LONGITUDINAL GENOMIC SURVEILLANCE OF SURFACE WATER AS A PROXY FOR ANTIMICROBIAL RESISTANCE IN A RESOURCE-LIMITED COMMUNITY AND ITS PROXIMATE CLINICAL FACILITY



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Longitudinal Genomic Surveillance of Surface Water as a Proxy for Antimicrobial Resistance in a Resource-Limited Community and its Proximate Clinical Facility

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A thesis submitted to the School of Laboratory Medicine & Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Medical School, for the degree of Doctor of Philosophy in Medicine (Medical Microbiology).

This is a thesis in which the chapters are written as a set of discrete research manuscripts submitted or intended for submission to internationally recognised peer-reviewed journals with an overall introduction and final summary.

This is to certify that the content of this thesis is the original research work of Mr. Etando Ayukafangha, carried out under our supervision at the Antimicrobial Research Unit (ARU), Discipline of Pharmaceutical Sciences, School of Health Sciences, College of Health Sciences, Westville campus, University of KwaZulu-Natal (UKZN), Durban, South Africa.

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DECLARATION

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DEDICATION

To my mother, **Mrs Constance Ayuk**, the woman whose sacrifices cannot be fully captured in words. You gave so much of yourself emotionally, financially, spiritually and even your own education so that we, your children, could have a safe future. Your prayers became my covering, your love became my comfort, and your resilience became my example. I dedicate this thesis to you with deep gratitude and love.

And

To my grandmother, the late **Mrs Takor Anastasia**, who played a foundational role in my upbringing and in shaping my values, character, and strength. Your sacrifices, guidance, and training helped make me who I am today. I deeply wish you were here to see this moment and to share in the joy of what your love helped produce. May you continue to rest in peace.

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2. Does AMR surveillance of surface water reflect AMR in humans? A genomic comparison of ESBL-producing *Escherichia coli* from surface water, healthy residents, and a clinical facility [Submitted to Environmental Research (Manuscript Number: ER-26-9361].

3. Monitoring Extended-spectrum-β-lactamases-producing *Klebsiella pneumoniae*: Do Surface Water Phenotypic Patterns Reflect Community and Clinical Resistance? [Submitted to Environmental Monitoring and Assessment (Ref: Submission ID daab4231-0147-49dd-bb9a-7b9428fe3332].

4. Evaluating Surface Water for Genomic Surveillance of Community-Linked Antimicrobial Resistance in ESBL-Producing *Klebsiella pneumoniae* [Environment International (ENVINT-D-26-02964)].

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LIST OF ABBREVIATIONS AND ACRONYMS

AIARMS	Alternate Antimicrobial Resistance Monitoring System
AMR	Antimicrobial Resistance
ARG	Antibiotic Resistant Gene
ARU	Antimicrobial Research Unit
AST	Antimicrobial Susceptibility Testing
AWaRe	Access, Watch, and Reserve
CARD	Comprehensive Antibiotic Resistance Database
CDC	Centre for Disease Control
CLSI	Clinical and Laboratory Standards Institute
CL	Clinical Isolates
DNA	Deoxyribonucleic Acid
DoHA	Departments of Health and Agriculture
E. coli	*Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EMB	Eosin methylene blue
EPEC	Enteropathogenic *E. coli*
ESBL *E. coli*	Extended-Spectrum βeta-Lactamase Producing *Escherichia coli.*
ESBL Ec	Extended-Spectrum βeta-Lactamase Producing *Escherichia coli.*
ESBL *K. pneumoniae*	Extended-Spectrum βeta-Lactamase Producing *Klebsiella pneumoniae.*
ESBL Kp	Extended-Spectrum βeta-Lactamase Producing *Klebsiella pneumoniae.*
ESBL	Extended-Spectrum βeta-Lactamase
ESBL-E	Extended-Spectrum βeta-Lactamase Producing Enterobacterales
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ExPEC	Extraintestinal Pathogenic *Escherichia coli*

FAO	Food and Agriculture Organization of the United Nations
GHS	General Household Survey
GLASS	Global Antimicrobial Resistance Surveillance System
HR	Healthy Residents
IHME/GRAM	Institute for Health Metrics and Evaluation/Global Research on Antimicrobial Resistance
InPEC	Intestinal pathogenic *Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
KZN	KwaZulu-Natal
LMICs	Low- and Middle-Income Countries
MAAP	Mapping Antimicrobial Resistance and Antimicrobial Use Partnership
MDR	Multidrug Resistance
MGE	Mobile Genetic Elements
MIC	Minimum Inhibitory Concentration
MLST	Multi-locus Sequence Typing
NASEM	National Academies of Sciences - Engineering and Medicine
NCBI	National Center for Biotechnology Information
NDoH	National Department of Health
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
NMEC	Neonatal Meningitis-associated *Escherichia coli*
OH	One Health
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational Multivariate Analysis of Variance
SA	South Africa

SARS CoV 2	Severe Acute Respiratory Syndrome Coronavirus 2
SNP	Single Nucleotide Polymorphisms
SSA	Sub-Saharan Africa
STATS SA	Statistics South Africa
ST	Sequence Types
SW	Surface Water
UKZN	University of KwaZulu-Natal
UMDP	uMgungundlovu District Municipality
UNEP	United Nations Environment Programme
UNICEF	United Nations Children's Fund
UPEC	Uropathogenic *Escherichia coli*
USA	United States of America
WBE	Wastewater-Based Epidemiology
WGS	Whole Genome Sequencing
WHO	World Health Organisation
WOAH	World Organization for Animal Health
WWTP	Wastewater Treatment Plants
cgSNP	Core-genome single-nucleotide polymorphism
HGT	Horizontal Gene Transfer

ABSTRACT

Background: Conventional antimicrobial resistance (AMR) surveillance based on clinical and laboratory-based surveillance remains constrained in many resource-limited settings. Wastewater-based surveillance provides an alternative and/or complementary AMR surveillance approach, but requires structured sewer infrastructure, which is often absent in informal communities. In such settings, surface water may provide an alternative matrix for tracking AMR.

Aim: This study investigated the prevalence, phenotypic, and genomic AMR profiles of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-Ec) and *K. pneumoniae* (ESBL-Kp) from surface water (SW), healthy residents (HR) residing in an informal settlement, and a clinical facility (CL) to determine whether SW is a suitable proxy for human-associated AMR.

