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Community and Clinical Resistomes and Mobilomes: A Correlation

A dissertation submitted in fulfilment of the requirements for the degree of Master of Medical Science (Medical Microbiology) in the School of Laboratory Medicine and Medical Science, College of Health Sciences, , University of KwaZulu-Natal.

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This dissertation is presented in manuscript format and includes an overall introduction and final summary of the research.

This dissertation is hereby certified as the original research work conducted by Khanyisa Mahonisi, under the supervision of:

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DEDICATION

I dedicate this study to my late mother and father, Tinstswalo Alice and Eric Mbudana Mahonisi. The memories we shared will forever be cherished. To my unwavering support system and greatest cheerleaders, Thembi and Nyiko Mahonisi, and my brother-in-law, Pandelani Mafhanga, thank you for your constant encouragement, love, and prayers. God has truly blessed me with the most amazing siblings, and I am grateful for your lives. May God continue to bless you abundantly.

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"Bless the Lord, O my soul, and all that is within me, bless His holy name. Bless the Lord, O my soul, and forget not all His benefits." (Psalm 103:1-2)

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LIST OF ABBREVIATIONS

AMC	Amoxicillin–Clavulanic Acid
AMK	Amikacin
AMP	Ampicillin
AMR	Antimicrobial Resistance
ARB	Antimicrobial-Resistant Bacteria
ARG	Antibiotic Resistance Gene
AST	Antimicrobial Susceptibility Testing
BREC	Biomedical Research Ethics Committee
BSI	Bloodstream Infection
CAI	Community-Acquired Infection
CARD	Comprehensive Antibiotic Resistance Database
CAZ	Ceftazidime
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
ColRE	Colistin-Resistant Enterobacterales
CPM	Cefepime
CRE	Carbapenem-Resistant Enterobacterales
CRKP	Carbapenem-Resistant *Klebsiella Pneumoniae*
CRO	Ceftriaxone
CTX	Cefotaxime
CXM	Cefuroxime
DOR	Doripenem
ECC	*Escherichia Coli*/Coliforms
ERT	Ertapenem
ESBL	Extended-spectrum β-lactamase
Etest	Epsilometer test
EV	Extracellular Vesicles
FOX	Cefoxitin
gDNA	Genomic DNA
GEN	Gentamicin
GI	Genomic Island
GNB	Gram-Negative Bacteria
HAI	Healthcare-Associated Infection
HGT	Horizontal Gene Transfer

ICE	Integrative And Conjugative Element
ICU	Intensive Care Unit
IMP	Imipenem
IS	Insertion Sequence
iTOL	Interactive Tree of Life
MDR	Multidrug-Resistant
MEM	Meropenem
MGE	Mobile Genetic Element
MLST	Multilocus Sequence Typing
NCBI	National Centre For Biotechnology Information
NDoH	National Department Of Health
NHLS	National Health Laboratory Service
NICD	National Institute For Communicable Diseases
NIT	Nitrofurantoin
OPD	Outpatient Department
PCR	Polymerase Chain Reaction
PGAP	Prokaryotic Genome Annotation Pipeline
PHREC	Provincial Health Research Ethics Committee
PLSDB	Plasmid Sequence Database
QRDR	Quinolone Resistance–Determining Region
RND	Resistance–Nodulation–Division
SCAI	Simmons' Citrate Agar With Inositol
ST	Sequence Type
SXT	Trimethoprim–Sulfamethoxazole
T4CP	Type IV Coupling Protein
T4SS	Type IV Secretion System
TGC	Tigecycline
Tn	Transposon
TOB	Tobramycin
TZP	Piperacillin–Tazobactam
USA	United States of America
UTI	Urinary Tract Infection
VGT	Vertical Gene Transfer
WGS	Whole Genome Sequencing
WHO	World Health Organization

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ABSTRACT

Bacteria acquire antibiotic resistance genes (ARGs) through horizontal gene transfer or mutations. Colonization of healthy individuals with multidrug-resistant (MDR) bacteria therefore poses a public health concern, as these individuals may serve as reservoirs of antimicrobial resistance (AMR). This study compared the resistomes, mobilomes, and phylogenetic relationships of *Escherichia coli* and *Klebsiella pneumoniae* isolates from a regional hospital and nearby healthy community dwellers in uMgungundlovu District, using whole genome sequencing (WGS) and bioinformatics to assess AMR transmission directionality. Stool and clinical samples were collected, and presumptively identified isolates underwent antimicrobial susceptibility testing, extended-spectrum β-lactamase (ESBL) screening, and WGS. Bioinformatic analyses were conducted using RAST, ResFinder, CARD, PlasmidFinder, INTEGRALL, ISFinder, PGAP, MLST, BV-BRC, and iTOL. This study reports on 32 *E. coli* isolates (15 clinical and 17 community) and 14 *K. pneumoniae* isolates (6 clinical and 8 community) confirmed by WGS. Seven *E. coli* isolates (3 clinical and 4 community) and one community *K. pneumoniae* isolate were identified as ESBL producers, with *E. coli* from both settings sharing multiple resistance genes, including $bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1B}$, *dfrA17*, *sul1*, *sul2*, *aph(3")-Ib*, *aph(6)-Id*, *qnrS1*, *mph(A)*, *catA1*, and *tet(A)*, while *K. pneumoniae* isolates commonly carried $bla_{SHV\text{-}1}$, $bla_{TEM\text{-}1B}$, *sul1*, *sul2*, *aph(3")-Ib*, *aph(6)-Id*, *qnrS1*, and *fosA5*. Most of these ARGs were associated with insertion sequences and transposons in both settings. Among *E. coli*, the most common sequence types (STs) in both clinical and community isolates were ST131, ST10, and ST1193, while ST17 was shared among *K. pneumoniae* isolates. Phylogenetic analysis revealed clustering between clinical and community isolates, although clustering was less pronounced for *K. pneumoniae*. In conclusion, this study revealed the possibility of bidirectional AMR transmission between clinical and community settings. These findings highlight healthy individuals as potential reservoirs of resistance and strengthen the need to include community settings in AMR mitigation strategies.

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Antimicrobial resistance (AMR) is defined as the ability of bacteria, fungi, viruses, and parasites to survive exposure to antimicrobial drugs using a variety of genetic processes, making it difficult to prevent and treat infections. The processes involved are intricate, and there are many ways that bacteria can acquire these traits (Prestinaci et al., 2015; Slizovskiy et al., 2020). The dissemination of AMR is increasingly a global threat to disease control, resulting in prolonged illness, more antibiotic exposure, and increased mortality (Musoke et al., 2021). AMR exists and can be transferred in the form of antimicrobial resistant bacteria (ARB) and antibiotic resistance genes (ARGs) together with their associated mobile genetic elements (MGEs) among humans, animals, and the environment. MGEs capture, accumulate, and distribute ARGs (Partridge et al., 2018 ; Sridhar et al., 2021). The accumulated ARGs and MGEs can be transferred to other bacteria of the same or different species (Velazquez-Meza et al., 2022).

The resistome is all the genes in a genome that encodes for AMR, whereas a microorganism's mobilome is the collection of all MGEs (Singh et al., 2019). As novel resistance mechanisms develop and spread, this problem will continue to exacerbate (Brinkac et al., 2017). Hence, AMR surveillance is vital for determining the nature and extent of resistance, establishing strategies to improve the appropriate use of antimicrobial drugs, and reducing AMR selection pressure. It also enables the detection of emerging strains or species and the identification of changes in antimicrobial susceptibility patterns through continuous monitoring of pathogens (Fuhrmeister & Jones, 2019). Bacteria can acquire AMR through horizontal gene transfer (HGT) of MGEs or mutations in the bacterial genome (Von Wintersdorff et al., 2016). MGEs are distinct DNA segments that can travel between and within bacterial cells and are classified based on their traits and genetic makeup (Johansson et al., 2021). Furthermore, MGEs interact to construct an intricate structure that can round up and spread ARGs across a bacterial population, which plays a critical role in disseminating AMR. To understand the epidemiology of AMR, it is essential to recognize and distinguish MGEs because they bring about phenotypic variance in microbes when present in genomic DNA (Abbas et al., 2024; Johansson et al., 2021).

MGEs play a vital role in the evolution and spread of drug-resistant bacteria by facilitating the exchange of ARGs among bacteria. Integrons, transposons (Tns), insertion sequences (ISs), plasmids, integrative conjugative elements (ICEs), bacteriophages, and genomic islands (GIs) are examples of these MGEs (Durrant et al., 2020; Stokes & Gillings, 2011; Wang, P., et al., 2022). MGEs replicate together with their host cell and confer traits that improve the adaptive fitness of their host, which in return benefits their adaptive fitness (Hall et al., 2022; Rocha & Bikard, 2022). Integrons are flexible genetic scaffolds that reorganize multiple gene segments through site-specific recombination of gene cassettes via integrase (*IntI*) activity (Deng et al., 2015; Fonseca & Vincete, 2022; Shetty et al., 2023). Tns and ISs

are reoccurring DNA segments that can randomly mobilize themselves with their associated ARGs from one DNA molecule to another (Gao et al., 2015; Partridge et al., 2018). Plasmids are self-replicatory MGEs, and they differ in their gene content, how they replicate, and their shapes, with most being circular (Robertson et al., 2020). ICEs are integrated into the host chromosome and replicate through chromosomal replication and cell division (Johnson & Grossman, 2015). Phages carry virulent genes and disseminate them through HGT. Phages carrying ARGs have been detected in various environments, ranging from the human gut to ready-to-eat food (McCarthy et al., 2012; Jebri et al., 2021). Lastly, GIs are DNA segments acquired from other microorganisms through HGT, harboring genes that increase the host cell's fitness by offering functions like AMR, pathogenesis, symbiosis, metabolic activities, and other traits that help them adapt to various environmental conditions (Bellanger et al., 2014; Rao et al., 2020).

HGT is mainly carried out via conjugation, transformation, and transduction processes. Bacteria acquire MGEs carrying ARGs through two main processes: conjugation and transduction (MacLean & Millan, 2019; Tao et al., 2022). Conjugation is an HGT mechanism in which the DNA from a donor bacterium is transferred to a recipient bacterium through direct contact with the aid of the pili (Virolle et al., 2020; Patkowski et al., 2023), and it occurs in a diverse array of environments, such as soil, plant surfaces, medical devices, and notably, within the gut. The gut is a particularly significant reservoir for gene exchange among bacteria, especially those of considerable clinical importance (Frankel et al., 2023). Transformation is when a bacterium actively absorbs free DNA from its surroundings and either incorporates it into its genome or converts it into extrachromosomal DNA molecules (Winter et al., 2021). Transduction is the transfer of ARGs/DNA (chromosomal/ plasmid) by phages from a bacterium into another bacterium (Von Winterdorff et al., 2016).

There are two types of ARGs in the gut microbiome: intrinsic and acquired resistance genes (Singh et al., 2019). Intrinsic genes are naturally hereditary, non-mobile, and provide resistance to specific antibiotic classes even when no prior exposure is present. These intrinsic genes sometimes get captured by MGEs and become mobile-resistant genes. Mobile ARGs, conversely, are made up of genes encoded within MGEs that allow them to move and expand among various microbial communities (Paul & Das, 2022). Whole genome sequencing (WGS) reads an entire genome down to individual nucleotides, enabling the detection of almost all genetic variations in coding and noncoding regions. It can identify AMR determinants, including ARGs and MGEs (Davies et al., 2022; Waddington et al., 2022; Schlieben & Prokisch, 2023).

The acquisition and carriage of multidrug resistant (MDR) bacteria in healthy individuals pose a significant public health threat, as colonized individuals can serve as reservoirs of AMR. Therefore, understanding AMR dynamics in healthy populations is essential for mitigating its spread (Farrell et al., 2023; Neut, 2021). This study investigates the resistome and mobilome of *Escherichia coli* and

Klebsiella pneumoniae from hospital patients and healthy community dwellers using WGS and bioinformatic analyses to better understand the emergence and spread of AMR in the community compared with clinical settings in uMgungundlovu.

1.2. Literature review

1.2.1. Antimicrobial resistant bacteria

1.2.1.1. The dissemination of antimicrobial resistant bacteria in community and hospital settings

The carriage of ARB is rising in community settings, and recognition of asymptomatic carriers underscores the need for expanded AMR surveillance beyond healthcare facilities (Jamrozik & Selgelid, 2019). Dissemination occurs in both community and hospital environments, and monitoring ARB colonization is essential to assess the true burden of resistance (Kumar et al., 2023). In developing countries, community-level AMR dynamics remain poorly understood, despite their key role in shaping resistance patterns (Ingle et al., 2018). Both community and hospital settings are necessary to study because, to tackle AMR, it is crucial to identify the transmission patterns of ARB in each setting. A lack of information about either setting could result in poor AMR control methods (Knight et al., 2018). Healthcare facilities are a rich habitat of ARB and ARGs. Additionally, ARB and ARGs are ubiquitous and can be quickly disseminated. The exposure of healthcare workers, patients, and visitors to drug-resistant bacteria or contact with contaminated surfaces creates a web of AMR dissemination (Ding et al., 2023). The global dissemination of AMR is aided by the mobilization (travel) of healthy human populations (Findlater Bogoch, 2018). Arcilla et al. (2017) reported that travelers have a high potential of acquiring ARB, which can persist for about 12 months after traveling. However, colonization by ARB does not always lead to infection. Supporting this, Borer et al. (2012) examined risk factors for clinical infection among patients initially colonized with carbapenem-resistant *K. pneumoniae* (CRKP) in a hospital setting. Of 464 colonized patients, only 42 patients developed clinical infection. Progression of infection was more likely in patients with diabetes, solid tumors, tracheostomy, urinary catheters, prior invasive procedures, or recent treatment with antipseudomonal penicillins. This indicates that specific patient characteristics and medical interventions increase the likelihood of colonized individuals developing symptomatic infections, highlighting the importance of infection control and careful antibiotic management in healthcare settings.

Studies from multiple regions illustrate the prevalence and transmission of ARB across settings. Chowdhury et al. (2023) conducted their study in 2019 in Bangladesh, analyzing stool and nasal samples from hospitalized and community-dwelling adults. Isolates were identified using selective media and tested for antimicrobial susceptibility (AST) with the Vitek 2 system. They reported that extended-spectrum cephalosporin-resistant Enterobacterales were highly prevalent in both groups (78% in the community, 82% in hospitals), while carbapenem-resistant Enterobacterales (CRE) were more frequent

among hospitalized patients (37% vs. 9%). Furthermore, colonization with colistin-resistant Enterobacterales (ColRE) occurred in 11% of community participants and 7% of hospital patients, and methicillin-resistant *Staphylococcus aureus* (MRSA) rates were similar across settings (22% vs. 21%). Similarly, Hilty et al. (2012) studied ESBL-producing *E. coli* (ESBL-Ec) and ESBL-producing *K. pneumoniae* (ESBL-Kp) among hospitalized patients and their household contacts in Switzerland, reporting low hospital transmission (4.5% for ESBL-Ec, 8.3% for ESBL-Kp) but higher household transmission (23% and 25%, respectively). Both studies highlight that hospitals can serve as hotspots for highly resistant strains, while community and household environments also play a significant role in the spread of ARB. Hospitals struggle to control healthcare-associated infections (HAIs) because asymptomatic patients can carry and spread ARB. Detecting these carriers is challenging without systematic screening on admission and during hospitalization (Pei et al., 2021). Limited data on asymptomatic carriers complicates understanding pathogenic ARB, including their prevalence, duration of colonization, and distinction from active infection. As a result, carriers can undermine infection control measures by exposing susceptible patients to ARB (Chisholm et al., 2018).

Singh et al. (2020) investigated ARB carriage in 42 households (376 participants) in Siem Reap, Cambodia, collecting 376 nasal swabs and 290 stool specimens. MRSA was isolated from 6.9% of specimens, ESBL-Ec from 92.8%, and ESBL-KP from 44.1%, with 42.4% co-colonized with ESBL-Ec. Carbapenemase-producing Enterobacterales were rare (2.1%). Importantly, no significant differences in MRSA, ESBL-Ec, or ESBL-Kp carriage were observed between hospital-associated households (household with recent child hospitalization >48 h) and community households (household on the same street, with no child hospitalizations in past 12 months). Similarly, Nagelkerke et al. (2017) evaluated the prevalence of ARB carried by in and outpatients in a secondary care hospital in Zambia. Nasal and rectal samples were randomly collected from 50 adult inpatients and 50 outpatients. Nasal samples were tested for the presence of *Staphylococcus aureus*, and AST was performed for oxacillin, gentamicin, and ciprofloxacin to detect MRSA. Rectal samples were tested for Enterobacterales non-susceptible to gentamicin, ciprofloxacin, and ceftriaxone, with ESBL production confirmed using Epsilometer tests (Etest) for ceftriaxone-resistant isolates. Nasal colonization with *S. aureus* was low (18% inpatients, 14% outpatients) with no MRSA detected. In contrast, rectal carriage of drug-resistant Enterobacterales (especially *E. coli* and *K. pneumoniae*) was high, observed in 90% of inpatients and 48% of outpatients, with gentamicin-resistant Enterobacterales detected in 78% and 32%, respectively. Finally, a systematic review and meta-analysis of commensal *E. coli* in low- and middle-income countries (LMICs) reported that 28% of isolates were MDR, 24% extensive drug-resistant, and 5% pan-drug-resistant in community settings (Nkansa-Gyamfi et al., 2019). Collectively, these studies emphasize that both hospital and community environments contribute significantly to the persistence and dissemination of ARB, highlighting the need for integrated surveillance and control strategies that address both settings.

1.2.1.2. Translocation of antimicrobial resistant bacteria from the gut microbiome

Bacterial translocation is a process in which pathogenic bacteria move from the intestinal barrier to the bloodstream and other organs. This occurrence has been connected to various illnesses like cancer, pancreatitis, and inflammatory bowel disease (Shu et al., 2023). A healthy microbiome possesses ARB; however, this can be disrupted when exposed to antimicrobials, leading to dysbiosis, defined as the alteration in the host microbiome, leading to ARB dissemination (Dahiya & Nigam, 2023). Wheatly et al. (2022) conducted a study on a patient admitted to an intensive care unit (ICU) in Badalona, Spain, over 30 days to test the significance of gut-to-lung translocation in *Pseudomonas aeruginosa* colonization and AMR. To test for translocation, they used phylogenetic approaches and studied the link between AMR and translocation using a combination of genomic and phenotypic methods. The patient was treated with meropenem for a suspected urinary tract infection (UTI) for ten days. About 52 *P. aeruginosa* isolates [12 endotracheal aspirate (ETA) samples and 40 peri-anal swabs] during the ICU stay. Their comparative genomic analysis showed that gut and lung isolates shared several single nucleotide polymorphisms (SNPs) and insertions/deletions (indels), indicating that they were closely related. Phylogenetic analyses further showed that these related lineages appeared first in the gut and later in the lung, providing evidence consistent with gut-to-lung translocation within the patient. Similarly, Tuomisto et al. (2014) showed bacterial translocation from the gut to sterile body sites. They investigated alterations in gut microbiota composition in patients with alcoholic liver cirrhosis and the translocation of intestinal bacteria to sterile body sites, including the liver and ascitic fluid. They reported marked gut dysbiosis, characterized by an overrepresentation of Enterobacterales, alongside increased intestinal permeability resulting from impaired epithelial barrier function associated with inflammation and portal hypertension. This compromised gut barrier facilitated bacterial translocation from the intestine to the liver and ascites. They found that bacterial species identified in the liver and ascitic fluid closely mirrored those detected in the gut, indicating the gastrointestinal tract as the primary source of infection.

Bernard-Raichon et al. (2022) investigated the impact of antibiotic-associated gut dysbiosis on microbial translocation and bloodstream infections (BSIs) in hospitalized coronavirus disease 2019 (COVID-19) patients. Stool samples from patients were analyzed using 16S rRNA gene sequencing to characterize gut microbiome composition, and results were compared to clinical blood cultures. Their study found that bacteria detected in the blood of some patients matched taxa abundant in their gut microbiome, indicating translocation from the gut to the blood. Other studies have reported that the coexistence of many virulent genes is associated with a high rate of intestinal translocation (Sarowska et al., 2019). For example, Krawczyk et al. (2015) examined *E. coli* from the blood and stool of adults with hematologic malignancies and bacteremia to explore how gut bacteria move into the bloodstream. They found that blood isolates often matched stool isolates genetically, indicating direct translocation from the gut. Certain virulence genes (*papC*, *sfa*, *usp*, and *cnf1*) were more frequently present in strains

capable of causing bacteremia, suggesting that multiple virulence factors work together to enable translocation. Strains from pathogenic phylogenetic groups, particularly B2, and those carrying *dr*/*afa* adhesins, were more likely to invade the bloodstream. These results highlight that specific virulent *E. coli* strains, rather than random commensals, are responsible for translocation in immuno-compromised patients. These findings collectively highlight how gut dysbiosis promotes ARB translocation in immunocompromised patients. The gut microbiome serves as a reservoir for ARB and ARGs, and translocation can lead to infection while contributing to the dissemination of AMR determinants. Understanding these translocation mechanisms is therefore important for contextualizing possible AMR transmission dynamics across clinical and community settings.

1.2.2. Antimicrobial resistant genes

1.2.2.1. Healthcare and community settings can share similar resistome content

AMR strains and their associated resistomes can circulate between healthcare and community environments. For instance, Hounmanou et al. (2023) demonstrated that patients discharged from two tertiary hospitals in Uganda, who were still colonized with ESBL-Ec, could disseminate these strains and their resistomes to their household contacts, animals, and environments. They accomplished this by collecting rectal samples from patients, healthcare workers and caretakers and healthcare environment (surface, sinks, and wastewater swabs). After the patients were discharged, they did a follow-up in their households in which they collected household contacts, pets, and household environment samples. A total of 67 isolates were subjected to WGS of which revealed high clonal diversity, encompassing 42 sequence types (STs), suggesting limited clonal transmission across settings. Despite clonality differences, ARGs were largely similar, with $bla_{CTX\text{-}M\text{-}15}$ predominating in both healthcare and community *E. coli* isolates. Plasmid analysis identified IncF-type plasmids as the principal vectors carrying the $bla_{CTX\text{-}M\text{-}15}$ gene, with IncY and IncFIB plasmids also contributing to its AMR dissemination. Comparably, Dwiyanto et al. (2022) conducted a multi-setting epidemiological surveillance study in Segamat, Malaysia, to compare ESBL-Ec from community carriers and clinical patients using WGS. Faecal samples were collected from community dwellers and clinical ESBL-Ec isolates were obtained from a local hospital. Selected isolates from both settings (n = 40) underwent pan-genome comparison, cluster analysis, and resistome profiling to characterise shared and unique genetic features. IncFII and IncFIB plasmid replicons were the most prevalent in both settings. Several $bla_{CTX\text{-}M}$ variants dominated, including $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}65}$, and $bla_{CTX\text{-}M\text{-}15}$, and virulence markers for extraintestinal pathogenic *E. coli* (ExPEC), uropathogenic *E. coli* (UPEC), and enteroaggregative *E. coli* (EAEC) pathotypes were present in isolates from both settings. Although pan-genome comparison indicated that community and clinical isolates were not clonally related, cluster analyses based on plasmid profiles, ARGs, and phenotypic susceptibility identified four clusters with similar patterns across settings, pointing to shared resistome features and horizontal gene transfer as key drivers. The authors concluded that ESBL-Ec from community and clinical settings share similar resistome profiles,

likely due to frequent exchange of MGEs, underscoring the role of HGT in disseminating ARGs across settings.

