Collaborative approaches can help bridge these gaps and inform better practices. Policy development and implementation are vital for long-term impact. Advocacy efforts should focus on updating WHO and national guidelines to incorporate timely escalation and de-escalation of antimicrobial therapy based on local resistance patterns and patient risk factors. Policies prioritizing investments in healthcare systems are necessary to mitigate the impact of HAIs and AMR. By addressing these gaps, future research and interventions can significantly improve the management of HA-GNBSIs and reduce the burden of AMR among vulnerable paediatric populations in Africa.

Chapter 6 provides critical insights into the genomic epidemiology and AMR dynamics of *E. coli*, with a particular focus on ESBL-E and CRE. Among the key findings, common ESBL-E genotypes identified in both hospital and community settings included CTX-M-15, CTX-M-16, and CTX-M-27, while clustered occurrences of CTX-M-9 and CTX-M-55 were also observed a study done in East Africa and Malawi[9, 13-15]. CRE were not detected in community settings, but in hospitals and post-discharge environments, NDM-5 emerged as the most prevalent genotype, particularly in urban areas such as Nairobi. Plasmid-mediated CTX-M genes exhibited distinct differences from chromosomal genes, with CTX-M-107 being the most frequent plasmid-associated type, followed by CTX-M-15. Carriage of *E. coli* sequence types (STs) showed significant diversity, with ST38 and ST131 being the most common, both of which are associated with invasive disease[16]. Between hospital admission and discharge, the prevalence of ST167 increased, likely reflecting its origin in hospital reservoirs. The hospital environments plays a role in amplifying AMR transmission, driven by plasmid-mediated resistance patterns that contribute to the persistence and dissemination of resistant strains[17]. Key determinants of AMR acquisition include prolonged hospital stays, pre-hospital antibiotic exposure, malnutrition, and the use of invasive devices. ESBL-E carriage at discharge significantly increases the likelihood of readmissions, representing a substantial healthcare burden. Dominant AMR clones tend to cluster within hospital settings but often diminish in the community by six months post-discharge. Genomic data suggest that local strains are less persistent in community settings, with community transmission occurring but potentially mitigated by post-discharge mortality. *E. coli* and *K. pneumoniae* were identified as leading pathogens in chapter 3, with critical implications for neonatal sepsis prevention. Maternal vaccines targeting these pathogens show promise, supported by ongoing efforts from the

Bill & Melinda Gates Foundation and researchers such as Professor Katherine Holt at LSHTM, who are exploring combined vaccines for *E. coli* and *K. pneumoniae*[18]. Further genomic analysis, including O and H antigen profiling of *E. coli* strains will be done on my data to give more insights essential to guide vaccine development suitable for our setting. Future directions for this research include granular genomic analyses, such as high-resolution phylogenetic reconstructions using single nucleotide variant (SNV) distances, and comparative studies of resistance plasmids to elucidate mechanisms of plasmid- and mobile genetic element-mediated AMR dissemination. Longitudinal studies are needed to examine plasmid persistence and transmission, particularly the transition between hospital and community environments. Integrating genomic surveillance with routine public health measures is vital, focusing on high-risk clones such as ST38, ST131, and ST167 to refine early detection and intervention strategies[17]. Incorporating decolonisation of dominant AMR clones, STs and plasmids, complemented by antimicrobial stewardship and environmental decontamination may be useful in reducing transmission. Policy and public health efforts must incorporate these findings into vaccination strategies to reduce invasive infection rates and mitigate AMR threats. Collaborative efforts across healthcare and community settings will be essential to address AMR's multifaceted challenges. Innovative approaches, such as vaccines and probiotics, should also be explored to reduce colonization and transmission of resistant strains, alongside risk stratification strategies to facilitate early discharge of low-risk patients and minimize AMR acquisition. Integrating microbiome data with whole-genome sequencing could enhance understanding of AMR acquisition and loss dynamics. Expanding sample sizes and increasing longitudinal time points will provide more comprehensive analyses. Comparative genomic studies should contextualize these findings within broader African and global AMR patterns, leveraging international

consortia to improve data-sharing and capacity-building like the GRAM project and WHO GLASS initiative[19-22].