Method: Over months from May to December 2023, 80 SW, 1,978 HR stool, and 2,154 CL samples of hospitalised adults were collected (N = 4,212). These yielded 512 SW, 1,915 HR, and 588 CL *E. coli* isolates, and 462 SW, 1,167 HR, and 244 CL *K. pneumoniae* isolates. Isolates underwent ESBL screening and confirmation, antimicrobial susceptibility testing using a VITEK® 2 for phenotypic studies. DNA extraction, and a balance subset of 32 isolates per sources was subjected to whole-genome sequencing using an Illumina NextSeq 1000/2000 for comparative genomics. Bioinformatic analyses were performed to characterise antimicrobial resistance genes (ARGs), virulence determinants, phylogroups, serotypes, multilocus sequence types (MLST), and phylogenetic relatedness. Data were analysed in R.

Results: ESBL-Ec prevalence varied ($p < 0.05$) across sample sources, with the highest prevalence in HR (27.10%), followed by SW (16.8%), and CL (5.3%). Multidrug resistance (MDR) also varied across sample sources with the highest MDR observed in CL (64.5%), followed by HR (41.6%) and SW (5.7%) ($p < 0.05$). SW resistance more closely resembled HR than CL, particularly for β-lactams and trimethoprim-sulfamethoxazole, although antibiogram overlap was limited and mainly involved non-MDR patterns. ESBL-Ec genomic analysis identified three phylogroups (A, B1, and D) shared across all three sources; one (B2) SW-HR; none was shared by SW-CL. Of the 29 STs identified, ST10 and ST38 were shared across sources, whereas ST131, ST69, and ST75 were observed in both SW-HR isolates, and ST13823 was observed in both SW-CL isolates. Forty-five ARGs were identified: nine (*bla*$_{\text{CTXM-M-15}}$, *bla*$_{\text{TEM-1B}}$, *aadA5*, *qnrS*1, *mph(A), Sul*1, *Sul*2, *dfrA17*, *dfrA5*) core-shared, five (*bla*$_{\text{CTXM-M-27}}$, *bla*$_{\text{OXA-1,}}$ *OqxA/B*, *tetA/B*, d*frA1*) SW-HR and none shared by SW-CL. Among

the 285 distinct virulence genes identified, the highest proportion was detected in HR isolates (36.3%) and the lowest in CL isolates (29.7%) ($p < 0.05$). *E. coli* hybrid pathotypes (EPEC + UPEC + NMEC) predominated among HR and CL isolates, while pure pathotypes (EPEC) dominated in SW isolates. Phylogeny was ST-structured rather than source-structured, with high within-source clustering and a small subset showing closer SW-HR than SW-CL clustering.

ESBL-Kp prevalence varied significantly ($p < 0.05$) across sample sources, with the highest prevalence in CL (15.2%), followed by SW (13.0%) and HR (6.3%). MDR was highest in CL (94.6%) and lowest in SW (25.0%). SW isolates showed greater resistance similarities with HR isolates than with CL isolates, particularly across 3rd and 4th generation cephalosporins, gentamicin, and ciprofloxacin. Non-MDR pattern overlap was common to all sources, but MDR pattern overlap was limited to SW-HR. Genomic analysis of 96 ESBL-Kp isolates (32 per source) revealed 16 STs, of which ST983 was shared across all sources, whereas ST25 and ST219 were shared bySW-HR isolates and ST152 was unique to the SW-CL isolates.. Of the 79 ARGs detected, 27 were shared across all sources ($p < 0.05$). Twenty ARGs were observed in both HR-CL isolates, seven were observed in both SW-HR isolates, and two were observed in both SW-CL isolates. In addition, several ARGs were source-specific, with 14 unique to CL, nine unique to HR, and none to SW isolates. The SW-HR virulome was observed predominantly among isolates belonging to ST25/KL2 and ST219/KL114; a limited SW-CL link was observed through ST152/KL149, and source-restricted lineages were concentrated mainly in clinical and healthy resident isolates. Phylogenomic analysis revealed a lineage-driven rather than source-structured population, with SW isolates forming strongly supported subclades with HR isolates through ST25 and ST219, and 93.8% of SW isolates sharing STs with HR isolates compared to 9.4% with CL isolates.

Conclusion: Surface water did not fully reflect community and clinical ESBL-Ec and ESBL-Kp populations, but it did capture clinically relevant lineages and resistance determinants. These findings demonstrate the value of surface water as a complementary early-warning tool for detecting dominant community-linked ESBL-producing Enterobacterales and resistance determinants in poorly sewered informal settings while highlighting the need for integrated interpretation alongside clinical, community phenotypic and genomic data.

CHAPTER ONE - INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Antimicrobial resistance (AMR) is a major public health threat of the 21st century, occurring when microorganisms are unaffected by antimicrobial agents, making infections increasingly difficult to treat (Laxminarayan et al., 2013; WHO, 2024a). Although AMR can arise through natural evolutionary selection, inappropriate antimicrobial use across human, animal , and agricultural sectors has accelerated its emergence and spread (Iskandar et al., 2020). In 2021, bacterial AMR was associated with an estimated 4.71 million deaths globally, including 1.14 million deaths directly attributable to resistant infections, with low- and middle-income countries, particularly those in Sub-Saharan Africa, bearing a disproportionate burden (Naghavi et al., 2024). This reflects the combined effects of high infectious disease burdens, constrained health systems, inadequate sanitation, limited access to clean water, weak antimicrobial stewardship, poorly regulated antimicrobial use, and broader social and ecological drivers of transmission (Iskandar et al., 2020; Jonas et al., 2017; Klein et al., 2018; Sulis et al., 2022). Given this uneven burden, projected increases in AMR-attributable mortality by 2050, and continued constraints in antibacterial development, effective surveillance is essential for detecting, characterising, and responding to AMR at sufficient scale and speed (Naghavi et al., 2024; Theuretzbacher et al., 2019).

Surveillance is a central component of effective AMR containment, as it provides the operational link between AMR burden and public health response by generating data to monitor resistance trends, guide empiric treatment, support antimicrobial stewardship, and detect emerging threats (WHO, 2015). Globally, AMR surveillance remains largely clinical and laboratory-based, relying mainly on resistant organisms recovered from routine patient specimens such as blood, urine, stool, and cervical and urethral swabs and reported through

systems such as the WHO Global Antimicrobial Resistance Surveillance System (GLASS), which was launched in 2015 to standardise AMR data collection, analysis, and sharing (WHO, 2015). However, implementation remains uneven, particularly in LMICs, due to weak laboratory infrastructure, inadequate quality assurance systems, limited diagnostic capacity, shortage of trained personnel, and fragmented health information systems (Osena et al., 2025; Seale et al., 2017; Tornimbene et al., 2022). Even where clinical surveillance functions well, it captures only individuals who access healthcare and whose samples are collected, cultured, and reported, leaving asymptomatic carriers, underserved communities, and environmental reservoirs of resistant bacteria and resistance genes underrepresented (Lim et al., 2021; WHO, 2015). Consequently, conventional clinical and laboratory-based surveillance may underestimate the true AMR burden and provide limited insight into transmission pathways occurring beyond healthcare settings.