Resistance genes beyond ESBL genes are frequently shared between healthcare and community settings. Singh et al. (2023) examined MDR Enterobacterales in Cambodia by comparing recently hospitalized children (hospital-associated, admitted within the previous 14–28 days for at least 48 h) and their household members with community-associated children (not hospitalized in the past 12 months) and their household members, all of whom had also not been recently hospitalized. This study allowed the authors to assess both the impact of recent hospitalization on MDR carriage and potential transmission within households. *E. coli* (n = 276) and *K. pneumoniae* (n = 89) were the most prevalent species across both settings and were further characterized using WGS which revealed ARGs associated with resistance to several commonly used antibiotics, including ciprofloxacin, co-trimoxazole, chloramphenicol, gentamicin, and colistin. Ciprofloxacin resistance was predominantly mediated by *qnrS1*, while co-trimoxazole resistance was mainly associated with the sulfonamide resistance genes *sul1*, *sul2*, or their combination. Chloramphenicol resistance was mediated by *catI* and *catA1* exclusively in *E. coli*, whereas *floR* was detected in both *E. coli* and *K. pneumoniae*. Gentamicin resistance was primarily associated with *aac(3)-iid*. Colistin resistance was rare and detected only in *E. coli* mediated by *mcr-1* and *mcr-3* genes. These ARGs were commonly shared among isolates from both healthcare and community settings, highlighting substantial resistome overlap beyond ESBL determinants.

Carbapenem-resistant Enterobacterales (CRE) are a significant public health concern due to high mortality rates and limited therapeutic options (Yang et al., 2024). While CRE are often associated with healthcare settings, *E. coli* has also been linked to community-associated CRE (Bulens et al., 2023). Li et al. (2024) conducted a nationwide genomic study in China to investigate carbapenem-resistant *E. coli* in healthy individuals (n = 147) and compared them with clinical ICU isolates from a previous study by Zhang et al. (2023) (n = 113) using WGS. In healthy isolates, bla_{NDM-5} was the most prevalent carbapenemase gene, with ST224 being the most common sequence type, whereas clinical isolates predominantly carried bla_{KPC-2} and ST131. Beyond carbapenemase genes, both settings shared other ARGs including bla_{CTX-M}, *sul1*, *sul2*, *aadA*, *tet(X4)*, and *mcr-1*. Conserved plasmid replicons, notably IncX3 and IncHI2A, were detected in both groups. Clear differences included a broader diversity of plasmid replicons and more virulence factors in clinical isolates. Overall, this section exemplifies overlap between clinical and community resistomes, emphasizing the need for integrated AMR surveillance across both settings.

1.2.2.2. Human resistome and progression

Many factors that reshape the microbiome also have the potential to influence resistome progression (Crits-Christoph et al., 2022). The resistome encompasses all ARGs found in the microbiome of a

specific environment (Ma et al., 2021). The resistome and its differences in microbial communities are important not only for understanding the dissemination of AMR but also for characterizing pools of potentially novel resistance mechanisms (Despotovic et al., 2023). Recent research has shown that the gut microbiome plays a crucial role in hosting ARGs. These genes can be transferred between resident and temporary/transitory bacteria in the human intestinal microbiome. In a study by Hu et al. (2013), 1093 ARGs were identified in 162 faecal metagenomes collected from individuals in China, Denmark, and Spain. Another study by Kristoffer et al. (2013), focused on healthy individuals from seven countries, found ARGs related to 50 out of 68 antibiotic classes in 252 faecal metagenomes, averaging 21 ARGs per sample. It is essential to note that exposure to ARB or ARGs found in animal waste, food products, and water, exposure to antibiotics, can lead to the acquisition of ARGs within the human intestinal microbiome (Bich et al., 2019).

A meta-analysis by Fredriksen et al. (2023) re-examined gut microbiome metagenomic data from 26 human case-control studies to assess how disease status relates to the gut resistome. Although resistome composition was highly variable between individuals, patients with diseases commonly treated with antibiotics (such as cystic fibrosis and diarrhoea) showed significantly higher ARG abundances compared with healthy controls. Importantly, resistome expansion was observed even in some diseases not typically treated with antibiotics, suggesting ongoing ARG acquisition in disease-associated bacterial strains. Similarly, Shuai et al. (2022) analysed a large community-based cohort in China (n = 1,210) to examine the relationship between the gut resistome and host metabolic traits. Stool samples were profiled using shotgun metagenomic sequencing, with taxonomic and functional annotation and ARG identification performed through bioinformatic pipelines. Their integrative multi-omics analysis revealed that resistome composition and abundance increased progressively with disease severity, even in participants without recent antibiotic exposure. Notably, individuals with type 2 diabetes harboured greater diversity and abundance of ARGs compared with healthy controls, suggesting that disease-associated gut dysbiosis creates conditions that favour the persistence and expansion of resistance determinants.

The human gut resistome progresses with age, accumulating both in abundance and diversity over a lifetime (Tavella et al., 2021). An example of this is a metagenomic study on 246 healthy individuals [age groups Y20 (0–20 years), Y40 (21–40 years), Y60 (41–60 years), Y80 (61–80 years), Y100 (81–100 years), and Y120 (100–120 years)] from China, which investigated how the resistome changes with age (Wu et al., 2021). The study found that older age groups, particularly Y100, had the highest abundance and complexity of ARGs, while younger groups (Y20–Y60) harboured fewer ARGs. In the oldest group (Y120), ARG abundance declined slightly from the peak seen in Y100 but remained higher than the youngest groups, suggesting a cumulative effect of resistome progression over time. Across all ages, *tetQ* was the most prevalent ARG, predominantly carried by Bacteroides.

1.2.2.3. Antibiotics shape the abundance of resistant genes

The intestinal microbiome has a wide range of ARGs and the most described are those directed particularly against tetracycline, β-lactams, aminoglycosides, and glycopeptides. When antibiotics are used, they have different effects on the intestinal microbiome (Huang et al., 2022). In most cases, antibiotics reduce the number of commensal microorganisms in the intestine and increase the presence of ARGs (Baron et al. 2018). Raymond et al. (2016) demonstrated that short-term antibiotic treatment can cause predictable changes in the gut microbiome. Following a 7-day course of cefprozil in healthy individuals, consistent increases in *Lachnoclostridium bolteae* were observed, while a subset of participants showed enrichment of the opportunistic pathogen *Enterobacter cloacae*, particularly those with low baseline microbiome diversity. Antibiotic exposure further altered the resistome, with previously undetectable resistance genes, including mutations in $bla_{CfxA\text{-}6}$, emerging after treatment, highlighting the importance of baseline microbiome composition in shaping antibiotic responses. Antibiotics kill susceptible bacteria, but resistant bacteria survive because each time antibiotics are used, this selective pressure allows resistant bacteria to persist and spread (Yosef et al., 2016).

1.2.3. Modes of acquiring antimicrobial resistant genes

1.2.3.1. Modes of resistance

There are three ARB modes: intrinsic, acquired, and adaptive resistance (Lee, 2019). Intrinsic resistance is inherited through genes or chromosomes, and it is not dependent on exposure to antimicrobials. In contrast, acquired resistance is obtained through chromosomal DNA mutation or vertical gene transfer (VGT) and HGT, and it can either be a temporary or permanent acquisition. VGT is when a bacterium inherits ARGs from its ancestor, whereas HGT is a process in which bacteria transfer ARGs to related or unrelated bacterial species via MGEs (Cai et al., 2022; Fredriksen et al., 2023). Ultraviolet radiation, certain chemicals, stress such as starvation, and other factors can cause genetic mutations (Reygaert, 2018). Mutations that induce AMR are limited to specific categories of genes, including those responsible for drug targets, drug transporters, regulators that oversee drug transporters, and those that encode for enzymes that modify antibiotics (Martinez,2014). Adaptive antibiotic resistance involves changes in gene expression due to its growth state or environmental factors. When the circumstances that induce adaptive resistance cease to be present, bacteria return to its susceptibility state (Coleman et al., 2020).

1.2.3.2. Mechanisms of horizontal gene transfer

1.2.3.2.1. Conjugation

Conjugation is the key mechanism of HGT for ARG dissemination, occurring when bacterial cells connect through pili or adhesins to transfer DNA between them (Wintersdorff et al., 2016). Conjugation, carried out by plasmids and ICEs, uses two systems: mobility (MOB), which prepares DNA via the origin of transfer (*oriT*), relaxase, and type IV coupling protein (T4CP), and mating pair formation

(MPF), which delivers DNA through the type IV secretion system (T4SS) (Partridge et al., 2018; Getino & de la Cruz, 2018).

1.2.3.2.2. Transformation

Unlike conjugation, transformation does not require direct cell-to-cell contact (Liu et al., 2022). It involves the uptake of extracellular DNA from the environment into the bacterial genome, independent of MGEs (Blokesch, 2016). This process requires bacteria to be in a competent physiological state, which is often induced under stressful conditions, and more than 80 bacterial species are known to be naturally competent (Blokesch, 2016; Liu et al., 2022).

1.2.3.2.3. Transduction

Phages were discovered in the early 20th century, and their ability to move bacterial genes was recognized only decades later (Parkinson, 2016). There are three main types of transduction: i) generalized transduction, occurs when phages transfer any bacterial DNA, whether from the chromosome or a plasmid, to another bacterium; ii) specialized transduction moves DNA containing both viral and bacterial host DNA, usually during prophage excision and; iii) lateral transductions, is part of the normal phage life cycle and transfers large regions of bacterial DNA (Chiang et al., 2019).

1.2.3.2.4. Vesiduction and the role of extracellular vesicles

Extracellular vesicles (EVs) are tiny, spherical, consisting of a bilayer made of proteins and lipids with a typical size ranging from 20 to 200 nanometers and contain various cellular components (enzymes, toxins and nucleic acids). EVs serve multiple functions that help bacteria to survive. Other studies have suggested that EVs can protect bacteria from the harmful effects of antibiotics by either diverting the antibiotics or transferring ARGs but the exact mechanisms behind this protection are not characterized (Kim et al., 2020). EVs can be found in various body fluids, such as blood, urine, breast milk, saliva, ascites, and cerebrospinal fluid (Lee et al., 2023). Soler and Forterre (2020) proposed the term vesiduction as the fourth way of HGT mediated by EVs. Vesiduction is the process in which small membrane vesicles, released from a donor cell, transport DNA containing ARGs into the cytoplasm of a recipient cell by fusing with its cell membrane. This recently recognized mechanism transfer genetic material via EVs is particularly significant for the transfer of ARGs. In previous studies, it has been shown that the spread of carbapenem resistance in *Acinetobacter baumannii* can occur through EVs carrying carbapenemase genes and that EVs from *E. coli* can transfer colistin resistance genes to different species such as *P. aeruginosa* and *Acinetobacter radioresistens* (Jiang et al., 2022).

1.2.3.3. Mobilome: disseminate antimicrobial resistant genes

The mobilome refers to the collection of MGEs in a bacterial genome, including plasmids, integrons, Tns, ISs, ICEs, GIs, and phages. These elements facilitate movement and transfer between bacterial genomes, playing a central role in the emergence, dissemination, and persistence of ARGs, while also shaping bacterial genome evolution (Algarni et al., 2022). The transfer of ARGs from non-pathogenic

to pathogenic bacteria, together with human activities that promote ARG spread, contributes to the increasing abundance of MGEs (Zhang et al., 2022). Consequently, mobilome analysis must be contextualized within the bacterial host, and linking bacterial clones to their associated mobilome is essential for accurately identifying ARGs and understanding their transmission dynamics (Martínez et al., 2017).

1.2.3.3.1. Plasmids and bacteriophages

Plasmids are genetically diverse because they undergo frequent mutations and can contain similar genes that are found on the chromosome, which makes it challenging to identify them because they exhibit similarities in gene content (Tang et al., 2023). Wang et al. (2022) investigated ARGs of 2635 Enterobacterales genomes (*Escherichia*, *Klebsiella* and *Salmonella*) from the National Centre for Biotechnology Information (NCBI), in which they only analysed 416 ARGs and found that plasmids and chromosomes shared 33% of those genes. ARGs in Enterobacterales are carried on various plasmids, with IncF linked to resistance to cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones, IncI2, IncX4, and IncP associated with colistin resistance, and IncHI1/IncHI2 commonly found in MDR strains (Altayb et al., 2022).

Moreover, plasmids can either be classified as conjugative, mobilizable or non-mobilizable based on their mobility. A plasmid that encodes for its own MPF genes is called a conjugative plasmid which is a form of T4SS, which is a mating complex during the process of conjugation. When a plasmid utilises an MPF of another plasmid, it is called a mobilizable plasmid, whereas, non-mobilizable plasmids disseminate by either transformation or transduction (Smillie et al., 2010). Conjugative plasmids in Gram-negative bacteria (GNB), normally consist of and carry complete sets of genes required for genetic transfer, including *oriT*, relaxase protein, T4CP and T4SS, whereas mobilizable plasmids consists of *oriT*, relaxase, and at times T4CP (Shintani et al., 2015).

Phages are currently of interest and can either be lytic or lysogenic. Phages are of growing interest, with lytic phages explored for therapeutic use due to their ability to destroy bacteria, while lysogenic phages may worsen AMR by transferring virulence and resistance genes through transduction or lysogenic conversion, altering bacterial phenotypes and increasing pathogenicity in humans and animals (Hitchcock et al., 2023). Additionally, most prophages integrate into the chromosome, but some persist as phage-plasmids (P-Ps), which are widespread in Enterobacterales and other hospital pathogens and may play an important role in spreading ARGs more than other phages because they combine features of both plasmids and phages (Pfeifer et al., 2022).

1.2.3.3.2. Integrative conjugative elements and genomic islands

GIs are gene clusters that typically range in size from 10 to 200 kilo base pair and are classified according to their biological functions; metabolic islands (MIs) that consist of genes for proteins with metabolic properties, resistance islands (RIs) that consist of genes associated with AMR, and symbiotic

islands (SIs) that contain genes for proteins with symbiotic properties and can be found on either plasmids or chromosomes, but mostly on chromosomes (Finan, 2002; Juhas et al., 2009; Assaf et al., 2021). Notably, a significant number of GIs can excise from their chromosomal location, encode for HGT to another cell, and reintegrate into the target site on the new host's chromosome (Juhas et al., 2009). ICEs are chromosomal genetic elements that replicate with the host genome but can occasionally excise and transfer to other cells via the T4SS. They often carry beneficial cargo genes conferring antibiotic resistance, pathogenicity, or metabolic functions, as exemplified by ICE Tn916 which is known to confer tetracycline resistance and can transfer between bacterial cells through conjugation (Johnson et al., 2022).

1.2.3.3.3. Transposons and insertion sequences

ISs are transposable elements are tiny and self-contained, with short terminal inverted repeats (IRs), (Udaondo et al, 2022). They generally possess at least one Tn gene, which facilitates in the movement of DNA (transposition). Furthermore, they can be transferred between genomes through HGT, often being a component of bacteriophages and plasmids. ISs and Tn are grouped according to the amino acids found in their transposase, generally: aspartic (D), aspartic (D), and glutamic acid (E) (DDE); aspartic, glutamic, aspartic, and aspartic acid (DEDD); and two histidine residues separated by a large hydrophobic amino acid (HUH) motif. Transposition pathways of these elements are either conservative or replicative (Carr et al., 2023). Some ISs are flanked by distinct shorter direct repeat sequences known as target site duplications (TSDs). These TSDs occur because of the ISs target site being duplicated and they can be transferred between genomes through HGT (Razavi et al., 2020). ISs adapt to balance two goals: surviving within host cells and competing with other ISs. During transposition, they can form circular copies that allow them to regulate transposition rates ("bet-hedging") under different conditions, thereby increasing their chances of spreading to new hosts without harming the original host (Kanai et al., 2023).

Che et al. (2021) observed a higher prevalence and greater abundance of ISs on conjugative plasmids compared to plasmids in the other two categories (mobilizable and non-mobilizable). Their analysis, using the Kruskal-Wallis test with Dunn's multiple comparisons, revealed a significant difference ($P < 0.001$). Furthermore, they specifically examined plasmids carrying ARGs, which led them to find that ISs were more numerous on plasmids carrying ARGs than on those lacking them. ISs consists of common regions (ISCRs) and represents a potent gene-capturing system for incorporating DNA into different genomes. Unlike most IS elements, ISCRs use the rolling-circle replication mechanism for transposition, enabling them to transpose without requiring two intact copies and resulting in a high transposition rate. IS26, employing a replicative mechanism, plays a key role in plasmid variation carrying ARGs. Notably, IS26, prefers transposing within plasmids rather than chromosomes, promoting HGT of ARGs. This significantly contributes to the emergence of MDR phenotypes, especially in clinically important pathogens (Aminov, 2021).

A composite Tn function as a carrier of ARGs are flanked by ISs, and examples include, Tn903, Tn9, Tn10, and Tn5 are particularly significant in AMR, especially in *E. coli*. Tn5 is bounded by IS50R and IS50L (right and left, respectively) and contains kanamycin (kan^R), bleomycin (ble^R), and streptomycin (str^R) ARGs; Tn10 is flanked by IS10R and IS10L and contains genes such as *tetA* (produces the tetracycline efflux pump), t*etR* (responsible for a tetracycline-related repressive protein), *tetC* (involved in transcription regulation), and *tetD* ARGs; Tn10, containing tetracycline ARGs, has been detected in *Proteus*, *Pseudomonas*, *Salmonella*, *Klebsiella*, and *Vibrio*; Tn9 is a carrier for the *cat* ARGs, which confers chloramphenicol resistance, and is flanked by IS1, one of bacteria's earliest and smallest ISs, with a length of less than 770 bp; and Tn903 contains two IS903 elements and encodes the enzyme aminoglycoside 3'-phosphotransferase I (*aph-3'-I*) (Babakhani & Oloomi, 2018).

Non-composite Tn elements belong to the Tn3 family and play a crucial role in the evolution of bacterial genomes and AMR dissemination by inducing mutations and examples are Tn5053, Tn5041, Tn5652, Tn1013, Tn5036, Tn5541, Tn5090, Tn5060, Tn5051, Tn1331, Tn4430, Tn5044, Tn5563, and Tn402. Most of them are mercury ARGs (*merC*, *merA*, *merR*, *merT*, *merP*, *merE*, *merD*) carriers. Tn1721 carries ARGs against chloramphenicol, tetracycline, kanamycin, and streptomycin. Tn5403 was detected in carbapenem-resistant *K. pneumoniae*. Tn5044 carries *mer* ARGs and has been identified in the *E. coli* K12 strain. Tn1331 harbours *aac(6')-Ib*, *aadA1*, and bla_{OXA-9} ARGs, and these resistance genes are positioned as gene cassettes. Additionally, Tn1331 lacks the *intI* gene, and it is responsible for conferring resistance to amikacin and kanamycin in *K. pneumoniae* (Bebakhani & Oloomi, 2018).

1.2.3.3.4. Integrons

1.2.3.3.4.1. Mobility and structure

Integrons are not self-mobile because *intI* cannot excise itself from the chromosome (Gillings, 2014), instead, they rely on association with Tns or plasmids to facilitate the movement of gene cassettes between integrons or bacterial genomes (Deng et al., 2015; Sabbagh et al., 2021). Integrons typically consists of two conserved parts, known as the 5′-conserved region (5′-CS) and 3′-CS which are separated by a variable region containing gene cassettes. The 5′-CS section includes an *IntI* gene, a primary recombination site (*attI*), and a promoter (Pc) responsible for cassette gene transcription, whereas the 3′-CS region comprises a truncate *qacE*Δ*1* gene that confers resistance to quaternary ammonium compounds, and a sulfonamide ARG (*sul1*), and a sequence of unknown function (orf5) (Pérez-Etayo et al., 2018). The integrase *IntI* inserts gene cassettes at *attI* and the recombination site (*attC*) sites, with *attC* forming DNA structures that help *IntI* recognize the cassette and enable recombination (Zhang et al., 2020).

1.2.3.3.4.2. Classification of integrons

There are five notable classes of integrons (Ghaly et al., 2021), and classes I, II, and III integrons have been discovered to disseminate multiple ARGs in Enterobacterales (Kaushik et al., 2018). Class I

integron has an *intI1* capable of recombining with various *attC* sites, and it is a reservoir of most gene cassettes associated with AMR and have been associated with Tn402 and Tn3 (Deng et al., 2015; Okoh & Fadare, 2022). Class II integrons are found within Tn7 and its derivatives (Tn1825, Tn1826, and Tn4132), which carry the *attI2* site and Pc (Sabbagh et al., 2021; Okoh & Fadare, 2022). Tn7 associated with *intI2* have transposition modules (tns module) and the 3′-CS in *intI2* has tns that encode for tnsA, tnsB, tnsC, tnsD, and tnsE proteins that are essential for transposition (Hamza & Omran, 2023). The *intI2* gene harbours genes that confer resistance to trimethoprim (*dfrA*), streptothricin (*sat*), and streptomycin (*aadA*) (Fonseca & Vicente, 2022). Class III *intI3* is embedded in Tn402-like but in a reverse mode compared with *intI1* (Collis et al., 2002). The 3′-CS in *intI3* consists of *qacEΔ1*, *sul1*, *orf5*, and *orf6* genes, like that of class I integrons. What distinguishes them is that *intI3* does not have transcription genes and that the *intI3* gene possesses gene cassettes that encode metallo β-lactamase (MBL) enzymes and the *aacA4* gene that confers resistance to tobramycin (Sabbagh et al., 2021). Class IV and class V integrons are not commonly reported on and have been reported in *Vibrio* species (Kaushik et al., 2019; ; Okoh & Fadare, 2022).

1.2.4. Antimicrobial resistant *Escherichia coli*

E. coli is a Gram-negative, rod-shaped bacteria that belongs to the Enterobacterales subclass of Gamma proteobacteria and it is well known for its rapid growth under favorable conditions with a replication time of about 20 minutes (Jang et al., 2017). The true burden of *E. coli*-associated infections in Sub-Saharan Africa is largely unclear, while reports of prevalence rates ranging from 14.7% to as high as 82% have been reported in Nigeria, Tanzania, and South Africa (Egwu et al., 2023). *E. coli* is regarded as a reliable indicator of AMR and a significant reservoir of ARGs due to its ability to both acquire and transfer ARGs within bacterial communities (Jain et al., 2021). MDR *E. coli* is a major global concern in human medicine, as wild-type strains that are naturally susceptible to most clinically important antimicrobials can readily accumulate ARGs encoding ESBLs, carbapenemases, 16S rRNA methylases, plasmid-mediated quinolone resistance (PMQR) genes, and *mcr* genes, conferring resistance to cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, and polymyxins, respectively (Poirel et al., 2018). Mbelle et al. (2019) reported 20 MDR clinical *E. coli* isolates from Pretoria that were resistant to 3rd to 4th generation cephalosporins and carbapenems, with most also showing resistance to aminoglycosides (gentamicin and tobramycin) and ciprofloxacin, and four isolates additionally resistant to the polymyxin colistin.

1.2.4.1. Extraintestinal pathogenic *Escherichia coli* infections

ExPEC has been reported to cause a number of community-acquired infections (CAIs) and HAIs, like neonatal meningitis, peritonitis, and bloodstream and UTIs in all age groups, with *E. coli* accounting for approximately >75% UTIs. The most common extra-intestinal site colonized by these bacteria is the urinary tract, which in turn, is a common source for BSIs. *E. coli* strains are causative of a high number of hospital outbreaks worldwide that result in longer hospital stays, increased healthcare costs and high

mortality rates (Kocsis et al., 2022; Morales et al., 2023). ExPEC are distinguished from commensal strains by their capacity to cause disease beyond the host gut as a result of pathogenic virulence factors they possess (Dale & Woodford, 2015).