This research highlights the complex interplay between hospital and community reservoirs in AMR dissemination, emphasizing the critical role of genomic surveillance in identifying resistance determinants. Findings contribute essential data from underrepresented settings, aligning with regional and global trends, and providing actionable insights to inform public health interventions and policy. Current *E. coli* vaccines, which cover limited antigens, underscore the necessity of global surveillance to guide antigen selection. By contributing valuable genomic data, this thesis advances efforts to address these challenges.

This work has shown interconnectedness of hospital and community environments in driving AMR transmission among children in Kenya. By integrating phenotypic and genomic insights, this thesis provides a foundation for targeted interventions to combat AMR in resource-limited settings. Moving forward, sustained investments in research, healthcare infrastructure, and capacity-building are essential to safeguard the health of vulnerable paediatric populations and curb the global threat of AMR. Overall, much prior data on ESBL-E and CRE in the sub-Saharan Africa context is limited by the kinds of institutions able to undertake microbiology and publish results. In three Kenyan public hospitals, my findings primarily suggest circulation of ESBL-E between hospitals and communities, with substantial amplification in hospital, mostly with internationally common AMR genes. Carbapenem use is currently very limited in public hospitals, with CRE genes generally limited to Nairobi. Most children admitted to hospitals in sub-Saharan Africa and South Asia meet criteria for antibiotic initiation. However, prescribing guidelines do not fully consider AMR or mortality risk. As a result, antibiotic treatment is

too infrequently adjusted up or down beyond initial guideline recommendations. These results will help guide targeted antimicrobial stewardship, enhance IPC measures, and support the development of evidence-based guidelines to combat AMR.

REFERENCES

1. Russell NJ, Stöhr W, Plakkal N, Cook A, Berkley JA, Adhisivam B, Agarwal R, Ahmed NU, Balasegaram M, Ballot D: **Patterns of antibiotic use, pathogens, and prediction of mortality in hospitalized neonates and young infants with sepsis: A global neonatal sepsis observational cohort study (NeoOBS)**. *PLoS Medicine* 2023, **20**(6):e1004179.
2. Zelellw DA, Dessie G, Worku Mengesha E, Balew Shiferaw M, Mela Merhaba M, Emishaw S: **A Systemic Review and Meta-analysis of the Leading Pathogens Causing Neonatal Sepsis in Developing Countries**. *BioMed research international* 2021, **2021**(1):6626983.
3. Verani JR, Blau DM, Gurley ES, Akelo V, Assefa N, Baillie V, Bassat Q, Berhane M, Bunn J, Cossa AC: **Child deaths caused by Klebsiella pneumoniae in sub-Saharan Africa and south Asia: a secondary analysis of Child Health and Mortality Prevention Surveillance (CHAMPS) data**. *The Lancet Microbe* 2024, **5**(2):e131-e141.
4. Sands K, Carvalho M, Portal E, Thomson K, Dyer C, Akpulu C, Group B: **Characterization of antimicrobial-resistant Gram-negative bacteria that cause neonatal sepsis in seven low-and middle-income countries. Nat Microbiol. 2021; 6 (4): 512–23**. *DOI: https://doi org/101038/s41564-021-00870-7 PMID: https://www ncbi nlm nih gov/pubmed/33782558*:512-523.
5. Bassat Q, Blau DM, Ogbuanu IU, Samura S, Kaluma E, Bassey I-A, Sow S, Keita AM, Tapia MD, Mehta A: **Causes of death among infants and children in the child health and mortality prevention surveillance (CHAMPS) network**. *JAMA Network Open* 2023, **6**(7):e2322494-e2322494.
6. Labi A-K, Bjerrum S, Enweronu-Laryea CC, Ayibor PK, Nielsen KL, Marvig RL, Newman MJ, Andersen LP, Kurtzhals JA: **High carriage rates of multidrug-resistant gram-negative bacteria in neonatal intensive care units from Ghana**. In: *Open Forum Infectious Diseases: 2020*: Oxford University Press US; 2020: ofaa109.
7. Ndir A, Diop A, Faye PM, Cissé MF, Ndoye B, Astagneau P: **Epidemiology and burden of bloodstream infections caused by extended-spectrum beta-lactamase producing Enterobacteriaceae in a pediatric hospital in Senegal**. *PLoS One* 2016, **11**(2):e0143729.
8. Hasegawa S, Livorsi DJ, Perencevich EN, Church JN, Goto M: **Diagnostic accuracy of hospital antibiograms in predicting the risk of antimicrobial resistance in enterobacteriaceae isolates: a nationwide multicenter evaluation at the Veterans Health Administration**. *Clinical infectious diseases* 2023, **77**(11):1492-1500.
9. Turner RM, Omar RZ, Yang M, Goldstein H, Thompson SG: **A multilevel model framework for meta-analysis of clinical trials with binary outcomes**. *Statistics in medicine* 2000, **19**(24):3417-3432.
10. Lim C, Ashley EA, Hamers RL, Turner P, Kesteman T, Akech S, Corso A, Mayxay M, Okeke IN, Limmathurotsakul D: **Surveillance strategies using routine microbiology for antimicrobial resistance in low-and middle-income countries**. *Clinical Microbiology and Infection* 2021, **27**(10):1391-1399.
11. Gerber JS, Kronman MP, Ross RK, Hersh AL, Newland JG, Metjian TA, Zaoutis TE: **Identifying targets for antimicrobial stewardship in children's hospitals**. *Infection Control & Hospital Epidemiology* 2013, **34**(12):1252-1258.