These limitations have strengthened the case for monitoring AMR through a One Health lens, which recognises the interconnectedness of human, animal, and environmental health systems and the movement of AMR across these interfaces (Robinson et al., 2016). Within this framework, the environment is increasingly viewed not only as a recipient of resistant organisms and antimicrobial residues, but also as a reservoir and transmission route where resistance genes can persist, accumulate, and move among microbial communities through horizontal gene transfer (HGT) (Bengtsson-Palme et al., 2018; Larsson & Flach, 2021). Environmental AMR surveillance therefore offers a complementary source of population-level AMR information, particularly in settings where clinical surveillance is constrained and where human waste, agricultural runoff, untreated wastewater, contaminated water, food systems, and direct environmental exposure may contribute to AMR circulation (Balcázar, 2025; Bengtsson-Palme et al., 2018; WHO, 2025).

Environmental AMR surveillance can target several interconnected matrices, including wastewater, surface water, sediments, soil, agricultural runoff, and animal waste, where resistant bacteria, resistance genes, and antimicrobial residues may persist or circulate across human, animal, and ecological systems (Bengtsson-Palme et al., 2018, 2023). Among these approaches, wastewater-based epidemiology (WBE) has gained particular attention because sewered wastewater integrates biological and chemical signals from contributing populations, enabling population-level monitoring of pathogens, antimicrobial resistance genes (ARGs), antimicrobial residues, and other biomarkers without individual sampling (Hendriksen et al., 2019; Karkman et al., 2018). Applied to AMR, sewage metagenomics has shown promise for characterising community-level resistomes and, in some settings, demonstrating associations with clinical resistance patterns (Pärnänen et al., 2019). This makes WBE a valuable complement to conventional clinical surveillance, especially where clinical data are incomplete or poorly representative (Hendriksen et al., 2019).

However, conventional WBE depends on sewered catchments and identifiable sampling points, such as utility holes, pump stations, or wastewater treatment plants (WWTPs), where community wastewater is sufficiently consolidated for surveillance (Hamilton et al., 2024; Mac Mahon et al., 2022). This dependency creates limitations for less-connected populations typical of lower-income settings, where sanitation may be decentralised, informal, or incomplete. Globally, even substantial expansion of WBE to more than 109,000 WWTPs across 129 countries would cover only an estimated 34.7% of the global population, leaving approximately five billion people outside centralised sewerage-based monitoring (Adhikari & Halden, 2022). This limitation is relevant to South Africa, where access to sanitation remains unequal despite progress. The 2024 General Household Survey (GHS) reported that 76.3% of South Africans

had safely managed sanitation, while 19.9% shared sanitation facilities nationally, rising to 31.9% among urban residents (Stats SA, 2024). In high-density informal settlements, shared sanitation and limited sewerage infrastructure weaken the catchment assumptions that underpin conventional WBE, as community waste may not converge into a single representative wastewater stream. These gaps strengthen the rationale for considering alternative environmental matrices, such as surface water, to complement clinical and sewer-based AMR surveillance in underserved settings that lack centralised sewerage infrastructure or have incomplete or poorly connected systems (Karkman et al., 2018; Berendonk et al., 2015).

Surface water is a promising surveillance matrix because rivers, streams, drainage channels, and wetlands can receive mixed inputs from households, informal settlements, agricultural runoff, stormwater, wastewater effluent, and healthcare-associated discharges, thereby accumulating microbial and resistance signals from multiple sources (Amoah et al., 2020; Morole et al., 2023). South African studies have shown that urban rivers and sediments are vulnerable to contamination from informal settlements and wastewater-related sources, including in the Kaalspruit River in Tembisa and eThekwini Municipality (Amoah et al., 2020; Morole et al., 2023). Resistant bacteria and resistance genes, including ESBL-producing Enterobacterales carrying clinically important determinants such as *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, and plasmid-mediated quinolone resistance genes, have also been reported in surface waters (Beshiru et al., 2024; Kotzamanidis et al., 2024). However, because surface water is hydrologically less controlled than sewered wastewater, its ability to reflect AMR circulating in surrounding communities and nearby healthcare settings remains insufficiently studied.

South Africa offers an important setting for evaluating whether surface water can complement conventional AMR surveillance, because it combines a formal national AMR response with

uneven implementation and marked infrastructural inequalities. Informal settlements are characterised by dense living conditions, inadequate infrastructure and basic services, and environmental health risks that may limit the representativeness of both clinical and sewer-based surveillance (Weimann et al., 2020; Weimann & Oni, 2019). These conditions create a need to evaluate whether surface water from informal-settlement catchments captures AMR signals that correspond to those observed in surrounding communities and nearby clinical settings.

To address this gap, the current study used extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) as sentinel organisms for cross-compartment AMR surveillance. Both species are clinically important Enterobacterales, among the leading bacterial pathogens implicated in urinary tract, bloodstream, and lower respiratory tract infections and are associated with AMR-related mortality globally (Murray et al., 2022). Their relevance is particularly clear in South Africa, where *K. pneumoniae* is among the most common bloodstream pathogens, ESBL-producing *K. pneumoniae* has remained highly prevalent, and *E. coli* is a major Gram-negative pathogen linked to bloodstream and community-associated urinary tract infections (National Department of Health, 2024). ESBL-producing isolates commonly harbour b-lactamase genes such as $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM}, which confer resistance to extended-spectrum cephalosporins and are often carried on mobile genetic elements (MGEs) alongside other resistance determinants, promoting multidrug-resistant phenotypes (Castanheira et al., 2021). Their clinical importance, mobility of resistance determinants, and detection in environmental waters, including clinically relevant ESBL-producing *E. coli* lineages such as ST131 and ST10, support their use as indicator organisms in evaluating whether surface water reflects AMR circulating in nearby human and clinical populations (Castanheira et al., 2021; Rahmat Ullah et al., 2024).

Against this background, the present study investigated the phenotypic and molecular epidemiology of ESBL-producing *E. coli* and *K. pneumoniae* across three interconnected sources in an informal settlement in South Africa: healthy community residents, surface water flowing through the settlement, and a proximate healthcare facility. Using culture-based phenotypic testing, whole-genome sequencing, and bioinformatics analysis, the study compared resistomes and virulomes and examined phylogenetic relationships among isolates from these sources. In doing so, it aimed to determine the extent to which surface water reflects AMR circulating in surrounding community and clinical populations, and to assess its potential as a context-appropriate environmental surveillance matrix in a resource-limited, non-sewered setting.