1.2.4.2. Diarrheagenic *Escherichia coli*

One of the main bacterial causes of diarrhea worldwide is diarrheagenic *E. coli* (DEC) and the pathotypes are distinguished by their distinct virulence factors. DEC can be divided into enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and diffusely adhering *E. coli* (DAEC) based on various pathogenic mechanisms and virulence-associated genes, with ETEC frequently linked to traveler's diarrhea, while EAEC and EPEC were typically linked to child outbreaks of acute diarrhea (Mandal et al., 2017; Khairy et al., 2019; Zhou et al., 2021). Heine et al. (2024) identified 164 MDR *E. coli* isolates from children under five with diarrhea in the Vhembe District, Limpopo, reporting over 90% resistance to cephalosporins (cefuroxime, cefotaxime, ceftazidime, and cefepime) and predominance of the bla_{TEM} ARG detected by PCR, followed by $bla_{CTX\text{-}M\text{-}9}$, with EAEC and EPEC among the detected pathotypes.

1.2.5. Antimicrobial resistant *Klebsiella pneumoniae*

K. pneumoniae is a major opportunistic pathogen frequently associated with a broad spectrum of infections, including urinary tract infections, pneumonia, BSIs, and pyogenic liver abscesses (Vading et al., 2018). These infections occur predominantly in healthcare settings and disproportionately affect vulnerable populations such as premature infants, the elderly, and immunocompromised individuals, where *K. pneumoniae* can spread rapidly in high-risk environments including intensive care units and neonatal care facilities, leading to healthcare-associated outbreaks (Prestinaci et al., 2015). Globally, more than 30% of *K. pneumoniae* isolates are resistant to third-generation cephalosporins, with resistance rates exceeding 60% in some countries (World Health Organization [WHO], 2014). In South Africa, *K. pneumoniae* has been identified as the leading cause of BSIs in both public and private healthcare sectors, with approximately 70% and 40% of isolates demonstrating resistance to cephalosporins and carbapenems, respectively, between 2018 and 2022 (National Department of Health, 2024). More recently, the emergence of hypervirulent ST23 *K. pneumoniae* carrying carbapenemase ARGs has been reported in several countries, however the prevalence and distribution of this high-risk clone within African regions remains unknown (WHO, 2024). Nonetheless, CRKP has been reported across various African regions (Moloto et al., 2023). A study by Marais et al. (2024) characterized 85 CRKP isolates from hospitalized patients in Cape Town, with ST219, ST307, ST17, ST13, ST2497 being the most prevalent STs, and resistance predominantly associated with $bla_{OXA\text{-}48}$-like ARGs.

1.2.6. Methods used to characterize the resistome and mobilome

The identification of ARGs in bacteria has predominantly relied on molecular techniques such as traditional PCR, multiplex PCR, real-time PCR (qPCR), and Reverse Transcriptase PCR (RT-PCR), which have been developed and extensively employed in laboratories for rapid identification of ARGs through target gene amplification (Algarni et al., 2022). DNA microarray and hybridization methods have also been utilized to simultaneously identify numerous ARGs in a short timeframe, but have largely been supplanted by WGS approaches, which have become widely adopted and effective tools for detecting ARGs (Algarni et al., 2022). WGS data can be effectively analysed using a wide range of bioinformatic pipelines, like ARG Gene Annotation (ARG-ANNOT), ARG-Online Analysis Pipeline (ARGs-OAP), Antibiotic Resistance Identification by Assembly (ARIBA), Comprehensive Antibiotic Resistance Database (CARD), DeepARGs, Genomic Resistance Outcome Optimizer Tool (GROOT), KmerResistance, NCBI-AMRFinder, Pathosystems Resource Integration Centre (PATRIC), PointFinder, ResFinder, Short Read Sequence Typing 2 (SRST2), Search Engine for Antimicrobial Resistance (SEAR), Short, Better Representative Extract Dataset (ShortBRED), Sequence Search Tool for Antimicrobial Resistance (SSTAR), and many others (Yamin et al., 2023). The selection of an appropriate database or tool is determined by the specific objectives of each study (Lee et al., 2021).

ARGs are mainly disseminated on plasmids and Tns (Wintersdorff et al., 2016). Plasmid Sequence Database (PLSDB) is a database focusing on plasmids (Schmartz et al., 2022), PHAge Search Tool Enhanced Release (PHASTER) tool dedicated to bacterioprophages (Arndt et al., 2016), ICEberg database for ICEs (Liu et al., 2019), Pathogenicity Island Database (PAIDB) for handling pathogenicity islands (Yoon et al., 2015), the IslandViewer tool for GIs (Bertelli et al., 2017), TnCentral database for Tns (Ross et al., 2021), and the ISfinder database for ISs (Siguier et al., 2006), are just a selection of the many publicly accessible resources available for the identification of bacterial MGEs. Other plasmid tools are based on the type of plasmid present, for example, Chen et al. (2021) classified plasmids according to their mobility properties using Plascad, a bioinformatic pipeline designed to identify and visualize plasmids. Additional tools for MGE detection include MobileElementFinder, which identifies IS elements, transposons, and ICEs via BLAST searches against curated references, and MGEfinder, which performs integrative MGE detection from short-read data; however, MGEfinder's local implementation demands substantial bioinformatics expertise, potentially limiting accessibility (Wang, M., et al., 2022).

The rate of ARB carriage is increasing within communities, and since there has been an increased awareness of asymptomatic ARB carriers, it is crucial to develop AMR surveillance within community settings (Jamrozik & Selgelid, 2019). Both community and clinical play a role in AMR mitigation and policy development (Bertagnolio et al., 2024). Despite this, there is a paucity of South African studies delineating the prevalence of ARB, the underlying resistome and mobilome conferring resistance, or what clones are circulating among healthy individuals, while many studies have focused on clinical

settings (Thakur et al., 2025) . *E. coli* and *K. pneumoniae* were selected as key indicator organisms, as *E. coli* is a leading cause of CAIs, while *K. pneumoniae* is frequently associated with HAIs (Lin et al., 2019; Thuy et al., 2021) and both have been reported as the leading cause of deaths associated with AMR globally (Murray et al., 2022). A key unanswered question is: are resistance determinants, clones, and transmission pathways shared between community and clinical *E. coli* and *K. pneumoniae*? To address this gap, our study will compare isolates from healthy household community dwellers and hospital patients, delineating the resistome, associated mobilome, clonal lineages, and phylogenetic relationships across these settings. This integrated genomic approach will enable more understanding of AMR transmission pathways without neglecting either setting. These findings can inform and strengthen integrated AMR surveillance strategies and mitigation across both community and clinical settings.

1.3. Aim

This study aims to compare and contrast the resistome, mobilome, and phylogenetic relationships of *E. coli* and *K. pneumoniae* isolates from hospital patients and nearby healthy community dwellers in the uMgungundlovu District of KwaZulu-Natal, to ascertain potential transmission dynamics and directionality of AMR.

1.4. Study objectives

- To compare the phenotypic characteristics of AMR observed in *E. coli* and *K. pneumoniae* isolates from hospital patients and healthy community dwellers.
- To describe and compare resistomes and mobilomes conferring resistance in clinical and community isolates using WGS and bioinformatic analyses.
- To ascertain transmission dynamics and directionality through the potential genomic associations of resistomes with mobilomes, clonality and phylogeny.

1.5. Summary of methods

This study received ethical approvals from UKZN's Biomedical Research Ethics Committee (BREC) (**Appendix 1**)and KZN Department of Health's Provincial Health Research Ethics Committee (PHREC) (**Appendix 2**).

1.5.1. General methodology

Stool samples were collected from healthy community dwellers residing in an informal settlement in uMgungundlovu District, while clinical samples were obtained from the Medical Microbiology Department of the National Health Laboratory Service that processes microbiology samples for the District. Community stool samples were processed by a private laboratory , whereas clinical samples were provided and processed by the National Health Laboratory Service. Presumptively identified *E. coli* and *K. pneumoniae* isolates were subjected to antimicrobial susceptibility testing (AST) using the

VITEK® 2 automated system and screened for ESBL production. Confirmed isolates were subsequently subjected to WGS, and bioinformatic pipelines were used to characterize the resistome, mobilome, clonality, and phylogenetic relationships of the isolates.

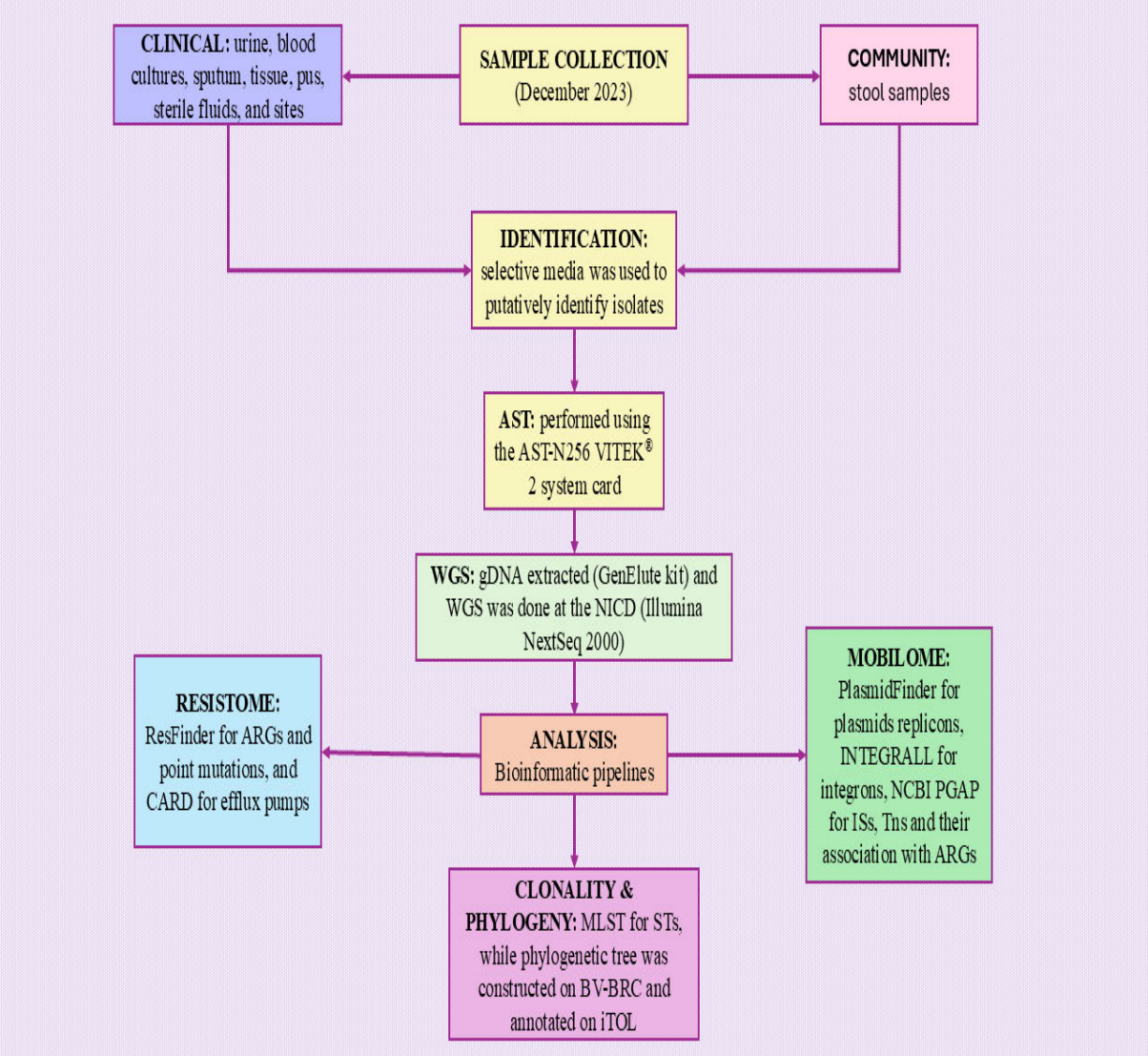

Figure 1: Overview of study methodology

1.6. Structure of the dissertation

This dissertation is structured into three chapters, as outlined below:

Chapter 1 (Introduction and Literature Review): outlines the burden of AMR in both clinical and community settings from the literature, provides an overview of shared resistomes across these settings, how antibiotics shape the resistome and describes the mechanisms underlying resistome dissemination. It further reviews antimicrobial resistance in *E. coli* and *K. pneumoniae*. The chapter concludes with the study rationale, presented in the final paragraph of the literature review, followed by the aims and objectives of the study.

Chapter 2 (Community and Clinical Resistomes and Mobilomes: A Correlation): comprises a manuscript submitted to *Scientific Reports* (**Submission ID:** c1a8b7fd-371a-42f9-921e-39cce210ae8a). It compares the resistomes, mobilomes, and phylogenetic relationships of clinical *E. coli* and *K. pneumoniae* isolates with those recovered from healthy community dwellers.

Chapter 3 (Conclusion, limitations and recommendations): summarises the main findings in relation to the study objectives and highlights the study's limitations and recommendations for future research.

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CHAPTER 2: MANUSCRIPT

This dissertation is presented in a manuscript format, in accordance with the requirements of the College of Health Sciences at the University of KwaZulu-Natal:

Community and Clinical Resistomes and Mobilomes: A Correlation

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Contributions:

Khanyisa Mahonisi (principal investigator) co-conceptualized the study, performed laboratory work and data analysis, and wrote the manuscript.

Joshua Mbanga (co-supervisor) co-conceptualized the study, assisted with laboratory protocols and data analysis, vetted results, and provided thorough critical review of manuscript.

Arshad Ismail assisted with data analysis, vetted results, and provided critical review of the manuscript.

Sabiha Y. Essack (principal supervisor) co-conceptualized the study, guided the literature review, obtained ethical approval and funding, vetted results, and provided a thorough and critical review of the manuscript.

Community and Clinical Resistomes and Mobilomes: A Correlation

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Abstract

Escherichia coli and *Klebsiella pneumoniae* are major contributors to antimicrobial resistance (AMR)-related mortality globally. The resistomes, mobilomes, clonalities, and phylogenies of 32 *E. coli* (15 clinical, 17 community) and 14 *K. pneumoniae* (6 clinical, 8 community) isolates from KwaZulu-Natal were compared to ascertain similarities and differences between healthcare-related pathogens and community commensals using whole genome sequencing (WGS) and bioinformatic pipelines. Resistome analysis revealed that $bla_{CTX\text{-}M\text{-}15}$ predominated in clinical isolates, whereas $bla_{TEM\text{-}1B}$ was more common in community *E. coli*, and all *K. pneumoniae* isolates carried a bla_{SHV} variant. Two clinical *E. coli* harboured $bla_{OXA\text{-}181}$, and one *K. pneumoniae* carried $bla_{OXA\text{-}181}$ and $bla_{NDM\text{-}5}$; none were detected in community isolates. IncF plasmid replicons were most frequent, and gene cassettes within class 1 integrons, insertion sequences, and transposons contributed to ARG mobilization across both settings. A broad range of sequence types (STs) were detected across both settings, with shared STs limited to ST131, ST10, and ST1193 in *E. coli*, and ST17 in *K. pneumoniae*. Phylogeny showed that clinical *E. coli* clustered with some community isolates, while *K. pneumoniae* showed limited clustering. The overlap of resistomes, mobilomes, and STs suggest potential AMR transmission between settings, highlighting the importance of AMR surveillance in community settings.

Keywords: community resistome, clinical resistome, carriage, *Escherichia coli*, *Klebsiella pneumoniae*, antimicrobial resistance

2.1. Introduction

Antimicrobial resistance (AMR) remains a persistent global public health threat, undermining the effective management of infectious diseases and imposing considerable constraints on medical progress [World Health Organization (WHO), 2024; Okeke et al., 2024]. Determining the true burden of AMR remains a significant challenge, particularly in settings where surveillance systems are weak and data availability is limited (Murray et al., 2022). Sub-Saharan Africa is projected to bear a high burden of AMR, with an estimated 6.63 million deaths (95% uncertainty interval 5.00-8.66) directly attributable to AMR expected to occur in the region from 2025 to 2050 (Naghavi et al., 2024). *Escherichia coli* and *Klebsiella pneumoniae* are opportunistic Enterobacterales pathogens (Yin et al., 2025). In 2019, they were among the six leading pathogens responsible for AMR-related deaths globally, in which *E. coli* was the leading cause of death (Murray et al., 2022).

Human-to-human interface plays a significant role in the transmission and acquisition of AMR in both healthcare and community settings (Salam et al., 2023). Other studies have reported a variety of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in *E. coli* and *K. pneumoniae* clinical isolates in South Africa conferring resistance to a wide range of antibiotics, such as aminoglycosides, β-lactams, polymyxins, and fluoroquinolones, among others (Newton-Foot et al., 2017; Mbelle et al., 2019; Msolo et al., 2020; Madni et al., 2021; Marais et al., 2024; Salvador-Oke et al., 2024; Hetsa et al., 2025; Masalane et al., 2025). The number of individuals colonized by antibiotic-resistant bacteria without symptoms far exceeds those with active infections, suggesting that AMR surveillance in healthy community dwellers could serve as a valuable early warning system for detecting emerging resistance mechanisms that contribute to the broader AMR burden (Smith et al., 2021). This is further supported by a longitudinal study by Hilty et al. (2012), who found that the transmission rates of extended-spectrum β-lactamase-producing (ESBL) *E. coli* and *K. pneumoniae* were significantly higher within households than in the hospital setting.

The acquisition of ARGs across bacterial species is largely attributable to MGEs (Partridge et al., 2018; Shang et al., 2025) with most ARGs located on MGEs rather than on the bacterial chromosome (Ye et al., 2025). Symptomatic patients and asymptomatic carriers are both reservoirs of AMR dissemination (Ye et al., 2025). Therefore, delineating the presence of ARGs along with their association to MGEs is essential for understanding the transmission dynamics of AMR in both clinical and community settings. This study compares the prevalence of AMR, the distribution of ARGs and MGEs, as well as the clonal and phylogenetic relationships with other South African isolates, among *E. coli* and *K. pneumoniae* isolates collected from patients at a regional hospital and community dwellers residing in proximity to the hospital in KwaZulu-Natal using whole genome sequencing (WGS) and bioinformatics analysis.

2.2. Methodology

2.2.1. Ethical approval and participant consent

This study was ethically approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Reference No. BREC 00003640-2021) and the Provincial Health Research Ethics Committee of the KwaZulu-Natal Department of Health (Reference No. KZ 202203 023). Written informed consent was obtained from all community participants before enrolment.

2.2.2. Study site and sample collection

This study is nested within a broader longitudinal AMR surveillance study that was conducted among 300 participants residing in informal settlements over a 12-month period. The study sites were informal settlements in Ward 22 in the uMgungundlovu District, which is one of the 11 districts in KwaZulu-Natal (Department of Cooperative Governance and Traditional Affairs, 2023). Stool samples were collected from healthy community dwellers monthly from which *E. coli* and *K. pneumoniae* were selectively isolated. The National Health Laboratory Services (NHLS) processed and provided the clinical samples that were collected from inpatients and outpatients at a regional hospital near the informal settlement. The hospital departments from which the samples were obtained included the intensive care unit (ICU), outpatient department (OPD), emergency department, gynaecology OPD, orthopaedic, paediatric, surgical, and other medical wards. The clinical specimen types included urine, blood cultures, sputum, tissue, pus, and other sterile fluids and sites. Isolates collected in December 2023 constituted the sample for this study, with the total sample size of 35 clinical isolates (22 *E. coli* and 13 *K. pneumoniae*) was matched with a proportionate number of randomly-selected isolates from the community (44 *E. coli* and 26 *K. pneumoniae*).

2.2.3. Bacterial identification and antibiotic susceptibility profiling

E. coli isolates were cultured on CHROMagar™ *E. coli*/Coliforms (ECC) plates (Paris, France) plates, incubated for 24 hours at 37°C. *K. pneumoniae* were cultured on Simmons' Citrate Agar (Merck, Darmstadt, Germany) plates that were supplemented with Inositol (SCAI), adjusted to pH 7.2, and incubated at 37°C for 48 h. Antibiotic susceptibility was assessed using the AST-N256 VITEK® 2 system card (bioMérieux, Durham, North Carolina, USA) on the VITEK® 2 automated system (bioMérieux, Marcy-l'Étoile, France). The following antibiotic panel was used: amikacin (AMK), amoxicillin–clavulanic acid (AMC), ampicillin/amoxicillin (AMP/AMX), cefepime (CPM), cefotaxime/ceftriaxone (CTX/CRO), ceftazidime (CAZ), cefuroxime (CXM), ciprofloxacin (CIP), ertapenem (ERT), gentamicin (GEN), imipenem (IMP), meropenem (MEM), piperacillin-tazobactam (TZP), tigecycline (TGC), and trimethoprim-sulfamethoxazole/co-trimoxazole (SXT). In addition, cefoxitin (FOX) and nitrofurantoin (NIT) were included in the antibiotic panel for clinical isolates, while doripenem (DOR) and tobramycin (TOB) were included for community isolates. Results were

interpreted as either susceptible (S), intermediate (I), or resistant (R) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020). Community isolates were screened for ESBL production using CHROMID® ESBL agar (bioMérieux, Marcy-l'Étoile, France) plates, incubated at 37°C for 24 h. Clinical isolates were screened at the NHLS using the VITEK® 2 automated system (bioMérieux, Marcy-l'Étoile, France), which applies the Advanced Expert System (AES) to detect potential ESBL-producers based on cephalosporin minimum inhibitory concentration patterns. A total of 60 putatively identified isolates were selected for WGS based on phenotypic resistance to one or more antibiotics. Following WGS, 14 isolates were excluded from this study due to cross-contamination with other bacterial species.

2.2.4. WGS and annotation

Genomic DNA was extracted using the GenElute™ Bacterial Genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. The NanoDrop Lite Plus Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to assess the quality and quantity of the gDNA. WGS was performed at the National Institute of Communicable Diseases (NICD). Paired-end libraries (2 × 300 bp) were prepared and multiplexed using the Illumina® DNA Prep Kit (Illumina, San Diego, CA, USA), and sequencing was conducted on the Illumina NextSeq 2000 System. Raw reads were trimmed using Trim-Galore v0.6.10 (https://github.com/FelixKrueger/TrimGalore), and *de novo* assembly was performed using SKESA v2.3.0 (https://github.com/ncbi/SKESA). The assembled genomes were submitted to the National Center for Biotechnology Information (NCBI) GenBank database under the BioProject PRJNA1183563, with individual accession numbers provided in Supplementary **Table S1**.

2.2.5. Genome, resistome, mobilome and clonal analysis

RAST SEEDVIEWER (https://rast.nmpdr.org/seedviewer.cgi) was used to determine the genome characteristics. Resistome (ARGs, chromosomal point mutations and efflux pumps) and mobilome (plasmids, insertion sequences, integrons, gene cassettes, and transposons) were characterized using bioinformatics tools. ARGs and chromosomal point mutations were identified using ResFinder (Zankari et al., 2012; Bortolaia et al., 2020), at a 90% threshold ID and 60% minimum length. The Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/) was used to identify efflux pump genes (Sundaramoorthy et al., 2019). Multilocus Sequence Typing (MLST) and PlasmidFinder analyses were performed using tools available through the Centre for Genomic Epidemiology (MLST 2.0: https://cge.food.dtu.dk/services/MLST/; PlasmidFinder 2.1: https://cge.food.dtu.dk/services/PlasmidFinder/) at a 95% threshold ID and 60% minimum coverage for plasmid replicons. The INTEGRALL database (http://integrall.bio.ua.pt/) was used to identify integrons and gene cassettes. Insertion sequences (ISs) were delineated using ISFinder (https://isfinder.biotoul.fr/). The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) annotation

files (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1183563) were used to assess the synteny of ISs and transposons (Tns) with ARGs.