12. America IDSo: **Combating antimicrobial resistance: policy recommendations to save lives**. *Clinical Infectious Diseases* 2011, **52**(suppl_5):S397-S428.
13. Lewis JM, Mphasa M, Banda R, Beale MA, Mallewa J, Anscome C, Zuza A, Roberts AP, Heinz E, Thomson NR: **Genomic analysis of extended-spectrum beta-lactamase (ESBL) producing Escherichia coli colonising adults in Blantyre, Malawi reveals previously undescribed diversity**. *Microbial Genomics* 2023, **9**(6):001035.
14. Lewis JM, Lester R, Garner P, Feasey NA: **Gut mucosal colonisation with extended-spectrum beta-lactamase producing Enterobacteriaceae in sub-Saharan Africa: a systematic review and meta-analysis**. *Wellcome open research* 2019, **4**.
15. Burgaya J, Marin J, Royer G, Condamine B, Gachet B, Clermont O, Jaureguy F, Burdet C, Lefort A, de Lastours V: **The bacterial genetic determinants of Escherichia coli capacity to cause bloodstream infections in humans**. *PLoS Genetics* 2023, **19**(8):e1010842.
16. Ling W, Paterson DL, Harris PN, Furuya-Kanamori L, Edwards F, Laupland KB: **Mortality, hospital length of stay, and recurrent bloodstream infections associated with extended-spectrum beta-lactamase-producing Escherichia coli in a low prevalence region: A 20-year population-based large cohort study**. *International Journal of Infectious Diseases* 2024, **138**:84-90.
17. Garcia-Fernandez A, Villa L, Bibbolino G, Bressan A, Trancassini M, Pietropaolo V, Venditti M, Antonelli G, Carattoli A: **Novel insights and features of the NDM-5-producing Escherichia coli sequence type 167 high-risk clone**. *MSphere* 2020, **5**(2):10.1128/msphere. 00269-00220.
18. Kumar CK, Sands K, Walsh TR, O'Brien S, Sharland M, Lewnard JA, Hu H, Srikantiah P, Laxminarayan R: **Global, regional, and national estimates of the impact of a maternal Klebsiella pneumoniae vaccine: A Bayesian modeling analysis**. *PLoS medicine* 2023, **20**(5):e1004239.
19. **Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2016-2017** [https://www.who.int/publications/i/item/9789241513449]
20. Organization WH: **GLASS manual for antimicrobial resistance surveillance in common bacteria causing human infection**: World Health Organization; 2023.
21. Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, Aguilar GR, Mestrovic T, Smith G, Han C: **Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050**. *The Lancet* 2024, **404**(10459):1199-1226.
22. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, Han C, Bisignano C, Rao P, Wool E: **Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis**. *The lancet* 2022, **399**(10325):629-655.