1.2 Literature Review

1.2.1 AMR Surveillance Systems and Their Limitations

The global escalation of AMR has resulted in an urgent need for surveillance systems capable of detecting, characterising, and monitoring resistant organisms across human, animal, and environmental compartments. This need is particularly important in low-resource settings, where the AMR burden may be substantial but diagnostic capacity, quality-assured laboratory systems, and integrated health information platforms are often limited (Iskandar et al., 2020). More so populations and environments that remain poorly represented in conventional clinical surveillance systems, particularly informal settlements where healthcare access and sanitation infrastructure may be constrained.

1.2.1.1 Overview and Rationale for AMR Surveillance

Effective containment of AMR depends on the capacity to detect, characterise, and monitor resistant organisms and their determinants across time and space. AMR surveillance serves this

function by systematically collecting, analysing, and interpreting data on resistant organisms and resistance patterns, thereby supporting the early detection of those of public health significance, guiding empiric treatment decisions, informing antimicrobial and diagnostic stewardship programmes, and strengthening the investigation and response to outbreak and emerging AMR threats (Diallo et al., 2020; WHO, 2024b). Globally, Diallo et al. (2020) conducted a mapping study of AMR surveillance systems, systematically identifying 71 surveillance systems across 35 countries and categorising them according to scope, sectoral coverage, and data sources. The majority of these systems were national in scope and focused primarily on human health, with limited integration of animal and environmental components. This heterogeneity in design, coverage, and reporting structures highlights a fragmented global AMR surveillance landscape with substantial variability in implementation across regions and sectors. Although this landscape reflects growing investment in AMR monitoring, it also illustrates a fragmented global surveillance response, with systems differing in scope, data sources, reporting structures, and sectoral coverage . Despite the apparent diversity of systems, the dominant paradigm remains clinical and laboratory-based surveillance anchored in healthcare settings, leaving important gaps in population-level, community-based, and environmental AMR monitoring (Bengtsson-Palme et al., 2023; van Leth & Schultsz, 2023).

1.2.1.2 Surveillance Approaches Used in AMR Monitoring

AMR surveillance systems can be operationalised through several overlapping approaches, including passive, active, case-based, syndromic, and sentinel surveillance designs (Murray & Cohen, 2017).

Passive surveillance relies on routinely generated data, usually from patients who seek care and have specimens collected for diagnostic purposes. It is relatively low-cost and can generate continuous data streams; however, it is constrained by inconsistent sampling, reporting,

variable data quality, selective specimen submission, and dependence on healthcare-seeking behaviour, which may underrepresent populations with limited access to healthcare (Bertagnolio et al., 2023; Lim et al., 2021; Murray & Cohen, 2017).

Active surveillance, by contrast, involves proactive case finding or deliberate data collection beyond routine reporting and can generate more complete metadata; however, it is resource-intensive and difficult to sustain at scale, particularly in resource-limited settings (Murray & Cohen, 2017).

Case-based AMR surveillance links microbiological results to patient-level clinical and epidemiological data, often using defined infectious syndromes or clinical conditions as the unit of surveillance rather than laboratory isolates alone. This improves the clinical interpretability of AMR data and can better inform empiric treatment guidelines, but it requires strong clinical documentation, laboratory capacity, and data linkage systems (Lim et al., 2021; Ryu et al., 2019).

Syndromic surveillance identifies suspected infections or unusual patterns based on signs and symptoms, often before laboratory confirmation, and may support early warning for healthcare-associated infections (HAIs) in resource-limited settings, although it is less specific than laboratory-confirmed surveillance (Mwanja et al., 2024).

Sentinel surveillance restricts activities to selected facilities, laboratories, or populations to generate useful trend data where universal coverage is impractical. Although this approach is common in AMR surveillance systems in LMICs because it reduces resource demands, its value depends critically on the representativeness, diagnostic capacity, and data quality of selected sentinel sites (Stone & Horney, 2017).

Sentinel surveillance contrasts with population-based surveillance, which aims to estimate AMR burden within a defined population or catchment by linking microbiological results to patient-level data and appropriate denominator information. Compared with sentinel surveillance, population-based surveillance can provide more robust estimates of AMR incidence and burden, and greater representativeness because it is anchored to a defined population denominator. However, it requires reliable healthcare access, routine microbiology testing, linked clinical and laboratory data, robust health information systems, and accurate population denominators. These requirements are often difficult to meet in LMICs, where underuse of microbiology, selective specimen collection, limited laboratory capacity, weak data systems, and uneven healthcare utilisation can bias facility-based AMR estimates. Consequently, sentinel surveillance is often more feasible, but it may offer only a partial view of AMR circulation if selected sites do not adequately represent underserved communities (Lim et al., 2021; Murray & Cohen, 2017).

1.2.1.3 Laboratory-based AMR Surveillance

Underpinning many AMR surveillance approaches is laboratory-based detection, i.e., the process by which microorganisms are isolated and identified from clinical specimens and their antimicrobial susceptibility is characterised. The WHO launched the Global Antimicrobial Resistance and Use Surveillance System (GLASS) in 2015 to provide harmonised standards for AMR surveillance and global reporting. GLASS is built around microbiology data generated from routinely collected clinical specimens and, where possible, linked to patient information, recognising that laboratories provide the primary evidence through which resistant bacterial pathogens are identified and their susceptibility profiles systematically assessed (Lim et al., 2021; WHO, 2015).

1.2.1.4 Culture-based Methods: Bacterial Isolation and Phenotypic AST

At the core of laboratory-based AMR surveillance is culture-based bacterial detection and phenotypic antimicrobial susceptibility testing (AST). Conventional laboratory surveillance relies on clinical specimens such as blood, urine, sputum, wound swabs, and stool samples being processed for microbial isolation, identification, and susceptibility testing (Bayot & Bragg, 2026; WHO, 2023). Once a bacterial isolate is identified, AST determines which antimicrobials inhibit its growth, generating results that are directly actionable for patient management and, when aggregated, can inform population-level resistance trends (Bayot & Bragg, 2026; Gajic et al., 2022). The principal phenotypic AST methods include disk diffusion, in which antimicrobial-impregnated discs are placed on agar inoculated with the test organism and inhibition zones are interpreted against standardised breakpoints, and minimum inhibitory concentration (MIC) determination using agar dilution or broth microdilution or testing, which determines the lowest antimicrobial concentration required to inhibit visible bacterial growth (EUCAST, 2025; Gajic et al., 2022). These methods are interpreted using standards developed by bodies such as the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), forming the basis on which resistance phenotypes are defined, categorised, and reported for clinical care and surveillance (CLSI, 2020; EUCAST, 2020).