2.2.6. Phylogenetic relationships

Publicly available South African clinical whole genome sequences of *E. coli* (n = 50, 2016-2018, 2020 and 2023) and *K. pneumoniae* (n = 131, 2019-2024) isolates curated on the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) platform (https://www.bv-brc.org/), were retrieved and compared with genomes sequenced in this study to construct phylogenetic trees. Phylogenetic analysis was performed on the BV-BRC platform, and the resulting trees were visualized, annotated, and edited using the Interactive Tree of Life (iTOL) online tool (https://itol.embl.de/) (Letunic & Bork, 2024).

2.3. Results

2.3.1. Prevalence of confirmed isolates and participant demographics

This study reports on 32 *E. coli* (n = 15 clinical; n = 17 community) and 14 *K. pneumoniae* (n = 6 clinical; n = 8 community) isolates that were confirmed by WGS. Clinical isolates were sourced from various hospital departments, including the medical ward (n = 5), paediatric ward (n = 1), emergency department (n = 4), gynaecology OPD (n = 3), surgical ward (n = 3), and other inpatient departments (n = 5). Clinical specimen types included urine (n = 13), blood cultures (n = 4), tissue (n = 2), and the remaining two isolates were from catheter tips and sterile sites. All community isolates were obtained from stool samples. The age distribution of community participants (excluding two with unknown age) ranged from 22 to 65 years, with a mean age of 40.9. For clinical participants, age ranged from 0 to 92 years, with a mean age of 39.5 years. The demographic details of the participants and bacterial isolate characteristics are shown in Supplementary **Table S1**, while the clinical isolate source by hospital department is detailed within the main text.

2.3.2. Antibiotic resistance profile of isolates

All *E. coli* and *K. pneumoniae* were susceptible to amikacin and ertapenem. Among the 32 *E. coli* isolates, 31 (96.9%) exhibited resistance to at least one β-lactam antibiotic, including penicillins (AMP/AMX and AMC), 2nd to 4th generation cephalosporins (CXM, CTX/CRO, CAZ, and CPM), and cephamycin (FOX). Resistance was also observed to non-β-lactam antibiotics, including aminoglycoside (GEN), β-lactamase inhibitor combination (TZP), fluoroquinolone (CIP), and trimethoprim-sulfonamide (SXT). High resistance to AMP/AMX (100% clinical, 94.1% community) was observed, followed by SXT (86.7% clinical, 82.4% community), CXM (73.3% clinical, 23.5% community), CIP (73.3% clinical, 17.6% community), and CTX/CRO (60% clinical, 23.5% community). One clinical isolate exhibited intermediate susceptibility to both IMP and MEM, while one community isolate was resistant to MEM (**Table 1**).

All *K. pneumoniae* isolates exhibited 100% resistance to AMP/AMX. Clinical *K. pneumoniae* isolates showed high resistance to CTX/CRO (83.3%), CXM (83.3%), CAZ (83.3%), SXT (83.3%), AMC (66.7%), TZP (66.7%), CIP (66.7%), GEN (66.7%), and FOX (50%). In contrast, community *K. pneumoniae* isolates showed narrower resistance profiles, with resistance to TGC (50%) being most prominent, and moderate resistance to TZP, CIP, and SXT (25%) was observed. One clinical isolate showed resistance to IMP and MEM, while one community isolate showed resistance to MEM (**Table 1**). Overall, clinical isolates exhibited higher and broader resistance profiles, which can be attributed to more antibiotic exposure within hospitals.

Table 1: Antimicrobial susceptibility of clinical and community *E. coli* and *K. pneumoniae* isolates

Antibiotic panel	***E. coli* clinical (n= 15)**			***E. coli* community (n= 17)**			***K. pneumoniae* clinical (n= 6)**			***K. pneumoniae* community (n= 8)**		
	S	**I**	**R**	**S**	**I**	**R**	S	I	R	S	I	R
AMK	15 (100)	0 (0)	0 (0)	17 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	8 (100)	0 (0)	0 (0)
GEN	13 (86.7)	0 (0)	2 (13.3)	17 (100)	0 (0)	0 (0)	2 (33.3)	0 (0)	4 (66.7)	8 (100)	0 (0)	0 (0)
TOB	–	–	–	17 (100)	0 (0)	0 (0)	–	–	–	8 (100)	0 (0)	0 (0)
AMP/AMX	0 (0)	0 (0)	15 (100)	1 (5.9)	0 (0)	16 (94.1)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	8 (100)
AMC	11 (73.3)	2 (13.3)	2 (13.3)	14 (82.4)	1 (5.9)	2 (11.8)	2 (33.3)	0 (0)	4 (66.7)	6 (75.0)	1 (12.5)	1 (12.5)
TZP	13 (86.7)	0 (0)	2 (13.3)	12 (70.6)	0 (0)	5 (29.4)	2 (33.3)	0 (0)	4 (66.7)	6 (75.0)	0 (0)	2 (25.0)
CPM	13 (86.7)	0 (0)	2 (13.3)	13 (76.5)	0 (0)	4 (23.5)	2 (33.3)	2 (33.3)	2 (33.3)	7(87.5)	0 (0)	1 (12.5)
CTX/CRO	6 (40.0)	0 (0)	9 (60.0)	13 (76.5)	0 (0)	4 (23.5)	1 (16.7)	0 (0)	5 (83.3)	7(87.5)	0 (0)	1 (12.5)
FOX	13 (86.7)	0 (0)	2 (13.3)	–	–	–	3 (50.0)	0 (0)	3 (50.0)	–	–	–
CXM	4 (26.7)	0 (0)	11 (73.3)	13 (76.5)	0 (0)	4 (23.5)	1 (16.7)	0 (0)	5 (83.3)	6 (75.0)	1 (12.5)	1 (12.5)
CAZ	10 (66.7)	1 (6.7)	4 (26.7)	13 (76.5)	0 (0)	4 (23.5)	1 (16.7)	0 (0)	5 (83.3)	7(87.5)	0 (0)	1 (12.5)
CIP	3 (20.0)	1 (6.7)	11 (73.3)	14 (82.4)	0 (0)	3 (17.6)	2 (33.3)	0 (0)	4 (66.7)	5 (62.5)	1 (12.5)	2 (25.0)
SXT	2 (13.3)	0 (0)	13 (86.7)	3 (17.6)	0 (0)	14 (82.4)	1 (16.7)	0 (0)	5 (83.3)	6 (75.0)	0 (0)	2 (25.0)
DOR	–	–	–	17 (100)	0 (0)	0 (0)	–	–	–	8 (100)	0 (0)	0 (0)
ERT	15 (100)	0 (0)	0 (0)	17 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	8 (100)	0 (0)	0 (0)
IMP	14 (93.3)	1 (6.7)	0 (0)	17 (100)	0 (0)	0 (0)	5 (83.3)	0 (0)	1 (16.7)	8 (100)	0 (0)	0 (0)
MEM	14 (93.3)	1 (6.7)	0 (0)	16 (94.1)	0 (0)	1 (5.9)	5 (83.3)	0 (0)	1 (16.7)	7(87.5)	0 (0)	1 (12.5)
TGC	15 (100)	0 (0)	0 (0)	17 (100)	0 (0)	0 (0)	6 (100)	0 (0)	0 (0)	4 (50.0)	0 (0)	4 (50.0)
NIT	15 (100)	0 (0)	0 (0)	–	–	–	2 (33.3)	2 (33.3)	2 (33.3)	–	–	–

Data is expressed as the number of isolates with the percentage in parentheses. The (-) symbol indicates that a particular antibiotic was not included in the respective antibiotic panel. **Key:** AMK= amikacin, GEN= gentamicin, TOB= tobramycin, AMP/AMX= ampi/amoxicillin, AMC= amoxycillin-clavulanic acid, TZP= piperacillin/tazobactam, CPM= cefepime, CTX/CRO= cefotaxime/ceftriaxone, FOX= cefoxitin, CXM= cefuroxime, CAZ= ceftazidime, CIP= ciprofloxacin, SXT= trimethoprim-sulfamethoxazole/co-trimoxazole, DOR= doripenem, ERT= ertapenem, IMP= imipenem, MEM= meropenem, TGC= tigecycline, NIT= nitrofurantoin, S= susceptible, I= intermediate, R= resistant

2.3.3. Genome characteristics

The genomic features of the sequenced isolates, such as coverage, GC content (%), genome size, L50, N50, and RNA count, are shown in Supplementary **Table S1**.

2.3.4. Resistome analysis

Resistome analysis showed that all clinical *E. coli* isolates (100%) and 15 (88.2%) community isolates harboured β-lactamase ARGs. A total of seven *E. coli* isolates were identified as ESBL-positive (20% clinical; 23.5% community) (**Table 2**). Notably, we detected the *bla*$_{CTX-M}$ (10/15) in clinical *E. coli* isolates but 3/10 were identified as ESBL-producers by the VITEK 2 AES. In clinical isolates, we detected *bla*$_{CTX-M-15}$ (8/15), *bla*$_{TEM-1B}$ (4/15), *bla*$_{CTX-M-27}$ (1/15), *bla*$_{CTX-M-231}$ (1/15), *bla*$_{TEM-1C}$ (1/*15),* and *bla*$_{OXA-1}$ (1/15) ARGs. In addition, the carbapenemase *bla*$_{OXA-181}$ ARG was detected in two clinical *E. coli* isolates (EC07 and EC19). EC07 exhibited no phenotypic resistance to carbapenems, whereas EC19 showed intermediate resistance to IMP and MEM. Among community isolates, the most prevalent β-lactamase ARGs were *bla*$_{TEM-1B}$ (12/17), followed by *bla*$_{CTX-M-15}$ (2/17), *bla*$_{CTX-M-231}$ (1/17), *bla*$_{CTX-M-27}$ (1/17), *bla*$_{TEM-216}$ (1/17), and *bla*$_{LAP-2}$ (1/17). Trimethoprim ARGs in clinical isolates included *dfrA17* (12/15), *dfrA1* (1/15), dfrA5 (1/15), and *dfrA12* (1/15). Sulfonamide resistance was conferred either by *sul1* and *sul2* or a combination of both in clinical and community isolates. Among community isolates, *dfrA14* (6/17) was most common, followed by *dfrA17* (3/17), *dfrA7* (3/17), *dfrA1* (2/17), and *dfrA5* (1/17).

Aminoglycoside ARGs were detected in both clinical and community isolates. In clinical isolates, *aadA5* (10/15) was most common, followed by *aph(3'')-Ib* and *aph(6)-Id* (6/15 each). Other detected genes were *aph(3')-Ia*, *aac(3)-IIe*, *aac(3)-IId*, *aadA1*, and *aadA3*. Additionally, EC19 was resistant to GEN without detectable aminoglycoside genes. This discordance is likely due to the activity of efflux pumps (**Table 2**). In community isolates, *aph(3'')-Ib* and *aph(6)-Id* were most prevalent (9/17 each), followed by *aadA1* (2/17) and *aadA5* (2/17). Fluoroquinolone resistance was mainly mediated by *qnrS1*, detected in 4/15 clinical and 6/17 community isolates. *Aac(6')-Ib-cr* was also identified in one clinical isolate (EC13). Several *E. coli* isolates (7/15 clinical; 2/17 community) exhibited phenotypic resistance to CIP despite the absence of corresponding ARGs, suggesting that resistance may be mediated by chromosomal mutations in the quinolone resistance-determining regions (QRDRs) of DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE). Fluoroquinolone-associated point mutations were analysed in all CIP resistant *E. coli* isolates. Mutations within the QRDRs of gyrA (S83L and D87N) and parC (S80I and E84V) were the most frequently detected across isolates of both settings (Supplementary **Table S2**). Only two clinical isolates (EC14 and EC19) lacked detectable QRDR mutations. The absence of QRDR mutations in EC14 and EC19 suggests that CIP resistance in these isolates may be mediated by the AcrAB–TolC efflux pump (**Table 2**). Notably, QRDR mutations were

observed in isolates both with (clinical 4/15) and without (clinical 7/15; community 2/17) fluoroquinolone ARGs.

Two antibiograms were shared between clinical and community *E. coli* isolates, with AMP/AMX–SXT being the predominant shared pattern (2/15 clinical; 9/17 community), followed by AMP/AMX–CIP–SXT in one clinical (EC01) and one community (EC22) isolate (**Table 2**). Although these isolates were not genotypically identical, WGS revealed overlapping ARGs. Other detected ARGs in clinical isolates included genes conferring resistance to macrolides [*mph(A)*], phenicols (*catA1* and *catB3*), and tetracycline [*tet(A)*]. Community isolates also harboured *catA1*, *mph(A)*, *tet(A)*, and *tet(B)* genes. Resistome analyses generally corresponded with phenotypic resistance.

Table 2: Sequence types, antibiograms, ESBL production, resistome, efflux pump genes, and plasmid replicons of clinical and community *E. coli* isolates

Isolate (MLST)	Setting	Antibiogram	ESBL +/-	Resistome	Efflux pump genes associated with resistance	Plasmid replicons
EC01 (ST3337)	Clinical	AMP/AMX-CIP-SXT	-	*bla$_{TEM-1B}$, dfrA17, sul1, sul2, aadA5, aph(3')-Ia, aph(3'')-Ib, aph(6)-Id, catA1, mph(A), tet(B)*	mdtH, kdpE, msbA, mdtN, mdtM, evgS, evgA, H-NS, marA, AcrA, cpxA, gadX, qacEΔ1, leuO, CRP, rsmA, mdfA, baeR, AcrAB-TolC, tetR	IncQ1
EC02 (ST1193)	Clinical	AMP/AMX-CTX/CRO-CXM-CIP-SXT	-	*bla$_{CTX-M-27}$, dfrA17, sul1, sul2, aadA5, aph(3'')-Ib, aph(6)-Id, mph(A), tet(A)*	leuO, H-NS, cpxA, gadW, evgA, qacEΔ1, emrR, AcrA, rsmA, gadX, CRP, emrY, evgS, marA, kdpE, mdfA, AcrAB-TolC, baeR	Col(BS512), Col156, ColpVC, IncFIA, IncFIB(AP001918)
EC03 (ST131)	Clinical	AMP/AMX	-	*bla$_{CTX-M-15}$, dfrA17, sul1, aadA5, mph(A)*	H-NS, leuO, gadW, cpxA, evgA, qacEΔ1, kdpE, ermB, CRP, gadX, AcrA, evgS, baeR, AcrAB-TolC	IncFIA, IncFIB(AP001918), IncFII(pRSB107)
EC04 (ST38)	Clinical	AMP/AMX-CPM-CTX/CRO-CAZ-CXM-CIP-SXT	-	*bla$_{CTX-M-15}$, dfrA5, dfrA12, sul1, sul2, aadA3, aac(3)-IIe, mph(A)*	marA, H-NS, emrR, evgA, cpxA, qacEΔ1, leuO, CRP, kdpE, mdfA, evgS, gadX, AcrA, AcrAB-TolC, baeR	Col156, IncFIB(AP001918), IncFII
EC05 (ST38)	Clinical	AMP/AMX-SXT	-	*bla$_{TEM-1C}$, dfrA1, sul1, aadA1, aph(3'')-Ib, aph(6)-Id, tet(A)*	marA, emrR, H-NS, evgA, cpxA, qacEΔ1, leuO, CRP, rsmA, AcrA, evgS, gadX, AcrAB-TolC, baeR	IncFIA, IncFIB(AP001918), IncFIC(FII)
EC07 (ST3572)	Clinical	AMP/AMX-CTX/CRO-CXM-CIP-SXT	-	*bla$_{OXA-181}$, dfrA17, sul1, sul2, aph(3'')-Ib, aph(6)-Id, mph(A), tet(A)*	marA, kdpE, emrY, evgA, evgS, AcrA, gadX, qacEΔ1, H-NS, CRP, leuO, AcrAB-TolC, baeR	Col(BS512), Col(MG828), Col156, ColKP3, ColpEC648, IncFIA, IncM1
EC08 (ST69)	Clinical	AMP/AMX-SXT	-	*bla$_{TEM-1B}$, dfrA17, sul2, aadA5, aph(3'')-Ib, aph(6)-Id, mph(A), tet(B)*	emrY, evgA, mdtN, marA, mdtM, H-NS, evgS, rsmA, CRP, gadX, leuO, kdpE, AcrA, AcrE, AcrAB-TolC, baeR, tetR	Col156, Col(MG828), IncFIB(AP001918), IncFII(29), IncQ1
EC09 (ST131)	Clinical	AMP/AMX-CTX/CRO-CAZ-CXM-CIP-GEN-SXT	+	*bla$_{TEM-1B}$, bla$_{CTX-M-15}$, dfrA17, sul1, sul2, aadA5, aac(3)-IId, aph(3'')-Ib, aph(6)-Id, mph(A), tet(A)*	leuO, cpxA, evgA, H-NS, gadW, AcrD, qacEΔ1, mdfA, kdpE, CRP, TolC, emrY, evgS, marA, AcrA, gadX, baeR, AcrAB-TolC	Col156, ColpVC, IncFIA, IncFIB(AP001918), IncFII(pSE11), IncFII(pRSB107), IncQ1
EC10 (ST131)	Clinical	AMP/AMX-CTX/CRO-CXM-CIP-SXT	-	*bla$_{CTX-M-15}$, dfrA17, sul1, aadA5, mph(A)*	H-NS, leuO, gadW, cpxA, evgA, qacEΔ1, mdfA, kdpE, ermB, CRP, gadX, TolC, AcrA, evgS, baeR, AcrAB-TolC	IncFIA, IncFIB(AP001918), IncFII(pRSB107)
EC13 (ST131)	Clinical	AMP/AMX-CXM-CIP-SXT	-	*bla$_{CTX-M-15}$, bla$_{OXA-1}$, dfrA17, sul1, aadA5, aac(6')-Ib-cr, catB3, mph(A), tet(A)*	evgA, leuO, cpxA, gadW, H-NS, qacEΔ1, mdfA, kdpE, AcrA, TolC, CRP, gadX, AcrAB-TolC, baeR	Col(BS512), IncFIA, IncFII

EC14 (ST10)	Clinical	AMP/AMX-CTX/CRO-CAZ-CXM-CIP-SXT	+	*bla$_{CTX-M-15}$, dfrA17, sul1, aadA5, mph(A), qnrS1*	H-NS, AcrA, kdpE, emrY, evgA, evgS, gadX, cpxA, qacEΔ1, TolC, CRP, leuO, mdfA, baeR, AcrAB-TolC	Col(BS512), ColpVC, IncFII
EC15 (ST13823)	Clinical	AMP/AMX-CTX/CRO-CXM-SXT	-	*bla$_{CTX-M-15}$, dfrA17, sul1, aadA5, qnrS1*	evgA, AcrA, marA, gadX, H-NS, qacEΔ1, evgS, CRP, leuO, kdpE, cpxA, mdfA, baeR, AcrAB-TolC	IncFIB(AP001918)
EC16 (ST1193)	Clinical	AMP/AMX-CXM-CIP	-	*bla$_{TEM-1B}$, bla$_{CTX-M-231}$, mph(A), qnrS1*	cpxA, H-NS, evgA, leuO, gadW, kdpE, emrB, CRP, evgS, AcrA, marA, gadX, baeR, AcrAB-TolC	Col(BS512), ColpVC, IncFIA, IncFIB(AP001918)
EC18 (ST13823)	Clinical	AMC-AMP/AMX-CPM-CTX/CRO-FOX-CAZ-CXM-CIP-TZP-SXT	+	*bla$_{CTX-M-15}$, dfrA17, sul1, aadA5, qnrS1*	marA, evgA, AcrA, gadX, H-NS, qacEΔ1, evgS, CRP, leuO, kdpE, cpxA, AcrAB-TolC, baeR	Col(MG828), ColpVC, IncFIB(AP001918), pXuzhou21
EC19 (ST3572)	Clinical	AMC-AMP/AMX-CTX/CRO-FOX-CXM-CIP-GEN-TZP-SXT	-	*bla$_{OXA-181}$, dfrA17, sul1, mph(A), tet(A)*	marA, emrY, evgA, evgS, AcrA, kdpE, H-NS, cpxA, gadX, AcrD, qacEΔ1, leuO, CRP, baeR, AcrAB-TolC	ColKP3, IncM1
EC21 (ST1722)	Community	AMP/AMX-CXM-CPM-CTX/CRO-CAZ-AMC-SXT-TZP	+	*bla$_{CTX-M-15}$, bla$_{TEM-216}$, dfrA1, sul1, aadA1, qnrS1*	kdpE, marA, emrR, cpxA, evgA, H-NS, qacEΔ1, leuO, AcrA, TolC, CRP, gadX, evgS, AcrAB-TolC, baeR	Col(BS512), Col(MG828), IncB/O/K/Z
EC22 (ST1193)	Community	AMP/AMX-CIP-SXT	-	*bla$_{TEM-1B}$, dfrA17, sul2, aph(3")-Ib, aph(6)-Id, mph(A), tet(B)*	leuO, gadW, H-NS, evgA, cpxA, kdpE, mdfA, AcrA, gadX, emrY, evgS, marA, baeR, AcrAB-TolC	Col(BS512), Col156, IncFIA, IncFIB(AP001918), IncQ1
EC23 (ST10)	Community	AMP/AMX-SXT	-	*bla$_{TEM-1B}$, dfrA14, sul2, aph(3")-Ib, aph(6)-Id, tet(A)*	AcrA, kdpE, cpxA, marA, emrY, evgA, H-NS, leuO, CRP, mdfA, evgS, AcrAB-TolC, baeR	Col(MG828), IncFII, IncFII(pCoo), IncFII(pHN7A8), IncX1
EC24 (ST1380)	Community	SXT	-	*dfrA14, sul2, aph(3")-Ib, aph(6)-Id, tet(A)*	marA, emrY, evgA, H-NS, gadX, kdpE, evgS, leuO, AcrA, mdfA, gadX, AcrAB-TolC, baeR	Col(MG828), IncB/O/K/Z, IncFIB(AP001918), IncFII, IncFII(pCoo), IncFII(pHN7A8), IncFII(pRSB107), IncX1
EC25 (ST10)	Community	AMP/AMX-SXT	-	*bla$_{TEM-1B}$, dfrA14, sul2, aph(3")-Ib, aph(6)-Id, tet(A)*	AcrA, kdpE, cpxA, marA, emrY, evgA, H-NS, leuO, CRP, mdfA, evgS, AcrAB-TolC, baeR	Col(MG828), IncFII, IncFII(pCoo), IncFII(pHN7A8), IncX1