For ESBL detection specifically, phenotypic screening and confirmatory methods remain widely used in routine laboratory practice. Screening is typically based on reduced susceptibility to extended-spectrum cephalosporins and related indicator agents, including cefotaxime, ceftazidime, ceftriaxone, cefpodoxime, and aztreonam, while confirmation is commonly achieved using combination disc or double disc synergy tests that demonstrate clavulanic acid-mediated inhibition of ESBL activity (CLSI, 2020; Drieux et al., 2008; Jacoby

et al., 2006). These methods provide an essential first step in characterising ESBL-producing isolates before downstream molecular analysis, generating the resistance phenotype against which genotypic findings can subsequently be interpreted (Lob et al., 2016).

Culture-based methods retain important advantages as the foundation of AMR surveillance because they generate viable isolates for further phenotypic and genotypic characterisation, define the expressed resistance phenotype under standardised laboratory conditions, and remain the basis for internationally standardised reporting systems such as GLASS (WHO, 2020, 2023). In the current study, culture-based isolation and phenotypic AST profiling therefore served as the first phase of cross-source characterisation, providing phenotypically confirmed ESBL-producing isolates from community, surface water, and clinical sources for subsequent WGS and comparative genomic analysis.

1.2.1.5 Automated Phenotypic AST Systems and Limitations of Culture-Based Surveillance

Culture-based phenotypic surveillance has important limitations that constrain its utility as a comprehensive surveillance tool. Conventional culture-based workflows often require 48 to 72 hours from specimen receipt to final susceptibility results, delaying clinical decision-making and epidemiological reporting (Gajic et al., 2022). Some resistance phenomena, including low-level resistance, hetero-resistance, and resistance mediated by conditionally regulated genes, may be difficult to detect reliably using routine disk diffusion or MIC-based testing alone, even though they may influence treatment outcomes (Gajic et al., 2022). Inconsistency in result interpretation across laboratories, together with variation in adherence to breakpoint standards or differences between CLSI and EUCAST criteria, can further affect the comparability of aggregate surveillance data (CLSI, 2020; EUCAST, 2020; van der Bij et al., 2012). In response to some conventional culture-based limitations, many laboratories have adopted automated

AST platforms, including the VITEK® family of systems (bioMérieux), Phoenix™ (BD), and Sensititre™ (Thermo Fisher Scientific) to improve throughput, standardisation, and turnaround time (Gajic et al., 2022). While these systems can improve reproducibility and efficiency in well-resourced settings, their implementation in many LMIC laboratories remains constrained by cost, maintenance requirements, technical expertise, quality assurance needs, and reagent supply chains (Sharma et al., 2022).

1.2.1.6 Molecular Approaches for AMR Surveillance

The limitations of phenotypic detection become especially significant in epidemiological surveillance aimed at understanding AMR transmission dynamics across human and environmental compartments. Phenotypic profiles alone cannot establish whether resistant organisms from different sources are genetically related, whether resistance is spreading through clonal expansion or HGT, or which specific resistance determinants are involved (Zankari et al., 2013). Two isolates may share an identical resistance phenotype while being phylogenetically unrelated, reflecting convergent resistance acquisition rather than direct transmission, or may carry similar resistance determinants on different genomic backgrounds. Distinguishing between these scenarios requires genomic characterisation capable of resolving strain relatedness, identifying resistance genes and MGEs, and differentiating clonal spread from HGT (Djordjevic et al., 2024; Köser et al., 2014; Lerminiaux & Cameron, 2019).

Molecular surveillance techniques have been developed to address some of these limitations and are increasingly integrated into AMR surveillance systems to complement phenotypic AST. Polymerase Chain Reaction (PCR)-based methods allow rapid and specific detection of defined resistance genes, including beta-lactamase gene families such as *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{NDM}}$, and *bla*$_{\text{OXA}}$, as well as non-beta-lactamase resistance determinants (Anjum et

al., 2017). Multiplex PCR platforms enable simultaneous detection of multiple resistance determinants in a single reaction, thereby improving throughput and reducing the need for multiple single-target assays (Anjum et al., 2017). However, although PCR-based methods can provide information relevant to the detection of specific resistance determinants and, in some contexts, epidemiological relatedness, they offer lower resolution than WGS and are less able to resolve fine-scale phylogenetic relationships or closely related transmission events (WHO, 2020; European Centre for Disease Prevention and Control, 2016).

Whole genome sequencing (WGS) has emerged as one of the most comprehensive and discriminatory tools for AMR surveillance and epidemiological investigation (Yamin et al., 2023). By capturing the complete or near-complete genomic sequence of bacterial isolates, WGS enables simultaneous characterisation of the resistome, including resistance genes and mutations, the virulome, the MGSs associated with HGT, and the phylogenetic structure of isolate populations (Mbelle et al., 2019; WHO, 2020). WGS-derived MLST allows the identification of STs and clonal lineages with epidemiological significance, while comparative genomic analysis can infer whether isolates recovered from different sources are closely related or share recent common ancestry. This capability is essential for cross-compartment AMR surveillance studies such as the present investigation, which aimed to compare resistant organisms across community, clinical, and environmental sources. The application of WGS and associated bioinformatic tools in this study is addressed in detail in Section 1.1.7.

1.2.1.7 Implementation Gaps in AMR Surveillance Methods

Despite the expanding toolkit of AMR surveillance methods, a fundamental gap persists between methodological capability and implementation capacity. Some of the most informative approaches, including case-based or syndromic surveillance with comprehensive clinical data

linkage, automated phenotypic AST, and WGS-based molecular epidemiology, also require substantial laboratory infrastructure, technical expertise, data systems, quality assurance, and sustained financial investment (Lim et al., 2021). As the following section examines, this gap is especially acute in LMICs, where high AMR burden intersects with weak laboratory capacity, limited health information systems, constrained technical expertise, and insufficient resources, producing some of the most consequential shortfalls in the global AMR surveillance landscape (Gandra et al., 2020; Iskandar et al., 2021).

1.2.2 AMR Surveillance Gaps in LMICs and Resource-Limited Settings

According to the World Bank, LMICs include low-income, lower-middle-income, and upper-middle-income economies, classified annually using gross national income per capita thresholds. Middle-income countries alone account for a large share of the global population and economic activity, but poverty and service inequities remain substantial across many LMIC settings (The World Bank Group, 2024). Although LMICs are highly heterogeneous, many experience overlapping pressures that amplify AMR, including high infectious disease burden, inadequate water and sanitation infrastructure, limited access to quality healthcare, weak antimicrobial regulation, and antimicrobial use across human, animal, and agricultural sectors (Iskandar et al., 2020; Otaigbe & Elikwu, 2023).

1.2.2.1 The AMR Burden and Surveillance Capacity Paradox

The global AMR surveillance architecture is built on a profoundly unequal foundation. Settings with some of the highest burdens of resistant infection are often those with the weakest capacity to detect, characterise, and respond to AMR. This inverse relationship between AMR burden and surveillance capacity reflects broader structural and health-system constraints that also contribute to the emergence and persistence of resistance (Gandra et al., 2020; Murray et al.,

2022). As a result, AMR data from many LMICs remain sparse, fragmented, and unevenly representative, creating a self-reinforcing cycle in which limited evidence undermines the policy responses and investments needed to generate better surveillance data (Gandra et al., 2020).

1.2.2.2 Laboratory Infrastructure and Workforce Gaps

The structural determinants of this surveillance gap are well documented. Weak laboratory infrastructure remains one of the most pervasive constraints, encompassing inadequate physical facilities, unreliable power supply, insufficient cold chain capacity, limited diagnostic equipment, and inconsistent access to quality-assured consumables. In rural Rwanda, for example, Umutesi et al. (2021) found major equipment and supply gaps at district hospitals, with an eight-month delay between laboratory needs assessment and sample collection because essential supplies and equipment were not regionally available. Establishing essential microbiology capacity for three district hospitals required US$245,871, while the projected cost of processing 600 samples ranged from US$29,500 for manual testing to more than US$92,000 using an automated VITEK 2-based model . Similar capacity constraints have been reported in Zambia, where laboratory-based AMR surveillance assessments found that selected hospitals lacked full capacity to conduct AMR surveillance, underscoring persistent gaps in diagnostic infrastructure, quality management, and surveillance readiness (Yamba et al., 2024). South African evidence indicates that these constraints are not confined to lower-resource African settings. Despite comparatively stronger laboratory-linked surveillance infrastructure, surveillance capacity remains uneven across healthcare institutions. Singh-Moodley et al. (2018) noted that surveillance programme quality varies across institutions and that standardised surveillance systems are absent in most healthcare facilities, due to challenges with data systems, infrastructure, and trained personnel. Similarly, Dramowski et al. (2016)

found that available resources and local practice context influenced surveillance approaches in a South African paediatric hospital. These infrastructure deficits are compounded by shortages of trained personnel, including microbiologists, laboratory technicians, and data analysts with the skills required to perform standardised AST, interpret results correctly, and manage surveillance data systems. In Cambodia, Mao et al. (2023) identified workforce capacity, laboratory quality systems, data management, coordination, and sustainable financing as key barriers to implementing a national laboratory-based AMR surveillance system. Where trained personnel are available, staff turnover, inadequate continuing education, weak quality management systems, and limited participation in external quality assurance programmes can further erode the consistency and reliability of laboratory outputs. Evidence from Pakistan illustrates the depth of these deficits. A self-assessment across 30 microbiology laboratories in three cities found scores at or below 60% for readiness for AMR surveillance, quality assurance, and detection of specific organisms in both public and private laboratories, even in relatively urbanised settings with nominally functional laboratory systems (Saeed et al., 2017).

1.2.2.3 AMR Data Management Gaps

Data management represents a distinct but equally significant dimension of the AMR surveillance gap. In many LMIC settings, AMR data are generated across multiple disconnected sources, including hospital laboratories, private diagnostic facilities, national reference laboratories, hospital and community pharmacies, and national antimicrobial consumption datasets, but are often recorded in non-interoperable formats, stored in paper-based or fragmented systems, and insufficiently consolidated for national analysis and policy use (Africa CDC, 2023; Iskandar et al., 2021). This problem is particularly consequential in sub-Saharan Africa, where AMR-attributable mortality rates are among the highest globally (Murray et al., 2022). Yet, the systems required to generate complete, linked, and policy-

relevant surveillance data remain weak. The Mapping Antimicrobial Resistance and Antimicrobial Use Partnership (MAAP) initiative highlights the magnitude of this challenge in Africa. The project retrospectively assembled AMR data from 205 laboratories across 14 countries, alongside antimicrobial consumption data from 327 hospital and community pharmacies and 16 national-level datasets. Despite this substantial effort, clinical and treatment data were rarely linked to laboratory findings, underscoring persistent gaps in integrated surveillance systems and constraining the interpretation of AMR trends and drivers (Africa CDC, 2023). The same initiative identified a consistent cluster of barriers to effective surveillance, including limited access to advanced bacteriology testing, weak capacity to isolate and correctly identify resistant organisms, missing patient clinical profile information such as diagnosis and infection origin, and limited antimicrobial use data needed to contextualise resistance patterns against prescribing and consumption trends (Africa CDC, 2023). In the peer-reviewed MAAP analysis, only 12% of AMR records were linked to patient clinical information, and inadequate digital infrastructure for data collection and reporting was identified as a major barrier to estimating the true AMR burden in the region (Osena et al., 2025). The consequence is data that are simultaneously abundant and analytically constrained: scattered across institutions, inconsistently formatted, weakly linked to patient and treatment information, and insufficiently rich to support the integrated epidemiological inference required for effective AMR policy.

1.2.2.4 WHO GLASS Implementation Gaps

The WHO GLASS framework, launched in 2015 to standardise global AMR surveillance and create a coherent international dataset, has improved the architecture for global AMR reporting but has not resolved the structural barriers that limit surveillance implementation in many LMICs. GLASS encourages countries to combine microbiological, patient, and

epidemiological surveillance data to maximise the interpretability and public health utility of AMR surveillance outputs (WHO, 2015). In practice, however, implementation across LMICs remains uneven. A comparative review of eight LMICs in South and Southeast Asia (Bangladesh, India, Indonesia, Nepal, Pakistan, Thailand, Vietnam, and Cambodia) found variable progress but persistent challenges related to limited financial resources, technical capacity, laboratory readiness, data systems, and adherence to GLASS reporting requirements (Gandra et al., 2020). Reporting completeness also varied substantially: among the countries that submitted data to WHO GLASS for 2016 to 2017, only Thailand reported AMR rates for all requested specimens, pathogens, and antibiotics (Gandra et al., 2020). Even in Thailand, with comparatively stronger laboratory and health system capacity than many LMICs, implementation required pragmatic compromises. At Siriraj Hospital in Bangkok, Thailand, GLASS implementation relied on a locally developed web application to link microbiological and clinical data. Yet, the authors noted that the workload might require activating GLASS for only one six-month period every other year, a compromise that would limit temporal completeness and epidemiological utility (Sirijatuphat et al., 2018).