EC26 (ST131)	Community	AMP/AMX-CXM-CPM-CTX/CRO-CAZ-CIP-AMC-TZP	+	*bla$_{CTX-M-27}$*	evgA, gadW, leuO, cpxA, H-NS, emrY, evgS, marA, CRP, gadX, TolC, kdpE, AcrA, mdfA, rsmA, baeR, AcrAB-TolC	Col(MG828), IncFIA, IncFIB(AP001918),
EC30 (ST2758)	Community	AMP/AMX-SXT	-	*bla$_{LAP-2}$, bla$_{TEM-1B}$, dfrA14, sul2, aph(3")-Ib, aph(6)-Id, qnrS1*	evgA, cpxA, marA, gadX, H-NS, AcrA, leuO, CRP, mdfA, kdpE, TolC, emrY, evgS, baeR, AcrAB-TolC	IncFIB(K), IncFII
EC31 (ST2852)	Community	AMP/AMX-SXT	-	*bla$_{TEM-1B}$, dfrA14, sul2, tet(A), qnrS1*	H-NS, evgA, AcrA, marA, cpxA, emrY, evgS, CRP, gadX, leuO, kdpE, mdfA, baeR, AcrAB-TolC	IncY
EC32 (ST382)	Community	AMP/AMX-SXT	-	*bla$_{TEM-1B}$, dfrA7, sul2, aph(3")-Ib, aph(6)-Id, tet(B)*	evgA, AcrA, H-NS, marA, gadX, qacEΔ1, leuO, emrY, evgS, kdpE, CRP, cpxA, baeR, AcrAB-TolC	IncFIB(AP001918), IncQ1
EC33 (ST336)	Community	MEM-TZP	-	*bla$_{TEM-1B}$, dfrA14, sul2, tet(A), qnrS1*	H-NS, marA, evgA, gadX, leuO, kdpE, CRP, rsmA, AcrAB-TolC	Col(BS512), IncFII(pRSB107), IncY
EC35 (ST2178)	Community	AMP/AMX-SXT	-	*bla$_{TEM-1B}$, dfrA7, sul2, aph(3")-Ib, aph(6)-Id*	marA, evgA, H-NS, AcrA, cpxA, qacEΔ1, gadX, evgS, leuO, kdpE, mdfA, CRP, baeR, AcrAB-TolC	IncFIB(AP001918), IncFII
EC36 (ST101)	Community	SXT	-	*dfrA1, sul1, aadA1, aph(6)-Id, tet(A)*	marA, evgA, AcrA, H-NS, cpxA, qacEΔ1, emrY, evgS, mdfA, leuO, kdpE, CRP, gadX, AcrAB-TolC, baeR	IncFIB(AP001918), IncFII(pCoo), IncX1
EC37 (ST131)	Community	AMP/AMX-SXT	-	*bla$_{TEM-1B}$, dfrA17, sul1, sul2, aadA5, aph(3")-Ib, mph(A)*	evgA, H-NS, gadW, leuO, cpxA, qacEΔ1, mdfA, kdpE, evgS, AcrA, TolC, CRP, AcrAB-TolC, baeR	Col156, IncFIB(AP001918), IncFII(29), IncQ1
EC38 (ST4406)	Community	AMP/AMX-SXT	-	*bla$_{TEM-1B}$, dfrA7, sul2, aph(3")-Ib, aph(6)-Id, catA1, tet(A)*	kdpE, qacEΔ1, H-NS, cpxA, marA, AcrA, leuO, emrY, evgA, evgS, CRP, gadX, mdfA, TolC, AcrAB-TolC, baeR	IncFII(29)
EC39 (ST3570)	Community	AMP/AMX-SXT	-	*bla$_{TEM-1B}$, dfrA5, sul1, catA1*	marA, AcrA, evgA, cpxA, H-NS, qacEΔ1, evgS, leuO, gadX , CRP, mdfA, kdpE, baeR, AcrAB-TolC	Col(BS512), IncB/O/K/Z, IncFIB(AP001918), IncFII(pCoo)

EC40 (ST13823)	Community	AMP/AMX-CXM-CPM-CTX/CRO-CAZ-AMC-SXT-TZP	+	*bla*$_{CTX-M-15}$*, dfrA17, sul1, aadA5, qnrS1*	evgA, AcrA, marA, gadX, H-NS, qacEΔ1, evgS, CRP, leuO, kdpE, cpxA, mdfA, baeR, AcrAB-TolC	IncFIB(AP001918)
EC41 (ST210)	Community	AMP/AMX-CXM-CPM-CTX/CRO-CAZ-CIP-AMC-TZP	+	*bla*$_{TEM-1B}$*, bla*$_{CTX-M-231}$*, mph(A), qnrS1*	AcrA, evgA, marA, H-NS, CRP, gadX, leuO, evgS, mdfA, kdpE, ermB, AcrAB-TolC, baeR	IncB/O/K/Z, IncFII

With respect to *K. pneumoniae*, one community isolate (EC27) was identified as an ESBL-producer (**Table 3**). Clinical isolates predominantly harboured the $bla_{CTX\text{-}M\text{-}15}$ gene (5/6), frequently co-occurring with $bla_{SHV\text{-}1}$, $bla_{SHV\text{-}11}$, and $bla_{TEM\text{-}1B}$. One isolate (KP46) also harboured the carbapenemase $bla_{NDM\text{-}5}$ and $bla_{OXA\text{-}181}$ genes, while another (KP51) carried $bla_{TEM\text{-}34}$ and $bla_{SHV\text{-}75}$. The $bla_{SHV\text{-}1}$, $bla_{SHV\text{-}11}$, $bla_{SHV\text{-}27}$, and $bla_{SHV\text{-}61}$ were detected among community isolates. Two community isolates (KP57 and KP59) carried the $bla_{TEM\text{-}1B}$ gene, and one isolate (KP53) carried the $bla_{LAP\text{-}2}$ gene. Trimethoprim ARGs detected in clinical isolates included *dfrA12*, *dfrA14*, and *dfrA27*, whereas community isolates harboured *dfrA5*, *dfrA8*, and *dfrA14*. The *sul2* gene was the most prevalent sulfonamide ARG among all *K. pneumoniae* isolates. *Sul1* was detected in four clinical isolates and one community isolate. Moreover, a *sul3* gene was identified in one community isolate (KP53).

Aph(3")-Ib, *aph(6)-Id*, *aac(3)-IIe*, *aadA2* and *aadA16* aminoglycoside ARGs were detected in clinical *K. pneumoniae* isolates. Community isolates harboured *aph(3')-Ia*, *aph(3")-Ib*, *aph(6)-Id* and *aadA1* ARGs. Furthermore, we detected that fluoroquinolone resistance was mostly mediated by *aac(6')-Ib-cr* (4/6), which were frequently co-occurring with rifampicin (*arr-3*) genes, followed by *qnrB6* (3/6) and *qnrS1* (1/6) genes in clinical isolates (**Table 3**). In community isolates, *qnrS1* was identified in two isolates. Fosfomycin (*fosA5*) resistance genes were detected in all *K. pneumoniae* isolates. Additional resistance genes were detected in both clinical and community isolates. One clinical isolate carried *catA2* and *tet(D)*, conferring resistance to chloramphenicol and tetracyclines, while two clinical isolates carried *mph(A)*, conferring resistance to macrolides. Among community isolates, *mph(A)* was detected in one, and tetracycline [*tet(A)* or *tet(D)*] in two. Notably, most TGC-resistant isolates lacked corresponding ARGs, except for one carrying *tet(A)*, which may be due to overexpression of OqxA/B efflux pumps.

Table 3: Sequence types, antibiograms, ESBL production, resistome, efflux pump genes, and plasmid replicons of clinical and community *K. pneumoniae* isolates

Isolate (MLST)	Setting	Antibiogram	ESBL +/-	Resistome	Efflux pump genes associated with resistance	Plasmid replicons
KP43 (ST101)	Clinical	AMP/AMX-FOX	-	*bla_{SHV-1}, aph(3'')-Ib, aph(6)-Id, fosA5*	oqxA, oqxB20, H-NS, CRP, adeF, leuO, msbA, rsmA, KpnH, KpnG, KpnF, KpnE, emrA, emrB, emrR, adeF, acrA, marA, mdtN, AcrAB-TolC, baeR	IncFIA(HI1), IncFIB(K)(pCAV1099-114), IncFII(pKPX1), repB(R1701)
KP45 (ST348)	Clinical	AMP/AMX-CPM-CTX/CRO-CAZ-CXM-SXT	-	*$bla_{CTX-M-15}$, bla_{SHV-11}, dfrA14, sul2, aph(3'')-Ib, aph(6)-Id, catA2, fosA5, tet(D)*	oqxA, oqxB25, KpnF, KpnG, KpnH, KpnE, LptD, adeF, emrA, emrB, emrR, CRP, msbA, acrA, rsmA, H-NS, marA, leuO, AcrAB-TolC, baeR	IncFIB(K), IncFII(K)
KP46 (ST17)	Clinical	AMC-AMP/AMX-CTX/CRO-FOX-CAZ-CXM-CIP-GEN-IMP-MEM-TZP-SXT	-	*$bla_{CTX-M-15}$, bla_{NDM-5}, $bla_{OXA-181}$, bla_{SHV-11}, bla_{TEM-1B}, dfrA12, dfrA27, sul1, sul2, aadA2, aadA16, aac(3)-IIe, aac(6')-Ib-cr, aph(3'')-Ib, aph(6)-Id, arr-3, fosA5, mph(A), qnrS1*	KpnE, KpnF, KpnG, KpnH, oqxA, oqxB25, LptD, qacEΔ1, H-NS, msbA, adeF, acrD, acrA, emrA, ermB, emrR, rsmA, leuO, CRP, AcrAB-TolC, baeR	ColKP3, IncFIA(HI1), IncFIB(K), IncFII, IncFII(K), IncR, IncX3
KP47 (ST607)	Clinical	AMC-AMP/AMX-CTX/CRO-FOX-CAZ-CXM-CIP-GEN-NIT-TZP-SXT	-	*$bla_{CTX-M-15}$, bla_{SHV-1}, bla_{TEM-1B}, dfrA27, sul1, sul2, aadA16, aac(3)-IIe, aac(6')-Ib-cr, aph(3'')-Ib, aph(6)-Id, arr-3, fosA5, qnrB6*	oqxA, oqxB19, KpnF, KpnG, KpnH, KpnE, LptD, acrD, qacEΔ1, CRP, adeF, emrA, emrB, emrR, rsmA, marA, H-NS, acrA, leuO, msbA, AcrAB-TolC, baeR	ColRNAI, IncFIA(HI1), IncFIB(K), IncFII(K), IncR
KP48 (ST607)	Clinical	AMC-AMP/AMX-CTX/CRO-CAZ-CXM-CIP-GEN-NIT-TZP-SXT	-	*$bla_{CTX-M-15}$, bla_{SHV-1}, bla_{TEM-1B}, dfrA27, sul1, sul2, aadA16, aac(3)-IIe, aac(6')-Ib-cr, aph(3'')-Ib, aph(6)-Id, arr-3, fosA5, qnrB6*	oqxA, oqxB19, LptD, KpnF, KpnG, KpnH, KpnE, acrD, qacEΔ1, adeF, emrA, emrB, emrR, rsmA, CRP, H-NS, acrA, leuO, marA, msbA, baeR, AcrAB-TolC	ColRNAI, IncFIA(HI1), IncFIB(K), IncFII(K), IncR
KP51 (ST133)	Clinical	AMC-AMP/AMX-CPM-CTX/CRO-CAZ-CXM-CIP-GEN-TZP-SXT	-	*$bla_{CTX-M-15}$, bla_{SHV-75}, bla_{TEM-34}, dfrA27, sul1, sul2, aadA16, aac(3)-IIe, aac(6')-Ib-cr, aph(3'')-Ib, aph(6)-Id, arr-3, fosA5, mph(A), qnrB6*	LptD, oqxA, oqxB14, KpnE, KpnF, KpnH, KpnG, acrD, qacEΔ1, adeF, leuO, acrA, rsmA, H-NS, CRP, emrA, emrB, emrR, msbA, AcrAB-TolC, baeR	Col(pHAD28), IncFIA(HI1), IncFII, IncFII(pBK30683), IncI2, IncR
EC27 (ST716)	Community	AMP/AMX-CXM-CPM-CTX/CRO-CAZ-AMC-TZP	+	*bla_{SHV-27}, dfrA5, sul1, fosA5*	oqxA, oqxB19, KpnF, KpnG, KpnH, KpnE, LptD, qacEΔ1, adeF, H-NS, emrA, emrB, emrR, rsmA, msbA, acrA, marA, leuO, CRP, baeR, AcrAB-TolC	IncFIB(pQil), repB
KP53 (ST193)	Community	AMP/AMX-CIP-TGC	-	*bla_{SHV-61}, bla_{LAP-2}, sul2, sul3, aadA1, aph(3')-Ia, fosA5, qnrS1, tet(A)*	oqxA, oqxB25, cmlA1, KpnF, KpnG, KpnH, KpnE, mef(B), H-NS, adeF, emrA, emrB, emrR, rsmA, leuO, LptD, qacL, msbA, marA, CRP, acrA, baeR, AcrAB-TolC	IncFIB(pNDM-Mar)

KP54 (ST5396)	Community	AMP/AMX-TGC	-	*bla$_{SHV-1}$, fosA5*	KpnF, KpnE, KpnG, KpnH, oqxA, oqxB19, LptD, marA, CRP, msbA, adeF, emrA, emrB, emrR, rsmA, leuO, acrA, AcrAB-TolC, baeR	None
KP55 (ST17)	Community	AMP/AMX-TGC	-	*bla$_{SHV-11}$, fosA5*	oqxA, oqxB25, LptD, KpnE, KpnF, KpnG, KpnH, H-NS, adeF, emrA, emrB, emrR, rsmA, msbA, acrA, CRP, leuO, AcrAB-TolC, baeR	IncFIB(K), IncFII(K), IncR, repB(R1701)
KP56 (ST37)	Community	AMP/AMX-TGC	-	*bla$_{SHV-11}$, fosA5*	KpnF, KpnE, KpnG, KpnH, oqxA, oqxB, LptD, marA, rsmA, H-NS, msbA, CRP, acrA, AcrAB-TolC, baeR	Col440I, IncFIB(K), IncFII(K), IncR
KP57 (ST45)	Community	AMP/AMX-AMC-SXT-TZP	-	*bla$_{SHV-1}$, bla$_{TEM-1B}$, dfrA14, sul2, aph(3")-Ib, aph(6)-Id, fosA5, mph(A)*	LptD, KpnF, KpnG, KpnH, KpnE, KpnF, H-NS, emrR, rsmA, marA, acrA, CRP, leuO, msbA, oqxA11, oqxB, adeF	Col(pHAD28), IncFIB(K), IncFII(K), IncQ1
KP58 (ST873)	Community	AMP/AMX-SXT	-	*bla$_{SHV-27}$, dfrA8, sul2, fosA5, tet(D)*	KpnF, KpnE, KpnG, KpnH, LptD, marA, msbA, emrR, rsmA, adeF, acrA, H-NS, leuO, CRP, oqxA11, oqxB19, AcrAB-TolC, baeR	IncFIB(K), IncFII(K), IncR
KP59 (ST1863)	Community	AMP/AMX-CIP	-	*bla$_{SHV-1}$,bla$_{TEM-1B}$, fosA5, qnrS1*	KpnF, KpnE, KpnG, KpnH, oqxA, OqxB, LptD, H-NS, marA, CRP, adeF, emrA, emrB, emrR, rsmA, leuO, acrA, msbA, baeR, AcrAB-TolC	IncN

2.3.5 Mobilome analysis and associated ARGs

The IncF plasmid replicon family (IncFIA, IncFIB, IncFII, and IncFIC) was the most prevalent, detected in 87% of isolates. IncFIB was the most common replicon among *E. coli* (20/32, 62.5%) (**Table 2**) and *K. pneumoniae* (11/14, 78.6%) isolates (**Table 3**). IncFIA was detected in 8/15 clinical and 2/17 community *E. coli* isolates, as well as in 5/6 clinical *K. pneumoniae isolates*, but none in community *K. pneumoniae* isolates. IncFII plasmids were detected clinical (61.9%) and community (60%) isolates. IncFIC(FII) was only detected in one clinical *E. coli* isolate (EC05). The IncR plasmid replicon was present in clinical (4/6) and community (3/8) *K. pneumoniae* isolates. Clinical *E. coli* isolates exhibited a broader range of Col plasmid replicons, including Col(MG828), which was detected in isolates from both settings. Col plasmid replicons were also detected in *K. pneumoniae* isolates; however, none were shared between clinical and community settings. In both *E. coli* and *K. pneumoniae*, Col plasmid replicons commonly co-occurred with IncF plasmids. Notably, we detected ColKP3 replicons in all $bla_{OXA-181}$-harbouring clinical *E. coli* and *K. pneumoniae* isolates.

Synteny analysis revealed that most ARGs were co-localized with class 1 integrons, ISs, and Tn3 on the same contig, with most ARGs and MGEs encoded on plasmids (Supplementary **Table S3** and **S4**). The bla_{TEM-1} was commonly found with IS1 and Tn3 in clinical and community *E. coli* isolates. ISKpn19 and IS3000 commonly co-localized with the $bla_{OXA-181}$ ARG. $Bla_{CTX-M-15}$ was co-localized with Tn3 and IS3 in most clinical and community *E. coli* isolates along with ISKpn19 and *qnrS1* on the same contig. Notably, one clinical isolate (EC09) with a $bla_{CTX-M-15}$ ARG showed association with Tn3 and ISEcp1. The $bla_{CTX-M-27}$ resistant gene was associated with IS5 in both clinical (EC02) and community (EC26) isolates, while $bla_{CTX-M-231}$ (EC16 and EC41) was associated with IS26. The class 1 integron gene cassette *dfrA17-aadA5* was commonly co-localized with IS6100. In isolate EC07, *qacEΔ1* and *sul1* genes were found with IS4321, IS6100, and *mph(A)*. In community isolates, *aph(3")-Ib* and *aph(6)-Id* were associated with a range ISs (IS5075, IS91, IS26 and IS903B). IS5075 was the common one and was always co-localized with *aph(3")-Ib*, *aph(6)-Id*, *sul2*, and a Tn3. In contrast, *aph(3")-Ib* and *aph(6)-Id* in clinical *E. coli* isolates were not associated with MGEs, except for one isolate (EC05), which showed an association with TnAs1 and IS1133 (Supplementary **Table S3**). All detected *qnrS1* ARGs in *E. coli* isolates were co-localized with Tn3, ISKpn19, and IS3.

In *K. pneumoniae* clinical isolates, bla_{TEM-1} was predominantly associated with ISEcp1, and bla_{TEM-34} showed a similar association. In contrast, bla_{TEM-1} from community isolates was associated with IS6. Most $bla_{CTX-M-15}$ ARGs were co-localized with ISEcp1 (Supplementary **Table S4**). IS30 *and* ISCR1 flanked the bla_{NDM-5} ARG found in KP46. The bla_{LAP-2} ARG found in one isolate (KP53) was co-localized with ISKpn19, *qnrS1*, and IS3, while $bla_{OXA-181}$ found in KP46 was associated with Tn3 and IS3000. *Aac(3)-IIe,* detected only in clinical isolates, was commonly co-localized with IS3, ISKpn11,

and ISKpn12. *Aph(3'')-Ib* and *aph(6)-Id* in clinical isolates were co-localized with at least one insertion sequence (IS91 or ISPa38) and a *sul2* gene; however, isolate KP45 was additionally associated with IS5075. In KP48, *aph(6)-Id* was only co-localized with IS91. Aminoglycoside ARGs in community *K. pneumoniae isolates* were not associated with MGEs. Among *K. pneumoniae* community isolates, *sul2* was associated with ISVsa3, while *sul3* was co-localized with an *IntI1* gene, along with *mef(B)*, IS256, *qacL*, *aadA1*, *cmlA1*, and TnAs1. All detected *qnrB6* and *qnrS1* ARGs were associated with ISCR1 and ISKpn19, respectively.

Only class 1 integrons (*IntI1*) were detected among the isolates, present in 84.4% *E. coli* and 57.1% *K. pneumoniae* isolates (**Table 4**). The In54 integron was predominantly detected in clinical *E. coli* isolates, particularly those of ST131, and was associated with the *dfrA17-aadA5* gene cassette. The most prevalent integron in community *E. coli* isolates was In191, containing the *dfrA14* gene cassette. In addition, In183, associated with the *dfrA1* gene cassette was detected in two isolates. In clinical *K. pneumoniae*, In1021 was the predominant integron harbouring the *aacA4cr-arr-3-dfrA27-aadA16* gene cassette. Among community *K. pneumoniae*, the occurrence of class 1 integrons was less frequent, with only two isolates harbouring In13 (associated with *dfrA5*) and In191.

Table 4: Class 1 integrons and gene cassettes found in clinical and community *E. coli* and *K. pneumoniae* isolates

Sample ID	Species	Setting	Integron class	Integron	Gene cassette arrays			
					GC1	GC2	GC3	GC4
EC01	*E. coli*	Clinical	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC02	*E. coli*	Clinical	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC03	*E. coli*	Clinical	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC05	*E. coli*	Clinical	IntI1	–	–	–	–	–
EC07	*E. coli*	Clinical	IntI1	In987	*dfrA17*	–	–	–
EC08	*E. coli*	Clinical	IntI1	In987	*dfrA17*	–	–	–
EC09	*E. coli*	Clinical	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC10	*E. coli*	Clinical	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC13	*E. coli*	Clinical	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC14	*E. coli*	Clinical	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC15	*E. coli*	Clinical	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC18	*E. coli*	Clinical	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC19	*E. coli*	Clinical	IntI1	In987	*dfrA17*	–	–	–
EC21	*E. coli*	Community	IntI1	In183	*dfrA1*	–	–	–
EC22	*E. coli*	Community	IntI1	In987	*dfrA17*	–	–	–

EC23	*E. coli*	Community	IntI1	In191	*dfrA14*	–	–	–
EC25	*E. coli*	Community	IntI1	In191	*dfrA14*	–	–	–
EC30	*E. coli*	Community	IntI1	In191	*dfrA14*	–	–	–
EC31	*E. coli*	Community	IntI1	In191	*dfrA14*	–	–	–
EC32	*E. coli*	Community	IntI1	In22	*dfrA7*	–	–	–
EC33	*E. coli*	Community	IntI1	In191	*dfrA14*	–	–	–
EC35	*E. coli*	Community	IntI1	In22	*dfrA7*	–	–	–
EC36	*E. coli*	Community	IntI1	In183	*dfrA1*	–	–	–
EC37	*E. coli*	Community	IntI1	In54	*dfrA17*	*aadA5*	–	–
EC38	*E. coli*	Community	IntI1	In22	*dfrA7*	–	–	–
EC39	*E. coli*	Community	IntI1	In13	*dfrA5*	–	–	–
EC40	*E. coli*	Community	IntI1	In54	*dfrA17*	*aadA5*	–	–
KP45	*K. pneumoniae*	Clinical	IntI1	In191	*dfrA14*	–	–	–
KP46	*K. pneumoniae*	Clinical	IntI1	In1021	*aacA4cr*	*arr-3*	*dfrA27*	*aadA16*
KP47	*K. pneumoniae*	Clinical	IntI1	In1021	*aacA4cr*	*arr-3*	*dfrA27*	*aadA16*
KP48	*K. pneumoniae*	Clinical	IntI1	In1021	*aacA4cr*	*arr-3*	*dfrA27*	*aadA16*
KP51	*K. pneumoniae*	Clinical	IntI1	In1021	*aacA4cr*	*arr-3*	*dfrA27*	*aadA16*
EC27	*K. pneumoniae*	Community	IntI1	In13	*dfrA5*	–	–	–
KP53	*K. pneumoniae*	Community	IntI1	–	–	–	–	–
KP57	*K. pneumoniae*	Community	IntI1	In191	*dfrA14*	–	–	–

aacA4cr is synonymous with *aac(6')-br-cr* on the INTEGRALL database

2.3.6. Sequence Types and Phylogenomic Relationships

Overall, community isolates showed a higher clonal diversity compared to clinical isolates. Among all *E. coli* isolates, the most frequently detected sequence types (STs) were ST131, ST10, and ST1193, each identified in both clinical and community settings. ST131 predominated among clinical isolates (n = 4) and was also detected in two community isolates. ST10 was present in one clinical and two community isolates, while ST1193 was detected in two clinical and one community isolate (**Table 2**). Other sequence types detected in clinical isolates included ST38, ST1193, ST3572, and ST13823 (n = 2 each), as well as singletons ST69 and ST3337. Community *E. coli* isolates included several singletons (ST1193, ST1380, ST1722, ST2758, ST2852, ST382, ST336, ST2178, ST101, ST4406, ST3570, ST13823, and ST210). Despite differences in their antibiograms and plasmid profiles, the ST13823 isolates shared an identical resistome and the same synteny with MGEs, and all carried the IncFIB plasmid replicon. Similarly, community isolates belonging to ST10 had the same antibiogram, resistome content with the same MGE associations, and plasmid replicon profiles. Clinical *K. pneumoniae* isolates

belonged to five different STs, with ST607 identified in two isolates, while ST101, ST348, ST17, and ST133 were singletons (**Table 3**). The ST607 isolates shared similar antibiograms and displayed the same associations with MGEs and plasmid replicons. In *K. pneumoniae* community isolates, all eight isolates were assigned to distinct STs (ST17, ST37, ST45, ST193, ST5396, ST716, ST873, and ST1863).

Phylogenetic analysis revealed clustering of clinical and community isolates of this study with other South African isolates from Gauteng, Mpumalanga, Western Cape and KwaZulu-Natal based on their STs. Among *E. coli* isolates, all clinical isolates clustered with community isolates (**Figure 1**). Notably, six community isolates (EC35, EC39, EC41, EC31, EC33, and EC36) formed a distinct clade together with clinical isolates from the provinces listed above. In contrast, clinical *K. pneumoniae* isolates showed less frequent clustering with community isolates. KP46 clustered with community isolates KP53 and KP55 along with other clinical isolates from Western Cape and Gauteng (**Figure 2**). Similarly, KP45 clustered with community isolates KP57 and KP58 along with other clinical isolates from Gauteng, Western Cape and KwaZulu-Natal. The remaining clinical (KP51, KP53, KP47 and KP48) and community isolates (KP56, EC27, KP59 and KP54) formed clades with other clinical isolates from Gauteng, Western Cape and KwaZulu-Natal.

Figure 1: A phylogenetic tree illustrating the relationship between *E. coli* community (coded as purple) and clinical (coded as blue) isolates from this study and other South African isolates from diverse clinical sources (coded as yellow). The *E. coli* K12 MG1655 strain was used as a reference genome.

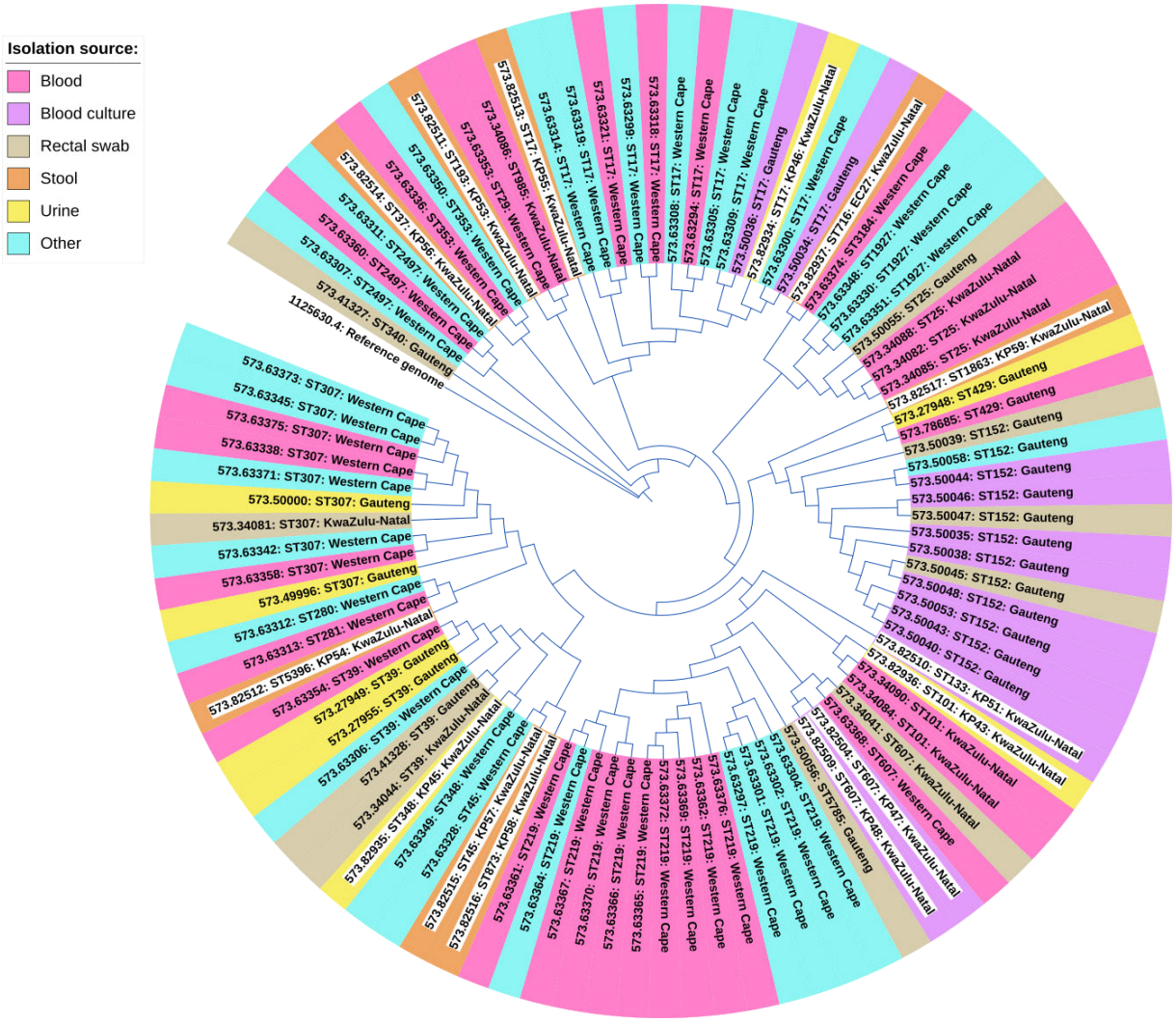

Figure 2: A phylogenetic tree illustrating the relationship between *K. pneumoniae* isolates from the community (stool) and clinical isolates (blood culture and urine highlighted in white) of this study, alongside other South African isolates from diverse clinical sources (blood, blood culture, rectal swab, urine and others). The *K. pneumoniae* HS11286 strain was used as a reference genome.

2.4. Discussion

There is a paucity of genomic data from healthy community dwellers in South Africa, which limits our understanding of the community resistome and its associated mobilome. This study addresses this critical gap by delineating and providing valuable genomic insights into the resistome, mobilome, clonalities, phylogenies, and potential transmission dynamics of *E. coli* and *K. pneumoniae* isolates obtained from stool samples of healthy community dwellers in uMgungundlovu alongside clinical isolates collected from a nearby hospital. Resistome analyses revealed a diverse array of ARGs conferring resistance to β-lactams, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, macrolides, fosfomycin, and tetracyclines. Several ARGs were located on plasmids in both community and clinical isolates, with many associated with transposons, integrons and insertion sequences. Notably, aminoglycoside ARGs detected in community *K. pneumoniae* isolates were not associated with any MGEs other than the plasmid replicons that were detected at the isolate level.

We observed that both clinical and community *E. coli* isolates were resistant to largely the same antibiotics, although resistance rates were lower in community isolates (**Table 1**). We observed high resistance prevalence of 86.7% and 82.4% to SXT in clinical and community *E. coli* isolates, respectively. This high prevalence is consistent with findings from other African studies reporting SXT resistance rates of 70% or higher in both healthy humans and clinical isolates (Aworh et al., 2019; Bumbangi et al., 2022; Medugu et al., 2022; Mbanga et al., 2023; Asare Yeboah et al., 2024; Kasanga et al., 2024; Kitaba et al., 2024; Yamba et al., 2024;). SXT prophylaxis is used to prevent opportunistic infections and malaria in people living with HIV (WHO, 2014). uMgungundlovu has a high HIV prevalence rate (Department of Cooperative Governance and Traditional Affairs, 2023), which can explain the high prevalence of SXT resistance in both clinical and community isolates. Furthermore, we observed a high resistance prevalence rate of 73.3% to CIP and CXM among clinical *E. coli* isolates. Similarly, Kitaba et al., (2024), reported overall resistance rates of 67.9% to CIP and 62% to CXM in clinical and environmental *E. coli* isolates in Zambia. In South Africa, CIP is routinely recommended for management of infections in both hospital and primary healthcare levels for various infections, including urinary tract infections (UTIs) and gastrointestinal conditions, whereas CXM is not (National Department of Health [NDoH], 2024a; 2024b), which may explain the elevated CIP resistance locally. In several isolates, CXM ARGs were located on the same contigs as CIP resistance determinants and were associated with ISs and Tns carried on plasmids. This co-localisation may promote co-selection, where resistance to one antibiotic helps maintain resistance to the other (Murray et al., 2024), which could partly explain the observed CXM resistance despite its limited clinical use. Naidoo et al., (2023), reported that *E. coli* accounted for 53% of uropathogens at a regional hospital in KwaZulu-Natal, with 38% of isolates resistant to CIP. Notably, most CIP and CXM resistant isolates in

this study were recovered from urine specimens (**Table 2** and Supplementary **Table S1**). The high prevalence of CIP and CXM resistance observed in this study is of concern and may reflect sustained antimicrobial selective pressure associated with their use in KwaZulu-Natal.

Our community *E. coli* isolates demonstrated a CXM and CIP resistance prevalence of 23.5% and 17.6%, respectively, which are comparable to previous community-based studies reporting concurrent CXM and CIP resistance, including 14% CXM and 19.3% CIP in commensal *E. coli* in China (Li et al., 2014) and 22.4% CXM and 12.2% CIP in commensal *E. coli* from children in India (Purohit et al., 2019). Most clinical and community *E. coli* isolates phenotypically resistant to CIP lacked corresponding ARGs but frequently harboured gyrA (S83L and D87N) and parC (S80I and E84V), regardless of ARG co-occurrence (Supplementary **Table S2**). Similarly, a WGS study by Golden et al. (2021), which analysed 636 ESBL-producing *E. coli* isolates from Canadian hospitals as part of the Canadian Ward Surveillance Study (2007-2018), reported that CIP resistance was predominantly associated with QRDR mutations in gyrA (S83L and D87N) and parC (S80I and E84V), occurring both with (n =187) and without (n =197) the *aac(6')-Ib-cr* gene. Collectively, this reinforces the role of chromosomal QRDR mutations as the primary mechanism driving CIP resistance in *E. coli* isolates of this study. Clinical isolate EC19 did not carry any QRDR mutations but harboured the AcrAB–TolC efflux pump. Previous studies have shown that overexpression of this efflux pump confers resistance to CIP (Sharma et al., 2019; Tewawong et al., 2025). Additionally, isolate EC19 did not harbour any ARGs conferring resistance to GEN but it carried the AcrD efflux pump (**Table 2**). AcrD, is a member of the Resistance-Nodulation-Division (RND) family and has been reported to mediate aminoglycoside resistance (Aires & Nikaido, 2005; Garneau-Tsodikova & Labby, 2016; Rihacek et al., 2023).

The prevalence of ESBL-producing *E. coli* in our study was 20% among clinical isolates and 23.5% among community isolates (**Table 2**). However, the $bla_{CTX\text{-}M}$ gene was detected by WGS in 66.7% of clinical *E. coli* isolates, yet only 20% were identified as ESBL-producers by VITEK2 AES. Similar to clinical *K. pneumoniae*, 83.3% isolates harboured the $bla_{CTX\text{-}M\text{-}15}$ but none were identified as ESBL-producers. In contrast, Carvalhaes et al. (2024) reported an AES specificity of 98.1% when detecting ESBLs in 447 clinical Enterobacterales, of which 103 were ESBL-positive by genotype and AES detected 101. This discrepancy highlights limitations of phenotypic methods and underscores the complexity of ESBL detection, particularly in the presence of co-existing resistance mechanisms (Chan and Leroi, 2021). Our phenotypic rates align with a recent meta-analysis across sub-Saharan Africa, which reported a pooled ESBL-producing *E. coli* prevalence of approximately 20.76% using the One Health approach (Olaitan et al., 2025). Their analysis further highlighted the variability by sub-region, with West Africa exhibiting the highest prevalence (22.8%) and Southern Africa the lowest (13.76%). Another meta-analysis by Bezabih et al. (2022) reported a concerning global increase in intestinal ESBL-producing *E. coli* carriage in both community and healthcare settings. Their analysis, covering

the period from January 2000 to April 2021, estimated a pooled prevalence of 21.1% in healthcare settings and 17.6% in community settings.

Both clinical and community *E. coli* isolates exhibited resistance to 3rd generation cephalosporins (CTX/CRO and CAZ). Resistance to CTX/CRO in clinical isolates was notably high at 60% (**Table 1**), exceeding the global median prevalence of 42% reported by the WHO (2023a), indicating a significant cephalosporin resistant *E. coli* burden in clinical settings within uMgungundlovu. The overall prevalence of CTX/CRO resistance in our *E. coli* community isolates was 23.5%. These rates represent combined resistance across both ESBL-producing and non-ESBL-producing isolates. Shirani et al. (2019) similarly reported high CTX and CRO resistance among clinical ESBL-producing *E. coli* isolates from inpatients in Iran (73.5% and 58.8%, respectively), with lower resistance observed in non-ESBL-producers (30.3% CTX, 27.3% CRO). Similarly, Ashraf et al. (2025) reported a high prevalence of CTX and CRO resistance (80.2%) among hospital-acquired uropathogenic *E. coli* (UPEC) isolates in Pakistan, alongside a lower prevalence among community-acquired UPEC (37.9%), which is comparable to the resistance observed in our community isolates. Consistent with these findings, Cheng et al. (2022) reported a 25.7% prevalence of CRO resistance among community carriage ESBL-producing *E. coli* isolates from hospitalized children in Taiwan, closely aligning with the CTX/CRO resistance observed in our community isolates (23.5%).

In South Africa, *K. pneumoniae* exhibits high resistance rates (>50%) to multiple antibiotic classes, with the notable exception of carbapenems (Yakobi and Nwodo, 2024). In the present study, the highest resistance prevalence among clinical *K. pneumoniae* isolates was observed for CTX/CRO, CXM, CAZ, and SXT (83.3%), followed by AMC, TZP, CIP, and GEN (66.7%), and FOX (50%) (**Table 1**). Comparatively, studies conducted in KwaZulu-Natal have reported even higher resistance prevalences, with up to 100% resistance to these antibiotics among clinical *K. pneumoniae* isolates (Madni et al., 2021; Hetsa et al., 2025; Masalane et al., 2025). The consistently high resistance levels observed across studies likely reflect sustained antimicrobial selective pressure in hospital settings and underscore the growing challenge of managing *K. pneumoniae* infections in KwaZulu-Natal due to the substantial burden of multidrug resistance (MDR). Interestingly, clinical *K. pneumoniae* isolates did not exhibit resistance to TGC, whereas a high resistance prevalence of 50% to TGC was observed among community isolates. This finding suggests the presence of non-clinical selective pressures or potential community-associated reservoirs contributing to TGC resistance among community dwellers in uMgungundlovu. The detection of high TGC resistance in healthy community carriers is concerning, given that TGC is classified as a last-resort antibiotic for the treatment of MDR infections (WHO, 2023b). Similarly, Salvador-Oke et al. (2025) reported an overall 30% resistance prevalence to TGC among rectal carriage carbapenemase-producing *K. pneumoniae* isolates from community hospitals across five South African provinces. Notably, we detected both OqxA and OqxB efflux pumps in all

TGC resistant isolates, irrespective of the presence of corresponding ARGs, and these efflux systems have previously been associated with reduced susceptibility to TGC (Zhong et al., 2014).

The clinical isolate KP46 harboured both *bla*$_{OXA-181}$ and *bla*$_{NDM-5}$ ARGs located on different contigs. The *bla*$_{NDM}$ gene has also been reported in several South African studies (Pedersen et al., 2018; Lowe et al., 2019; Osei Sekyere et al., 2024). Between 2015 and 2021, a 60% prevalence of *bla*$_{NDM}$ genes was observed in hospitals associated with the Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA) linked to the NHLS in KwaZulu-Natal (Ismail et al., 2025). All detected *bla*$_{OXA-181}$ genes were associated with Tn3, ISKpn19, and IS3000 (Supplementary **Tables S3** and **S4**), consistent with the IS3000-*bla*$_{OXA-181}$-repA-ISKpn19 genetic context described by Kim et al. (2021) in carbapenemase-producing *E. coli* isolates, and with findings in clinical *K. pneumoniae* reported by Madni et al. (2021) and Masalane et al. (2025), who identified Tn3:IS3000:*bla*$_{OXA-181}$:ISKpn19 associations. *Bla*$_{NDM-5}$ was associated with IS30 and ISCR1, aligning with the findings of Mapunda et al. (2025), who reported *bla*$_{NDM-5}$ in IncFIB(K), ColPK3–IncX3, and IncFIB(K)–IncFII(K) plasmids flanked by a similar genetic context consisting of IS26, IS30, and ISCR1. We detected ColKP3 replicons in all *bla*$_{OXA-181}$-harbouring isolates (**Tables 2** and **Table 3**). Similarly, Takei et al. (2024) reported that all *bla*$_{OXA-232}$ and *bla*$_{OXA-181}$ genes were carried on ColKP3 plasmids among MDR *K. pneumoniae* isolates recovered from two Nepalese hospitals. Notably, KP46 carried an IncX3 plasmid replicon, which has been reported to harbour *bla*$_{NDM-5}$ in Enterobacterales, including *K. pneumoniae*, from a children's hospital in China (Tian et al., 2020). The IncR replicon was detected in four clinical and three community *K. pneumoniae* isolates, co-occurring with IncF replicons. IncR plasmids have been reported to carry ARGs conferring resistance to β-lactams, SXT, fluoroquinolones, aminoglycosides, tetracyclines, and chloramphenicol, and their co-residence with other plasmid replicons facilitates HGT (Rozwandowicz et al., 2018; Zhang et al., 2025).

The *bla*$_{SHV-1}$ gene is typically chromosomally encoded in *K. pneumoniae* (Chaves et al., 2001) but was not detected in all isolates in this study, likely due to the emergence and diversification of other *bla*$_{SHV}$ variants (Tsang et al., 2024). The *bla*$_{CTX-M-15}$ ARG was predominantly detected in clinical isolates (8 *E. coli*, 5 *K. pneumoniae*), a globally dominant ESBL in *E. coli* (Peirano et al., 2025), in contrast with the higher occurrence of *bla*$_{TEM-1B}$ among community isolates (12 *E. coli*, 2 *K. pneumoniae*), suggesting differential resistance selection between hospital and community settings. Notably, community *K. pneumoniae* isolates did not harbour any *bla*$_{CTX-M}$ genes. Singh et al. (2023), observed a higher prevalence of the *bla*$_{CTX-M-15}$ gene among children with recent hospitalization (hospital arm) compared to children without recent hospitalization (community arm), as well as among their family members. Their study on MDR Enterobacterales showed that *bla*$_{CTX-M-15}$ was more predominant in the hospital arm. Consistent with our study, Belina et al. (2024) reported that *bla*$_{TEM-1B}$ was more frequently detected than *bla*$_{CTX-M-15}$ among diarrhoeagenic *E. coli* isolated from children under five years of age, their caretakers, and surrounding environments in Ethiopia. In *E. coli* isolates from both clinical and

community settings, *bla*$_{CTX\text{-}M\text{-}15}$ was frequently embedded within a *CTX-M-15*::Tn3:IS3:*qnrS1*::ISKpn19 genetic context and carried on plasmids (Supplementary **Table S3**). In contrast, previous studies from Pretoria and Ghana reported *bla*$_{CTX\text{-}M\text{-}15}$ in *E. coli* to be predominantly associated with IS1380-like ISs and Tn3 (Mbelle et al., 2019; Asare Yeboah et al., 2024). Consistent with these findings, one clinical isolate in this study (EC09) also harboured *bla*$_{CTX\text{-}M\text{-}15}$ in association with IS1380 (ISEcp1). The *bla*$_{CTX\text{-}M\text{-}15}$ among clinical *K. pneumoniae* isolates was associated with Tn3 and ISEcp1 (Supplementary **Table S4**). Interestingly, *K. pneumoniae* ST607 clinical isolates (KP47 and KP48) carried *bla*$_{CTX\text{-}M\text{-}15}$ flanked by Tn3 and ISEcp1 on both sides. Similar associations between *bla*$_{CTX\text{-}M\text{-}15}$, ISEcp1, and Tn3 have been reported by Masalane et al. (2025) among *K. pneumoniae* isolates from hospital patients in KwaZulu-Natal.

All gene cassettes detected were associated with *IntI1* in both clinical and community isolates. Gene cassettes *dfrA17–aadA5*, located in In54, was the most common among clinical *E. coli* isolates, particularly in ST131, whereas *dfrA14* in In191 predominated among community isolates (**Table 4**). Similarly, Mbelle et al. (2019) identified *dfrA17–aadA5* gene cassettes in In54 among MDR *E. coli* ST131 isolates in Pretoria. The In54 integron has also been detected in non-ST131 *E. coli*, and In191 in clinical isolates from patients and their hospital environments in Ghana (Asare Yeboah et al., 2024). Among clinical *K. pneumoniae* isolates, the *aacA4cr–arr-3–dfrA27–aadA16* gene cassettes within In1021 was most prevalent. In contrast, only three community isolates harboured *IntI1*, two of which carried *dfrA5* and *dfrA14* gene cassettes within In13 and In191, respectively. Both In1021 and In191 have been detected in other KwaZulu-Natal clinical studies (Hetsa et al., 2025; Masalane et al., 2025), while *dfrA5* gene cassette has been previously reported in *K. pneumoniae* isolates from urinary tract infections in Iran (Jahanbin et al., 2020).

Community isolates of both *E. coli* and *K. pneumoniae* showed a wide diversity of STs than clinical isolates, with 23 STs detected among community isolates and 13 STs among clinical isolates (**Table 2** and **Table 3**). A similar observation was reported by Raffelsberger et al. (2023), who investigated ESBL-producing *E. coli* and *K. pneumoniae* carriage in a Norwegian adult population. In their study, only *E. coli* carriage isolates were compared to clinical isolates collected through the Norwegian Surveillance System for Antimicrobial Drug Resistance (NORM), revealing 58 STs among carriage isolates and 27 STs among clinical isolates. The only common STs shared between clinical and community *E. coli* isolates were ST131, ST10, and ST1193, while for *K. pneumoniae* it was ST17. Clinical *E. coli* ST131 isolates in this study (EC03, EC09, EC10, and EC13) harboured *bla*$_{CTX\text{-}M\text{-}15}$, consistent with findings by Louka et al. (2021), who reported that ST131 *E. coli* predominantly carried *bla*$_{CTX\text{-}M\text{-}15}$ among hospitalized asylum seekers in the Netherlands. Notably, ST131 clinical *E. coli* isolates (EC09, EC10 and EC13) were resistant to CIP (Table 2). These findings agree with those of Ziadi et al. (2025), who reported that all clonal complex 131 (CC131) *E. coli* isolates in their study harboured *bla*$_{CTX\text{-}M\text{-}15}$ and were resistant to CIP. Of the two ST131 in our community isolates, only EC26 was phenotypically

resistant to CIP. ST1193 *E. coli* isolates have also been linked to CIP resistance in community-acquired infections across 30 county hospitals in China (Zhao et al., 2015) and in extraintestinal pathogenic *E. coli* in Vietnam (Nguyen et al., 2021). Consistently, ST1193 isolates in our study (2 clinical, 1 community) were also resistant to CIP, supporting the global association of this ST with fluoroquinolone resistance. All three isolates shared the same QRDR-associated point mutations (Supplementary **Table S2**). Musicha et al. (2025) conducted a community-based longitudinal One Health cohort study in Malawi (April 2019-December 2020) and Uganda (July 2020-August 2021), including human stool samples, and reported that *E. coli* ST131 and ST10 were dominant in Malawi and Uganda, respectively. Similarly, ST10 was predominant among community *E. coli* isolates in our study. These clinical and community isolates phylogenetically clustered together (**Figure 1** and **Figure 2**), suggesting possible transmission reservoir between the two settings.