South Africa has participated in GLASS through the National Institute for Communicable Diseases (NICD), whose Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses (CHARM) functions as both the WHO Collaborating Centre for AMR and the national coordinating centre specifically for WHO GLASS. During the early implementation period, South Africa was among twelve African countries that submitted AMR data across the three annual GLASS data calls between 2017 and 2019, reflecting meaningful progress in aligning national surveillance with global reporting systems (Tornimbene et al., 2022b). Nationally, AMR surveillance has been strengthened through collaboration between public and private laboratory services and incorporates laboratory-based surveillance from

sentinel sites alongside electronic surveillance drawing on the NHLS Laboratory Information System and data from major private laboratory providers (NDoH, 2024). However, significant gaps remain. The surveillance system focuses primarily on bloodstream isolates from the *Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species (ESKAPE) pathogen group, an approach that, while comprehensive within its scope, may not fully capture the broader burden of AMR across specimen types and care settings. The March 2024 national AMR surveillance report, which covers data from 2018 to 2022, documents several system-level limitations in its annexure on the existing surveillance framework, including non-standardised specimen collection practices, restricted access to microbiology services in some facilities, widespread reliance on empirical treatment without microbiological confirmation, variation in testing and data-capture methods across laboratories, and incomplete data resulting from missing cases or inconsistent diagnostic coding (NDoH, 2024). Therefore, while South Africa has made substantial progress in GLASS-aligned AMR surveillance, the current system retains important limitations regarding national representativeness, clinical linkage, and community-level coverage.

These examples suggest that GLASS participation alone does not guarantee complete, continuous, or nationally representative AMR surveillance, particularly where laboratory infrastructure, human resources, data linkage, and financing remain constrained (NDoH, 2024).

1.2.2.5 Surveillance Blind Spots in Informal and Underserved Communities

Beyond the infrastructure and data management challenges that characterise LMICs broadly, a further dimension of the surveillance gap concerns the underrepresentation of specific population subgroups within routine AMR surveillance systems. Conventional clinical AMR surveillance captures resistance mainly among individuals who access formal healthcare, have

a specimen collected, and have that specimen processed through a functioning laboratory linked to a reporting system. This cascade of requirements means that populations with limited healthcare access, who rely on informal providers, or who reside in underserved environments, may be poorly represented in facility-based AMR data. This concern is especially relevant for urban informal settlements, where healthcare seeking is shaped by geographic, financial, social, and structural barriers, and where poor service access may reduce the likelihood that infections are diagnosed, cultured, and reported through formal surveillance systems (Vicar et al., 2023; Weimann & Oni, 2019; Zerbo et al., 2020).

Urban informal settlements are particularly important in AMR epidemiology because they combine high exposure risk with weak surveillance visibility. Nadimpalli et al. (2020) described these settlements as potential hotspots for environmental AMR transmission, citing the proximity of humans, livestock, and diverse pests, frequent antibiotic misuse, and insufficient drinking water, drainage, and sanitation infrastructure as conditions that can facilitate AMR circulation. These conditions are not merely theoretical. Household-level research from urban informal settlements in Northern Ghana showed that antibiotic use is shaped by everyday access constraints and household decision-making, reinforcing the concern that antimicrobial exposure and selection pressure may occur outside formal diagnostic and stewardship systems (Vicar et al., 2023). In South Africa, the relevance of this concern is reinforced by persistent service inequities. Stats SA reported that 76.3% of South Africans had access to safely managed sanitation in 2024, leaving nearly one in four still without it (Stats SA, 2025).

The above evidence suggests that informal settlements may be important sites of community-level AMR carriage and transmission, while remaining underrepresented in routine clinical

surveillance. Residents are not necessarily absent from hospital laboratory databases, national AMR reports, or GLASS submissions, but they are less likely to be fully represented. This population-level surveillance blind spot has direct implications for countries with large informal settlement populations, including South Africa. If community AMR estimates are derived mainly from clinical isolates collected at healthcare facilities, they may be geographically, socioeconomically, and clinically unrepresentative of the wider population and may underestimate the community reservoir of resistance from which clinical infections can emerge (Ashley et al., 2019).

These gaps show that populations most affected by AMR are often least visible within current surveillance systems. Complementary One Health and environmental approaches, particularly surface water surveillance, may therefore help capture community-level AMR where conventional surveillance is weakest.

1.2.3 One Health and Environmental AMR Surveillance

1.2.3.1 The One Health Basis for AMR Surveillance

The recognition that conventional clinical or laboratory-based surveillance cannot capture the full scope of AMR transmission has driven a shift toward One Health approaches. One Health is an integrative approach that recognises that human, animal, plant, and ecosystem health are interconnected, and that threats operating across these interfaces cannot be fully understood or controlled through single-sector surveillance alone (Robinson et al., 2016; Zinsstag et al., 2011). AMR is widely regarded as a paradigmatic One Health challenge because resistant bacteria and the MGEs that carry resistance determinants circulate across human, animal, agricultural, and environmental compartments (Robinson et al., 2016). This multisectoral framing has been advanced internationally through WHO, FAO, and OIE/WOAH collaboration on AMR, including the Global Action Plan monitoring framework and

subsequent One Health strategic frameworks (WHO/FAO/OIE, 2019; WHO/FAO/OIE/UNEP, 2022). Environmental pathways are central within this framework. Resistant bacteria and resistance genes can move through human and animal waste, wastewater discharge, agricultural runoff, food systems, water bodies, and direct contact, creating transmission routes that clinical surveillance alone is not designed to capture comprehensively (Berendonk et al., 2015).

1.2.3.2 The Environment as an AMR Reservoir and Surveillance Interface

Within the One Health framework, the environment is increasingly recognised as a central component of AMR dynamics rather than merely a passive pathway between human and animal hosts. Environmental compartments such as wastewater systems, surface waters, sediments, soils, agricultural environments, and their associated microbial communities can act as reservoirs and transmission interfaces for resistant bacteria, antimicrobial residues, and resistance genes (Bengtsson-Palme et al., 2023; Berendonk et al., 2015; Larsson & Flach, 2022). These environments may also provide ecological conditions that support persistence, selection, and horizontal gene transfer, allowing resistance determinants to circulate across diverse bacterial populations before re-entering human and animal populations through water, food, waste, and direct environmental contact (Berendonk et al., 2015; Larsson & Flach, 2022).

Resistance enters environmental compartments through multiple interacting pathways. Human and animal faecal waste can introduce resistant bacteria and resistance genes into sewage systems, septic tanks, open drains, soils, and surface waters, with faecal pollution shown to be a major predictor of environmental ARG abundance (Karkman et al., 2019). Agricultural practices, including manure application, can introduce resistant bacteria, resistance genes, and antimicrobial residues into soils, from where they may be mobilised through runoff, irrigation, or leaching into aquatic systems (Chee-Sanford et al., 2009). Wastewater treatment plants are

particularly important convergence points because they receive domestic, healthcare, and industrial inputs and contain dense microbial communities exposed to antimicrobial residues and other selective agents. Treated effluent may still release residual resistant bacteria and ARGs into receiving water bodies (Gumede et al., 2021; Uluseker et al., 2021).