This study revealed the possibility of AMR transmission in both settings. Some of the limitations of this study were the relatively small sample size and short sampling period which does not fully capture the resistome and mobilome diversity and dynamics of AMR transmission or a shared reservoir within both settings. Nevertheless, the findings provide a snapshot of *E. coli* and *K. pneumoniae* AMR within both settings in uMgungundlovu.

2.5. Conclusion

Clinical isolates exhibited more extensive and diverse antibiograms than community isolates for both *E. coli* and *K. pneumoniae*. Notably, both clinical and community *E. coli* isolates were resistant to largely the same antibiotics. Diverse resistomes, mobilomes, and clonalities were identified in both settings, with several overlapping across clinical and community isolates. Although resistance levels were generally higher in clinical isolates, the presence of MDR genes and MGEs in community isolates underscores the potential of healthy carriers as silent reservoirs. Phylogenetic clustering further supports possible transmission routes between clinical and community isolates, highlighting that community settings should not be overlooked in AMR surveillance and mitigation efforts.

Data availability

All analysed data are included in the manuscript and its supplementary materials. The assembled genomes of this study are deposited in the NCBI GenBank database under the BioProject PRJNA1183563.

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Ethics Committee of the University of KwaZulu-Natal, ensuring that all procedures adhered to the highest standards for human subject research, including confidentiality and participant rights protection. The efforts of all involved have been vital to the success of this work.

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Competing interests

Professor SY Essack is the Chairperson of the Global Respiratory Infection Partnership and a member of the Global Hygiene Council, both supported by unconditional educational grants from Reckitt (Pty) Ltd, UK. All other authors declare no competing interests.

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Supplementary data

Table S1: Participant demographics and genomic characteristics of sequenced *E. coli* and *K. pneumoniae* isolates from clinical and community samples

Isolate ID	Accession no.	Species	Sex	Age (years)	Specimen type	Setting	Sequence size (bp)	No. of contigs	GC content (%)	N50	L50
EC01	SAMN44628981	*E. coli*	F	55	Urine	Clinical	4664742	192	50.7	74038	20
EC02	SAMN44628982	*E. coli*	F	81	Urine	Clinical	5470388	688	50.6	39368	45
EC03	SAMN44628983	*E. coli*	F	<1 year	Urine	Clinical	5480363	368	51.9	208575	9
EC04	SAMN44628984	*E. coli*	F	26	Tissue	Clinical	5215906	91	50.6	155771	10
EC05	SAMN44628985	*E. coli*	F	60	Urine	Clinical	5273316	104	50.6	120591	16
EC07	SAMN44628987	*E. coli*	M	92	Urine	Clinical	5744267	1468	50.7	17374	94
EC08	SAMN44628988	*E. coli*	F	21	Urine	Clinical	5239615	136	50.7	114193	14
EC09	SAMN44628989	*E. coli*	F	75	Urine	Clinical	5257170	346	50.7	77633	21
EC10	SAMN44628990	*E. coli*	F	33	Tissue	Clinical	4985625	82	50.8	225684	7
EC13	SAMN44628993	*E. coli*	M	41	Blood culture	Clinical	5255080	103	50.8	158933	10
EC14	SAMN44628994	*E. coli*	M	38	Blood culture	Clinical	4822768	129	50.8	95797	18
EC15	SAMN44628995	*E. coli*	F	17	Urine	Clinical	4932441	134	50.7	121143	12
EC16	SAMN44628996	*E. coli*	M	42	Urine	Clinical	5164990	311	50.6	92529	20
EC18	SAMN44628998	*E. coli*	F	56	Catheter tip	Clinical	4899046	86	50.6	121143	13
EC19	SAMN44628999	*E. coli*	M	76	Fluid-aspirate	Clinical	4721617	100	50.7	138046	10
EC21	SAMN44629001	*E. coli*	F	36	Stool	Community	4947919	90	50.6	258770	6
EC22	SAMN44629002	*E. coli*	F	62	Stool	Community	5118429	103	50.6	152654	11
EC23	SAMN44629003	*E. coli*	F	65	Stool	Community	5170098	141	50.4	110327	18
EC24	SAMN44629004	*E. coli*	M	27	Stool	Community	6179188	1251	50.5	23059	70
EC25	SAMN44629005	*E. coli*	F	50	Stool	Community	5163936	130	50.4	118612	16
EC26	SAMN44629006	*E. coli*	F	37	Stool	Community	5067166	87	50.7	159739	12
EC30	SAMN44629010	*E. coli*	F	55	Stool	Community	4809910	225	50.5	67628	22
EC31	SAMN44629011	*E. coli*	F	54	Stool	Community	4688321	59	50.8	182215	10
EC32	SAMN44629012	*E. coli*	M	31	Stool	Community	5080504	173	50.6	84590	20
EC33	SAMN44629013	*E. coli*	F	37	Stool	Community	5839137	2392	50.4	3859	402

EC35	SAMN44629015	*E. coli*	F	38	Stool	Community	4996882	92	50.5	215054	8
EC36	SAMN44629016	*E. coli*	N/A	N/A	Stool	Community	5008901	64	50.6	235774	8
EC37	SAMN44629017	*E. coli*	N/A	N/A	Stool	Community	5161183	66	50.7	195158	9
EC38	SAMN44629018	*E. coli*	F	39	Stool	Community	5135473	116	50.7	169840	12
EC39	SAMN44629019	*E. coli*	M	28	Stool	Community	5089382	142	50.7	126316	13
EC40	SAMN44629020	*E. coli*	F	31	Stool	Community	4916063	137	50.7	127336	12
EC41	SAMN44629021	*E. coli*	F	26	Stool	Community	5137122	143	50.7	140993	12
KP43	SAMN44629023	*K. pneumoniae*	M	20	Urine	Clinical	5697749	203	57.0	198204	10
KP45	SAMN44629025	*K. pneumoniae*	F	36	Urine	Clinical	5691941	208	57.0	156059	12
KP46	SAMN44629026	*K. pneumoniae*	F	50	Urine	Clinical	5725555	121	57.0	263913	7
KP47	SAMN44629027	*K. pneumoniae*	M	<1 year	Blood culture	Clinical	5556217	105	57.2	217065	9
KP48	SAMN44629028	*K. pneumoniae*	M	<1 year	Blood culture	Clinical	5678080	219	57.1	172178	10
KP51	SAMN44629031	*K. pneumoniae*	F	<1 year	Blood culture	Clinical	5645123	230	57.1	161736	12
EC27	SAMN44629007	*K. pneumoniae*	F	63	Stool	Community	5525333	69	57.0	367077	6
KP53	SAMN44629033	*K. pneumoniae*	M	27	Stool	Community	5563757	52	56.9	277243	7
KP54	SAMN44629034	*K. pneumoniae*	F	52	Stool	Community	5181281	34	57.6	397451	6
KP55	SAMN44629035	*K. pneumoniae*	F	63	Stool	Community	5502431	124	57.2	197398	11
KP56	SAMN44629036	*K. pneumoniae*	M	35	Stool	Community	5359929	95	57.3	142403	11
KP57	SAMN44629037	*K. pneumoniae*	F	35	Stool	Community	6387976	515	56.9	46189	37
KP58	SAMN44629038	*K. pneumoniae*	F	27	Stool	Community	5683501	74	57.1	208034	10
KP59	SAMN44629039	*K. pneumoniae*	F	22	Stool	Community	5284675	32	57.4	443289	5

Key: N50 = smallest contig of the size-sorted contigs that make up at least 50% of the respective assembly; L50 = number of contigs that make up at least 50% of the respective total assembly length

Table S2: Fluoroquinolone-associated point mutations detected in CIP resistant *E. coli* isolates

Isolate ID	Setting	Fluoroquinolone point mutations			
		GyrA	**GyrB**	**ParC**	**ParE**
EC01[Δ]	Clinical	S83L, D87N	E219K*	S80I, A56T, A620V*	-
EC02[Δ]	Clinical	S83L, D87N, D678E*, A828S*	A618T*	S80I	L416F, L254Q*
EC04[Δ]	Clinical	S83L, D678E*	-	D475E*, Q695L*	-
EC07[Δ]	Clinical	-	A618T*	-	-
EC09[Δ]	Clinical	S83L, D87N, A828S*	A618T*	S80I, E84V, A192V*, A471G*, D475E*, Q481H*	I529L, V136I*
EC10[Δ]	Clinical	S83L, A828S*	A618T*	S80I, E84V, A192V*, A471G*, D475E*, Q481H*	I529L, V136I*
EC13[#]	Clinical	S83L, D87N, A828S*	A618T*	S80I, E84V, A192V*, A471G*, D475E*, Q481H*	I529L, V136I*
EC14[#]	Clinical		-	-	-
EC16[#]	Clinical	S83L, D87N, D678E*, A828S*	A618T*	S80I	L416F, L254Q*
EC18[#]	Clinical	N652H*	A155V*, D639N*	-	-
EC19[Δ]	Clinical	-	-	-	-
EC22[Δ]	Community	S83L, D87N, D678E*, A828S*	A618T*	S80I	L416F, L254Q*
EC26[Δ]	Community	S83L, D87N, A828S*	A618T*	S80I, E84V, A192V*, A471G*, D475E*, Q481H*	I529L, V136I*

[Δ]CIP resistant isolates without corresponding ARGs; [#]CIP resistant isolates with corresponding ARGs; *Novel mutations

Table S3: Genomic distribution of MGEs and their association with ARGs in clinical and community *E. coli* isolates

Isolate (MLST)	Setting	Contig	Synteny of ARGs with MGEs	Plasmid/chromosomal sequence with closest nucleotide homology (accession number)
EC01 (ST3337)	Clinical	76	*CatA1::Tn3 (TnAs3):::IntI1:DfrA17:AadA5:QacEΔ1:sul1:::IS6100*	*E. coli strain* 800 chromosome (CP157958.1)
		100	*tet(B):::IS4 (ISVsa5)*	*E. coli* strain OXEC-466 plasmid unnamed (CP165369.1)
		118	*Transposase:TEM-1*	*E. coli* strain OXEC-478 plasmid unnamed (CP165316.1)
EC02 (ST1193)	Clinical	123	*IntI1:dfrA17:AadA5:QacEΔ1:sul1:::IS6 (IS6100):::Mph(A)*	*E. coli* 09-02E plasmid p1-09-02E DNA (AP022651.1)
		227	*Transposase:CTX-M-27:IS5 family transposase*	*E. coli* 09-02E plasmid p1-09-02E DNA (AP022651.1)
EC03 (ST131)	Clinical	52	*IntI1:DfrA17:AadA5:QacEΔ1:sul1::::IS6 (IS6100):::Mph(A)*	*E. coli* strain S1-IND-01-B plasmid p1-S1-IND-01-B (CP145656.1)
		74	*ermB::transposase*	*E. coli* strain S1-IVC-02-A plasmid p1-S1-IVC-02-A (CP145699.1)
EC04 (ST38)	Clinical	61	*IS6100:::Mph(A)*	*E. coli* strain W46 isolate China: Kunming chromosome (CP163115.1)
		66	*Transposase:CTX-M-15*	*E. coli* strain E305-2 plasmid punnamed2 (CP090252.1)
		62	*sul2:IS91 (ISVsa3 transposase)*	*E. coli* strain SCU-164 chromosome (CP054343.1)
		65	*IS3 family transposase::Aac(3)-IIe*	*E. coli* strain SX4 plasmid pSX4-2-ESBLs (CP142399.1)
EC05 (ST38)	Clinical	37	*IntI1:DfrA1:AadA1:QacEΔ1:sul1:::transposase:::::::::TEM-1::Tn3:::tet(A):::TnAs1:IS3(IS1133):Aph(3")-Ib:Aph(6)-Id*	*E. coli* strain OXEC-285 plasmid p2 (CP164926.1)
EC07 (ST3572)	Clinical	9	*Tn3 transposase:IS3000 transposase:OXA-181:::ISKra4(ISKpn19 transposase)*	*E. coli* strain Eco-15 plasmid pEco15-4 (CP047714.1)
		182	*QacEΔ1:sul1:IS110(IS4321)::IS6(IS6100):::Mph(A)*	*E. coli* strain 15.TR.026_OXA chromosome (CP032145.1)
		369	*DfrA17:IntI1*	*E. coli* strain SX23 plasmid pSX23-2-ESBLs (CP142790.1)

EC08 (ST69)	Clinical	92	*AadA3:DfrA17:IntI1*	*E. coli* strain SCU-482 plasmid pSCU-482-1 (CP053248.1)
		85	*DDE-type integrase:::Mph(A)*	*E. coli* strain GN4143 plasmid pGN4143-1 (CP142071.1)
		74	*tet(B)::IS4(ISVsa5)*	*E. coli* strain OXEC-466 plasmid unnamed (CP165369.1)
EC09 (ST131)	Clinical	93	*IntI1:DfrA17:AadA5:QacEΔ1:sul1:::IS6 (IS6100):::Mph(A):IS6 family transposase*	*E. coli* strain KFS-D30 plasmid pKFS-D30_1 (CP125005.1)
		153	*IS4(ISVsa5 transposase):Aac(3)-IId*	*E. coli* strain WTP01 plasmid pWTP-01 (OR287786.1)
		99	*IS1380 (ISEcp1 transposase):CTX-M-15::Tn3 family transposase*	*E. coli* strain 98214 chromosome (CP173519.1)
		104	*IS1 transposase: TEM-1*	*E. coli* strain EcPF18 plasmid p1 (CP054220.1)
EC10 (ST131)	Clinical	52	*IntI1:DfrA17:AadA5:QacEΔ1:sul1:::IS6(IS6100 transposase):::Mph(A)*	*E. coli* strain S1-IND-01-B plasmid p1-S1-IND-01-B (CP145656.1)
		61	*ermB:transposase*	*E. coli* strain S1-IVC-02-A plasmid p1-S1-IVC-02-A (CP145699.1)
EC13 (ST131)	Clinical	45	*IntI1:DfrA17:AadA5:QacEΔ1:sul1:::IS6(IS6100 transposase):::Mph(A)*	*E. coli* O25b:H4-ST131 strain 418 plasmid p418 (MK295833.1)
		61	*CTX-M-15::Tn3 transposase*	*E. coli* strain IVRI_FBI_607 chromosome (CP125922.1)
		57	*tet(A):::Tn3 transposase*	*E. coli* strain GN4143 plasmid pGN4143-1 (CP142071.1)
EC14 (ST10)	Clinical	68	*IntI1: DfrA17:AadA5:QacEΔ1:sul1:::IS6(IS6100 transposase):::Mph(A)*	*E. coli* strain 18AR0845 plasmid P18ar0845a (CP175692.1)
		60	*IS1 transposase:IS6(IS26 transposase):CTX-M-15::Tn3 transposase:IS3 transposase: QnrS1::ISKra4(ISKpn19 transposase)*	*E. coli* strain C51 plasmid pC5103 (CP127312.1)
EC15 (ST13823)	Clinical	57	*CTX-M-15::Tn3 transposase:IS3 transposase:QnrS1::ISKra4 (ISKpn19 transposase):::sul1:QacEΔ1:AadA5:DfrA17:IntI1*	*E. coli* strain 129130 plasmid pMRSN129130_p3 (CP158336.1)
EC16 (ST1193)	Clinical	62	*Mph(A):::IS6(IS6100 transposase)::TEM-1:IS1 transposase*	*E. coli* isolate 30345_1#221 genome assembly, plasmid: 2 (OZ039844.1)
		79	*ISKra4(ISKpn19 transposase)::QnrS1:IS3 transposase:Tn3 transposase::CTX-M-231:IS6 (IS26 family transposase)*	*E. coli* strain 123636 plasmid pMRSN123636_p3 (CP158326.1)
		100	*ermB:::transposase*	*E. coli* strain 5M plasmid pISV_IncFII_NDM-5 (MN218686.1)

EC18 (ST13823)	Clinical	57	*CTX-M-15::Tn3 transposase:IS3 transposase:QnrS1::ISKra4 (ISKpn19 transposase):::sul1:QacEΔ1:AadA5:DfrA17:IntI1*	*E. coli* strain 129130 plasmid pMRSN129130_p3 (CP158336.1)
EC19 (ST3572)	Clinical	29	*IS6(IS26 transposase):Tn3 transposase:Tn3(IS3000 transposase):OXA-181:::ISKra4(ISKpn19 transposase)*	*E. coli* strain Eco-15 plasmid pEco15-4 (CP047714.1)
		51	*Tn3(TnAs1 transposase):::IntI1:DfrA17:QacEΔ1:sul1:IS110(IS4321 transposase):::IS6(IS6100 transposase):::Mph(A)*	*E. coli* strain 15.TR.026_OXA chromosome (CP032145.1)
EC21 (ST1722)	Community	49	*QacEΔ1:AadA1: DfrA1:IntI1*	*E. coli* strain JKHS016 plasmid pJKHS016_2 (CP147060.1)
		32	*ISKra4 transposase::QnrS1:IS3 transposase:Tn3 transposase::CTX-M-15*	*E. coli* plasmid pWP8-S18-ESBL-07_2 DNA (AP022263.1)
		43	*TEM-216: TnAs1 transposase:IS91 transposase:sul2*	*E. coli* strain 786605 plasmid p786605_1 (CP091395.1)
EC22 (ST1193)	Community	67	*DDE-type integrase:::Mph(A)*	*E. coli* strain GN4143 plasmid pGN4143-1 (CP142071.1)
		59	*tet(B)::IS4(ISVsa5 transposase):IS1 transposase*	*E. coli* strain OXEC-466 plasmid unnamed (CP165369.1)
		71	*IS1 transposase:DfrA17:IntI1*	*E. coli* strain EcPF40 plasmid p1 (CP054215.1)
EC23 (ST10)	Community	71	*TEM-1:Aph(6)-Id:Aph(3")-Ib:sul2::IS110(IS5075 transposase):Tn3 transposase*	*E. coli* strain EC28 plasmid p2 (CP049102.1)
		86	*Tn3 transposase:::tet(A)*	*E. coli* strain IVRI_FBI_650 chromosome (CP125923.1)
		103	*DfrA14:IntI1*	*E. coli* strain AR Bank #0349 plasmid pAR349 (CP041997.1)
EC24 (ST1380)	Community	5	*sul2::DfrA14:Aph(3")-Ib:Aph(6)-Id:IS91 transposase*	*E. coli* strain 31CH-1 plasmid p31CH-1 (PP566049.1)
		235	*Tn3 transposase::tet(A):::Tn3 transposase*	*E. coli* strain IVRI_FBI_650 chromosome (CP125923.1)
EC25 (ST10)	Community	69	*TEM-1:Aph(6)-Id:Aph(3")-Ib:sul2::IS110(IS5075 transposase):Tn3 transposase*	*E. coli* strain EC28 plasmid p2 (CP049102.1)
		76	*Tn3(TnAs1 transposase):::tet(A)*	*E. coli* strain IVRI_FBI_650 chromosome (CP125923.1)
		90	*DfrA14:IntI1*	*E. coli* strain AR Bank #0349 plasmid pAR349 (CP041997.1)

EC26 (ST131)	Community	71	*CTX-M-27:IS5/IS1182 transposase*	*E. coli* O25b:H4-ST131 strain 4224 plasmid pMB6910_1 (CP174354.1)
EC30 (ST2758)	Community	82	*IS3 transposase:QnrS1::ISKra4 (ISKpn19 transposase):::IS91 transposase:Aph(6)-Id:Aph(3'')-Ib:sul2::IS110 (IS5075 transposase):Tn3 transposase*	*E. coli* strain 1008S19 plasmid p1008S19_1 (CP147051.1)
		118	*DfrA14:IntI1*	*E. coli* strain CFSAN061766 plasmid pCFSAN061766 (CP042872.1)
EC31 (ST2852)	Community	40	*Tn3 transposase:::ISKra4 (ISKpn19 transposase)::QnrS1:IS3 transposase:Tn3 transposase::TEM-1:Tn3 transposase:::tet(A)*	*E. coli* strain KFu021 plasmid pKFu021_1 (CP147102.1)
		43	*Tn3 transposase:IS110(IS5075 transposase)::sul2:IS91(ISVsa3 transposase)*	*E. coli* strain KFu021 plasmid pKFu021_1 (CP147102.1)
		49	*DfrA14:IntI1*	*E. coli* strain 98224 plasmid p98224-contig_3 (CP173471.1)
EC32 (ST382)	Community	98	*Aph(6)-Id:Aph(3'')-Ib:sul2:::IS6(IS26 transposase)*	*E. coli* strain OXEC-448 chromosome (CP165428.1)
		86	*TEM-1:::QacEΔ1:DfrA7:IntI1:::TnAs3 transposase*	*E. coli* O104:H4 strain FWSEC0009 chromosome (CP031902.1)
		95	*tet(B):::IS4(ISVsa5 transposase):IS1 transposase*	*E. coli* strain OXEC-466 plasmid unnamed (CP165369.1)
EC33 (ST336)	Community	19	*Tn3 transposase:::ISKra4 (ISKpn19 transposase)::QnrS1:IS3 transposase:Tn3 transposase::TEM-1:Tn3 transposase:::tet(A):::Tn3 transposase*	*E. coli* strain KFu021 plasmid pKFu021_1 (CP147102.1)
		251	*sul2:IS91(ISVsa3 transposase)*	*E. coli* strain KKa014 plasmid pKKa014_1 (CP147106.1)
		991	*DfrA14:IntI1*	*E. coli* strain 98224 plasmid p98224-contig_3 (CP173471.1)
EC35 (ST2178)	Community	56	*QacEΔ1:DfrA7:IntI1:::TnAs3 transposase*	*E. coli* strain SCU-164 chromosome (CP054343.1)
EC36 (ST101)	Community	34	*sul1:QacEΔ1:AadA1:DfrA1:IntI1:::IS1 transposase*	*E. coli* strain HB37 chromosome HB37 (CP053080.1)
		39	*IS5 transposase::IS5(IS903B transposase):Aph(6)-Id*	*E. coli* strain 58-3 plasmid pCD58-3-1 (CP050037.1)
		42	*TnAs1 transposase:::tet(A)*	*E. coli* strain WWCOL-236 plasmid pCOL236-1 (CP178553.1)

EC37 (ST131)	Community	39	*sul1:QacEΔ1:AadA5:DfrA17:IntI1*	*E. coli* strain SCU-482 plasmid pSCU-482-1 (CP053248.1)
		45	*DDE-type integrase:::Mph(A)*	*E. coli* strain GN4143 plasmid pGN4143-1 (CP142071.1)
EC38 (ST4406)	Community	2	*IS1(IS1B transposase):CatA1::TnAs3 transposase:::IntI1:DfrA7:QacEΔ1*	*E. coli* strain RHB23-C03 chromosome (CP057559.1)
EC39 (ST3570)	Community	51	*Tn3 transposase::TEM-1:IS1 transposase*	*E. coli* strain C012_chr chromosome (CP119577.1)
		53	*IS21(IS1326 transposase):IS21(IS1326 transposase)::sul1:QacEΔ1:DfrA5:IntI1:::TnAs3 transposase::CatA1:IS1 transposase*	*E. coli* strain PNUSAE013304 chromosome (CP034936.1)
EC40 (ST13823)	Community	56	*CTX-M-15::Tn3 transposase:IS3 transposase:QnrS1::ISKra4 (ISKpn19 transposase):::sul1:QacEΔ1:AadA5:DfrA17:IntI1*	*E. coli* strain 129130 plasmid pMRSN129130_p3 (CP158336.1)
EC41 (ST210)	Community	48	*Mph(A):::IS6(IS6100 transposase)::TEM-1:IS1 transposase*	*E. coli* isolate 30348_1#299 genome assembly, plasmid: 2 (OZ038863.1)
		55	*ISKra4(ISKpn19 transposase)::QnrS1:IS3 transposase:Tn3 transposase::CTX-M-231:IS6 (IS26 family transposase)*	*E. coli* strain 123636 plasmid pMRSN123636_p3 (CP158326.1)
		70	*ermB:::transposase*	*E. coli* strain 5M plasmid pISV_IncFII_NDM-5 (MN218686.1)

Table S4: Genomic distribution of MGEs and their association with ARGs in clinical and community *K. pneumoniae* isolates

Isolate ID (MLST)	Setting	Contig	Synteny of ARGs with MGEs	Plasmid/chromosomal sequence with closest nucleotide homology (accession number)
KP45 (ST348)	Clinical	47	*IS110(IS5075 transposase)::sul2:Aph(3'')-Ib:Aph(6)-Id:IS91 transposase*	*K. pneumoniae* subsp. *pneumoniae* strain ST101:960186733 plasmid p19-10 01 (CP023488.1)
		1	*IS3 transposase:integrase::::::OqxA:OqxB25:::::::::::: emrA:emrB*	*K. pneumoniae* strain ARLG-4837 chromosome (CP067750.1)
		57	*IS1 transposase::::IS6(IS6100 transposase):::DfrA14:IntI1*	*K. pneumoniae* strain AR_0109 plasmid unnamed6 (CP032212.1)
KP46 (ST17)	Clinical	46	*Aac(3)-IIe:IS3 transposase::IS3(ISKpn11 transposase)::IS5(ISKpn12 transposase)*	*K. pneumoniae* strain Kp202 plasmid pKp202_2 (CP041084.1)
		61	*AadA16:DfrA27:Arr-3:Aac(6')-Ib-cr5:IntI1*	*K. pneumoniae* strain KP09 plasmid pKP09-CTX-M (CP178566.1)
		42	*Tn3 transposase:IS3000 transposase:OXA-181:::ISKra4(ISKpn19 transposase):::::QnrS1:transposase*	*K. pneumoniae* strain 709 plasmid pKP709-OXA181 (MN227183.1)
		52	*IS30 transposase:NDM-5::::IS91(ISCR1 transposase)*	*K. pneumoniae* strain 47733 plasmid p47733_NDM_5 (CP050367.1)
		66	*IS1380(ISEcp1 transposase):CTX-M-15*	*K. pneumoniae* strain KP09 plasmid pKP09-CTX-M (CP178566.1)
		4	*IS3 transposase:::::::OqxA:OqxB25::::::::::::emrA:emrB*	*K. pneumoniae* strain NK H8 118 chromosome (CP152537.1)
KP47 (ST607)	Clinical	45	*AadA16:DfrA27:Arr-3:Aac(6')-Ib-cr5:IntI1:*	*K. pneumoniae* strain Bckp021 plasmid pBckp021-1 (CP050835.1)
		48	*IS5(ISKpn12 transposase)::IS3(ISKpn11 transposase)::IS3 transposase:Aac(3)-IIe:TEM-1*	*K. pneumoniae* strain MAKM-3381 chromosome (CP129122.1)
		54	*IS1380(ISEcp1 transposase):CTX-M-15::Tn3 transposase*	*K. pneumoniae* strain GN4146 plasmid pGN4146-1 (CP142061.1)
		56	*QnrB6::IS91(ISCR1 transposase)*	*K. pneumoniae* strain E16KP0115 plasmid unnamed (CP052638.1)
KP48 (ST607)	Clinical	46	*IS5(ISKpn12 transposase)::IS3(ISKpn11 transposase)::IS3 transposase:Aac(3)-IIe:TEM-1:::IS1380(ISEcp1 transposase):CTX-M-15::Tn3 transposase*	*K. pneumoniae* strain MAKM-3381 chromosome (CP129122.1)
		49	*AadA16:DfrA27:Arr-3:Aac(6')-Ib-cr5:IntI1*	*K. pneumoniae* strain Bckp021 plasmid pBckp021-1 (CP050835.1)

		71	*Aph(6)-Id:IS91 transposase*	*K. pneumoniae* strain C16KP0160 plasmid pC16KP0160-4 (CP052740.1)
		63	*QnrB6::IS91(ISCR1 transposase)*	*K. pneumoniae* strain E16KP0115 plasmid unnamed (CP052638.1)
KP51 (ST133)	Clinical	52	*AadA16:DfrA27:Arr-3:Aac(6')-Ib-cr5:IntI1*	*K. pneumoniae* strain Bckp021 plasmid pBckp021-1 (CP050835.1)
		54	*ISPa38 transposase:::::Aph(6)-Id:Aph(3'')-Ib:sul2*	*K. pneumoniae* strain E128606 plasmid pE128606.6 (CP136391.1)
		61	*IS3(ISKpn11 transposase):Aac(3)-IIe:TEM-34:::IS1380(ISEcp1 transposase)*	*K. pneumoniae* strain MAKM-3381 chromosome (CP129122.1)
		21	*emrB:emrA::::::::::::OqxB14:OqxA:::::integrase:IS3 transposase*	*K. pneumoniae* strain NK H15 032 chromosome (CP152854.1)
		69	*QnrB6::IS91(ISCR1 transposase)*	*K. pneumoniae* strain E16KP0115 plasmid unnamed (CP052638.1)
EC27	Community	50	*sul1:QacEΔ1:DfrA5:IntI1*	*K. pneumoniae* strain KP06_UID2108280006 plasmid unnamed1 (CP171581.1)
KP53 (ST193)	Community	33	*mef(B):::sul3:IS256 transposase:qacL:AadA1:CmlA1:::IntI1:::TnAs1 transposase*	*K. pneumoniae* strain SRY516 plasmid unnamed1 (CP138715.1)

		24	*ISKra4(ISKpn19 transposase)::QnrS1:IS3 transposase:LAP-2*	*K. pneumoniae* subsp. pneumoniae strain SCKP020079 plasmid pLAP2_020079 (CP029382.1)
		36	*Tn3 transposase:IS110(IS5075 transposase)::sul2:IS91(ISVsa3 transposase)*	*K. pneumoniae* strain KP20194c4 plasmid pKP20194c4-p3 (CP054747.1)
		38	*TnAs1 transposase:::tet(A)*	*K. pneumoniae* strain GN4147 plasmid pGN4147-2 (CP142055.1)
KP55 (ST17)	Community	2	*IS3 transposase:::::: OqxA:OqxB25:::::::::::: emrA:emrB*	*K. pneumoniae* strain SB1067 chromosome (CP084860.1) (99 %)
KP57 (ST45)	Community	169	*Mph(A):::IS6(IS6100 transposase):::DfrA14:IntI1*	*K. pneumoniae* subsp. pneumoniae strain R210-2 plasmid pR210-2-VIM (CP034084.1)
KP58 (ST873)	Community	50	*sul2:IS91(ISVsa3 transposase)*	*K. pneumoniae* strain 1675474 plasmid p1675474_1 (MK649827.1)
KP59 (ST1863)	Community	21	*TEM-1::IS6 transposase::::IS6(IS26 transposase):Tn3 transposase:IS3 transposase:QnrS1:ISKra4(ISKpn19 transposase)*	*K. pneumoniae* strain S166-1 plasmid pS166-1.4 (CP063949.1)

CHAPTER 3: CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

This study reveals differences and similarities in phenotypic profiles, resistomes, mobilomes, and phylogenetic relationships of *E. coli* and *K. pneumoniae* isolates from hospital patients and healthy community dwellers in uMgungundlovu District.

3.1. Conclusion

The main findings corresponding to each study objective are summarized as follows:

To compare the phenotypic characteristics of AMR observed in *E. coli* and *K. pneumoniae* isolates from hospital patients and healthy community dwellers:

- A total of 35 clinical isolates (22 *E. coli*, 13 *K. pneumoniae*) were matched with proportionate community isolates (44 *E. coli*, 26 *K. pneumoniae*).
- Clinical and community *E. coli* isolates in this study were resistant to largely the same antibiotics, including SXT (86.7% clinical; 82.4% community), CXM (73.3% clinical; 23.5% community), CIP (73.3% clinical; 17.6% community), and CTX/CRO (60.0% clinical; 23.5% community). AMP/AMX–SXT was the prevalent shared pattern (2 clinical, 9 community).
- Clinical *K. pneumoniae* isolates showed high resistance to CTX/CRO, CXM, CAZ, and SXT (each 83.3%), followed by AMC, TZP, CIP, and GEN (each 66.7%), and FOX (50.0%). In contrast, community *K. pneumoniae* isolates exhibited moderate resistance to TZP, CIP, and SXT (each 25.0%) and notable resistance to the last-resort antibiotic TGC (50.0%), while no resistance to TGC was observed among clinical isolates.

To describe and compare resistomes and mobilomes conferring resistance in clinical and community isolates using WGS and bioinformatic analyses:

Sixty isolates were selected for WGS, of which 32 *E. coli* (15 clinical, 17 community) and 14 *K. pneumoniae* (6 clinical, 8 community) were confirmed by WGS. Among these, seven *E. coli* isolates (3 clinical, 4 community) and one community *K. pneumoniae* isolate were identified as ESBL-producers. Resistome and mobilome analysis revealed diversity and substantial overlap of ARGs and MGEs between clinical and community isolates.

i. *E. coli*

- Resistome: β-lactamase ($bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1B}$), trimethoprim (*dfrA17*), sulfonamide (*sul1*, *sul2*), aminoglycoside [*aph(3")-Ib*, *aph(6)-Id*], fluoroquinolone (*qnrS1*), macrolide [*mph(A)*], and tetracycline [*tet(A)*] ARGs were detected in isolates from both settings. Community isolates shared this core resistome but showed a higher prevalence of trimethoprim resistance mediated by *dfrA14* rather than *dfrA17*. Most clinical isolates harboured $bla_{CTX\text{-}M}$ ARGs but were phenotypically non-ESBL producers, highlighting the importance of a molecular approach to delineate AMR. While

bla$_{CTX\text{-}M\text{-}15}$ is the predominant ESBL gene in *E. coli* worldwide, however, *bla*$_{TEM\text{-}1B}$ dominated in community isolates.

- Mobilome: IncFIA, and IncFII plasmid replicons were the most prevalent and widely shared. Gene cassettes associated with class 1 integrons (*intI1*) were detected in both settings, with In54 and In191 being most prevalent in clinical and community isolates, respectively. Most ARGs were plasmid-borne and in synteny with ISs and Tns on the same contigs. Common ISs across settings included IS1, IS3, IS26, ISVsa3, ISVsa5, and ISKpn19, with ARGs commonly carried on Tn3 transposons.

ii. *K. pneumoniae*

- Resistome: The *bla*$_{CTX\text{-}M\text{-}15}$ gene predominated, however, none of the clinical isolates harbouring this gene were phenotypically ESBL producers. Common ARGs in both settings included β-lactamase (*bla*$_{SHV\text{-}1}$, *bla*$_{SHV\text{-}11}$), trimethoprim (*dfrA14*), sulfonamide (*sul1*, *sul2*), aminoglycoside [*aph(3')-Ia*, *aph(3")-Ib*], fosfomycin (*fosA5*), macrolide [*mph(A)*], and tetracycline [*tet(D)*] resistance genes. Fluoroquinolone resistance was mainly attributed to *aac(6')-Ib-cr* in clinical isolates and *qnrS1* in community isolates.
- Mobilome: Plasmid replicons IncFIB, IncFII, and IncR were commonly detected in both settings. Most isolates from both settings were associated with the *intI1* gene, with In1021 predominating in clinical isolates, whereas In191 was common across both settings. ARGs were predominantly carried on plasmids and frequently associated with ISs and Tn3 transposons. IS3, IS5075, IS6100, and ISKpn19 were detected in both settings. Additionally, the IS91 family were identified in both settings, with ISCR1 unique to clinical isolates and ISVsa3 unique to community isolates.

To ascertain transmission dynamics and directionality through the potential genomic associations of resistomes with mobilomes, clonality and phylogeny:

- Clonality analysis revealed greater ST diversity among community than clinical *E. coli* and *K. pneumoniae* isolates. For *E. coli*, ST131, ST10, and ST1193 were shared across settings. Clinical-unique STs (ST38, ST3572, ST13823, ST69, ST3337) and community-unique STs (ST1380, ST1722, ST2758, ST2852, ST382, ST336, ST2178, ST101, ST4406, ST3570, ST210) were detected. All clinical *E. coli* isolates phylogenetically clustered with community isolates, suggesting close genetic relatedness and potential transmission between settings. In contrast, limited shared clonality was observed among *K. pneumoniae* isolates, with ST17 being the only ST shared between the two settings, represented by one clinical and one community isolate. Clinical isolates belonged to ST607, ST10, ST348, ST17, and ST133, whereas community isolates each exhibited distinct sequence types, including ST17, ST37, ST45, ST193, ST5396, ST716, ST873, and ST1863. Phylogenetic clustering between clinical and community *K. pneumoniae* isolates was less frequent, indicating more distinct strains.

Considering the resistome, mobilome, clonality, and phylogeny, clinical and community isolates revealed bidirectional AMR transmission potential across settings, indicating healthy individuals serve as key reservoirs, however, this study has several limitations.

3.2. Limitations

- A small sample size (clinical: 22 *E. coli* and 13 *K. pneumoniae*; community: 44 *E. coli* and 26 *K. pneumoniae*) may not fully capture AMR transmission dynamics in KwaZulu-Natal. The low number of clinical *E. coli* and *K. pneumoniae* isolates obtained from the NHLS during the sampling period contributed to the small clinical sample size.
- The short sampling period limits insight into AMR spread and evolution, and the findings, being limited to uMgungundlovu, may not be representative of other KwaZulu-Natal regions.
- Some isolates were excluded after WGS due to cross-contamination and misidentification, which led to reduced confidence in downstream analyses.
- Phenotype-genotype discordance in ESBL detection among clinical isolates highlights the limitation of relying solely on automated phenotypic testing, which may lead to underestimation of the true prevalence of ESBL-producing isolates.
- Lack of detailed patient and community participant metadata (e.g. antibiotic use, exposure, hospital stay or healthcare facility visitation) limited assessment of complete AMR transmission directionality.

3.3. Recommendations

- Studies should include larger sample sizes to better capture AMR transmission dynamics across KwaZulu-Natal.
- Longitudinal studies should be conducted to monitor trends and the evolution of AMR, and sampling should be expanded to multiple districts beyond uMgungundlovu to ensure findings are representative of broader KwaZulu-Natal
- Check culture purity immediately before gDNA extraction and implement additional identification methods, such as matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) or polymerase chain reaction (PCR) to minimize contamination and misidentification.
- Automated confirmatory phenotypic tests should be paired with manual methods, such as the double-disk synergy test (DDST) or Epsilometer test (E-test), to resolve genotype-phenotype discordance and accurately determine ESBL prevalence.
- Standardized questionnaires should be developed for participants to capture antibiotic history, healthcare visits, travel, and potential exposures.

APPENDICES

Appendix 1: UKZN BREC ethical approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

14 November 2025

Prof Sabiha Yusuf Essack (3951)
School of Health Sciences
Westville

Dear Prof Essack,

Protocol reference number: BREC/00003640/2021
Project title: One Health Alternate Antimicrobial Resistance Monitoring System in a Coronavirus World
Degree purposes: Non-Degree

RECERTIFICATION APPROVAL

Approved:	**27 March 2025**
Expiration of Ethical Approval:	**26 March 2026**

I wish to advise you that your application for Recertification for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 09 December 2025.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof S Singh
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor S Singh
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: https://research.ukzn.ac.za/research-office/ethics-overview/biomedical-research-ethics/
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix 2: KwaZulu-Natal's Department of Health PHREC ethical approval letter

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Postal Address: Private Bag X9050
Physical Address: 330 Langalibalele Str, PM Burg, 3201
Tel: 0333953189/3123/2805 Fax: 033-3943782
Email address: [redacted]
www.kznhealth.gov.za

DIRECTORATE:
Health Research &
Knowledge Management Unit

NHRD Ref: KZ_202203_023

Dear Professor S Essack
(UKZN)

Approval of research

1. The research proposal titled **'One Health Alternative Antimicrobial Resistance Monitoring System in a Coronavirus World'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

   The proposal is hereby approved for research to be undertaken at East Boom and Imbalenhle clinics, and Harry Gwala, Northdale and Greys hospitals, and protocol amendments as approved by the Biomedical Research Ethics Committee at UKZN (reference BREC/00003640/2021) are noted.

2. You are requested to take note of the following:

   a. *All research conducted in KwaZulu-Natal must comply with government regulations relating to Covid-19. These include but are not limited to: regulations concerning social distancing, the wearing of personal protective equipment, and limitations on meetings and social gatherings.*
   b. *Kindly liaise with the facility managers BEFORE your research begins in order to ensure that conditions in the facilities are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.*
   c. *Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.*
   d. *Provide an interim progress report and final report (electronic copy) when your research is complete to hrkm@kznhealth.gov.za*
   e. *Please note that the Department of Health shall not be held liable for any injury that occurs as a result of this study.*

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

[redacted]

Dr E Lutge
Chairperson, Health Research Committee
Date: 26/06/2023.

GROWING KWAZULU-NATAL TOGETHER

Appendix 3: Introduction to Research Ethics certificate

TRREE

Zertifikat

Certificado

Certificat

Certificate

Promouvoir les plus hauts standards éthiques dans la protection des participants à la recherche biomédicale
Promoting the highest ethical standards in the protection of biomedical research participants

Certificat de formation - Training Certificate

Ce document atteste que - this document certifies that

Clinical Trials Centre
The University of Hong Kong

Khanyisa Mahonisi

a complété avec succès - has successfully completed

Module 1 (2023) - Introduction to Research Ethics

du programme de formation TRREE en évaluation éthique de la recherche
of the TRREE training programme in research ethics evaluation

Release Date: 2024/01/31

CID : pcvsgGa0lH

Professeur Dominique Sprumont
Coordinateur TRREE Coordinator

APPROVED BY
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ISFM
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[REV : 20220217]

Appendix 4: Information sheet for participants and consent Form

One Health AlARMS in a COVID-19 World

Participant Information Sheet

My name is …….. (name of research assistant). Thank you for taking the time to listen to me about a study we are doing in this community.

You are being invited to consider participating in a study entitled One Health AlARMS in a COVID-19 World that focuses on bacteria. Bacteria are small living things/microorganisms that we can't see but they are found in our nose, throat, skin and in our gut. The bacteria are usually good bacteria and will not cause us harm but in some cases they can cause infection in other parts of our body if you don't, for example, wash your hands with soap and clean water before and after you use the toilet or before and after you handle or eat food. Some of these bacteria may be resistant to antibiotics, i.e. the bacteria are no longer affected by the antibiotics that used to kill the bacteria and cure the infection.

We want determine whether the bacteria we carry in our nose, throat and gut match the bacteria we find in the wastewater as well as the bacteria that make people sick so that they have to go to the clinic or hospital.

For us to be able to do this, we require your permission to take samples from your nose and throat and we would also like you to give us a sample of your stool. If you agree, we will collect these samples from you every month for a year. You will be given a shopping voucher worth R100 every time you provide these samples to compensate you for your time and any inconvenience. We will also be collecting wastewater samples to see whether the bacteria in your body is also found in the wastewater.

We would appreciate your contact details so that we can arrange to meet with you to collect the samples. This will be done by a research assistant who is trained to do this and legally and ethically bound to ensure that all your personal details are kept confidential. We will not share any of your information with anybody else.

When we collect your samples for the first time, we would appreciate it if you would take some time to answer questions regarding your household. These will include but not be limited to questions on who and how many people live in your household, the languages you speak, the education you have received, employment and income, the general health of everyone living in your household, the types of food you eat, the type of house you live in, i.e., whether you have electricity water and toilet facilities etc. The questionnaire will take approximately 1-2 hours to complete and we will only do this once. We will also be completing another document called an observation guide where we will take extra notes

on your household. A researcher will spend 4-8 hours at your house on a day most convenient for you. We would also like permission to vacuum your house to see if there are any germs that can cause infection in your house.

There are no risks to your participation. You may experience mild discomfort when samples are taken from your nose and throat, but please be assured that the research assistant is trained to take these samples. The swabs that will be used are sterile and the research assistant will change gloves for every person s/he takes samples from.

Your participation in this study may benefit you because we may be able to tell you if you are carrying any drug-resistant bacteria. Your participation will also help the Department of Health determine whether the bacteria making people sick is also found in wastewater and carried by healthy people living in this area. In this way, the Department of Health will know if they should monitor wastewater to know whether drug-resistant bacteria are increasing and whether wastewater should be monitored routinely throughout South Africa. Your participation will thus improve public health in South Africa.

Your participation is completely voluntary, i.e., you don't have to participate in this study if you don't want to. You can withdraw from the study at any time, again with no negative consequences. If you kindly agree to participate, we need you to sign this consent form giving us permission to (1) take nasal, throat and stool samples every month for a year, (2) complete the questionnaire and (3) allow us to observe and vacuum your household.

Please do not hesitate to contact the following person should you have any queries or concerns related to your voluntary participation:

Professor Sabiha Essack

B. Pharm., M. Pharm., PhD

South African Research Chair in Antibiotic Resistance & One Health

Antimicrobial Research Unit College
of Health Sciences

University of KwaZulu-Natal

Private Bag X54001

Durban

4000

South Africa

Telephone: +27(0)31 2607785

Telefax: +27(0)31 2607792

Email: essacks@ukzn.ac.za

We thank you for your invaluable time and your assistance.

One Health AlARMS in a COVID-19 World
Declaration of Consent

I _____ , Identity no. _____ residing at _____ have been informed about the study entitled "One Health AlARMS in a COVID-19 World" by the research assistant.

I understand the purpose and procedures of the study and I hereby agree to (1) provide nasal, throat and stool samples every month, (2) provide my contact details for future samples, (3) complete the questionnaire (3) allow the research assistant to observe my household, and, (4) allow the research assistant to vacuum my household. I acknowledge that all information will be used in the strictest confidence.

I have been given an opportunity to answer questions about the study and have had answers to my satisfaction. I have been informed about any available compensation.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without any consequences.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at the address below:

Professor Sabiha Essack

B. Pharm., M. Pharm., PhD

South African Research Chair in Antibiotic Resistance & One Health

Antimicrobial Research Unit College
of Health Sciences

University of KwaZulu-Natal

Private Bag X54001

Durban

4000

South Africa

Telephone: +27(0)31 2607785

Telefax: +27(0)31 2607792

Email: essacks@ukzn.ac.za

I also understand that for concerns related to my rights as participant or to the researcher, I can freely contact the Biomedical Research Ethics Committee at the address below:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus

Govan Mbeki Building

University of KwaZulu-Natal

Private Bag X 54001, Durban, 4000

KwaZulu-Natal, SOUTH AFRICA

Tel: +27(0) 31 2602486

Fax: +27 (0) 31 2604609

_____

Signature

Signed this _____ day of _____ 2016 at _____

Witness 1: _____ **Witness 2: _____**