In practice, environmental AMR surveillance has therefore been applied across several matrices, including wastewater, WWTP influent and effluent, surface water, sediments, soils, and agricultural environments. Wastewater has received the greatest attention because it aggregates human and industrial waste streams and can often be sampled at defined points, whereas surface waters such as rivers, streams, lakes, and drainage channels receive more diffuse inputs from wastewater effluent, agricultural runoff, stormwater, and direct faecal contamination, making them useful but hydrologically complex matrices for AMR monitoring (Kusi et al., 2022; Liguori et al., 2022). South African evidence further supports the relevance of environmental surveillance, with ARGs reported across clinical, environmental, and farm settings (Ekwanzala et al., 2018). Thus, different matrices capture different components of the AMR landscape and may complement clinical surveillance in specific epidemiological contexts.

The epidemiological relevance of environmental AMR surveillance is supported by studies showing that resistant organisms and clinically important resistance determinants can be detected in environmental waters. In Greece, ESBL-producing *E. coli* recovered from river water included ST131 O25 H30 isolates genetically linked to human clinical strains, while a Swedish study found that environmental ESBL-producing *E. coli* ST131 O25 H30 isolates were as closely related to clinical isolates as clinical isolates were to one another (Fagerström et al., 2019; Kotzamanidis et al., 2024). In sub-Saharan Africa, available studies are less

phylogenetically resolved but still demonstrate the presence of clinically important ESBL determinants in aquatic environments, including *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM}}$, and plasmid-mediated quinolone resistance genes in Nigerian surface waters and *bla*$_{\text{CTX-M-15}}$-producing Enterobacterales around Lake Victoria, Tanzania (Moremi et al., 2016). These findings support environmental surveillance as a complement to clinical monitoring for detecting reservoirs, transmission pathways, and resistant lineages that facility-based systems may not fully capture.

The critical question, however, is not simply whether environmental matrices harbour resistance, but whether they can generate interpretable and population-relevant information about AMR circulating in surrounding human populations (Larsson et al., 2018). This question is especially important in resource-limited and informal-settlement contexts, where clinical surveillance may be incomplete, sanitation-related environmental contamination may be high, and alternative surveillance strategies are urgently needed (Gholipour et al., 2023). Among environmental surveillance approaches, WBE has received the strongest attention because it can generate population-level signals from aggregated wastewater (Balcázar, 2025; Gholipour et al., 2023). This makes WBE the logical starting point for assessing environmental AMR surveillance, while also requiring careful consideration of its dependence on centralised sanitation infrastructure (Balcázar, 2025; Gholipour et al., 2023).

1.2.4 Wastewater-Based Epidemiology (WBE) and Its Infrastructure Dependency

1.2.4.1 Conceptual Basis and AMR Applications of WBE

Among environmental surveillance approaches within the One Health framework, WBE has attracted substantial and sustained research attention. The central premise of WBE is that wastewater flowing through a sewerage system contains biological, chemical, and

pharmacological signals excreted by the population it serves, creating a composite sample that can reflect health status, substance use, pathogen circulation, and microbial exposures at the catchment level without individual-level sampling (Choi et al., 2018; Lorenzo & Picó, 2019). By analysing wastewater at defined points in the sewerage network, particularly WWTP influent, WBE enables non-invasive population-scale surveillance and can capture signals from individuals who may be missed by clinical monitoring, including asymptomatic, oligosymptomatic, or untested individuals, provided their waste enters the sampled catchment (Bivins et al., 2020; Lorenzo & Picó, 2019). Historically, wastewater analysis was first applied to community-level monitoring of illicit drug use (Zuccato et al., 2005, 2008) and later extended to pathogen surveillance, including poliovirus and SARS-CoV-2, where wastewater monitoring has demonstrated value for early warning and trend tracking (Peccia et al., 2020; Zuccato et al., 2005).

Applied to AMR, WBE is based on the premise that resistant bacteria, resistance genes, and antimicrobial residues shed by individuals and communities within a sewered catchment can be detected in wastewater, providing an aggregate signal of resistance circulating in the contributing population (Hendriksen et al., 2019; Karkman et al., 2018). Metagenomic analysis of wastewater, which sequences total DNA extracted from a sample, enables culture-independent characterisation of the wastewater resistome, including the diversity and abundance of AMR genes present in the catchment, without requiring isolation of individual organisms (Hendriksen et al., 2019). In a landmark global study, Hendriksen et al. analysed untreated sewage from 79 sites in 60 countries and showed that wastewater metagenomics could map geographic variations in AMR gene abundance and diversity, supporting its use as a scalable approach for international AMR monitoring. Subsequent work by Karkman et al. (2020) showed that sewage resistome profiles correlated with clinical surveillance data for

invasive *E. coli*, although the authors cautioned that sewage metagenomics alone had limited resolution for distinguishing resistance patterns across specific antibiotic classes (Karkman et al., 2020). Alongside metagenomics, culture-based wastewater studies add an important isolate-level dimension by recovering viable resistant organisms for phenotypic testing, gene detection, sequence typing, and comparative genomic analysis. For example, Gómez-Sanz et al. (2023) used municipal wastewater to investigate the spatiotemporal dissemination of ESBL-producing Enterobacterales from community and hospital sources, while culture-based works in WWTP influent and effluent have been used to recover ESBL- and carbapenemase-producing organisms, including clinically relevant *E. coli* and *K. pneumoniae* lineages and carbapenemase genes (Gómez-Sanz et al., 2023; Gumede et al., 2021; Mbanga et al., 2021). Thus, metagenomic and culture approaches for WBE provide complementary information, whereby metagenomics offers broad resistome-level surveillance, whereas culture-based approaches confirm the presence of viable, phenotypically resistant organisms that can be further characterised genomically. This complementarity makes wastewater an information-rich AMR surveillance matrix where sewerage infrastructure is available.

1.2.4.2 WBE as a Population-Scale Surveillance Platform in Sewered Settings

The growing momentum behind WBE is most visible in high-income settings where wastewater surveillance has been incorporated into public health infrastructure, particularly during and after the SARS-CoV-2 pandemic. In the United States, the National Wastewater Surveillance System expanded from 209 sampling sites in September 2020 to more than 1,500 sites by December 2022, covering approximately 47% of the national population and demonstrating how sewered wastewater catchments can be scaled into a national surveillance platform (Adams et al., 2024). Similar experiences in Europe and Australia have reinforced the value of wastewater surveillance as a complement to clinical monitoring for tracking pathogen

circulation and population-level trends. For AMR, this infrastructure provides an opportunity to monitor aggregate resistance signals in sewered populations using metagenomic or culture-based approaches. However, the same feature that gives WBE its strength also defines its central limitation. Its population-integrating logic depends on sewerage networks that convey waste from households, healthcare facilities, and other sources into common sampling points. Where such infrastructure exists, WWTP influent can provide a composite signal from large catchment populations; where it is absent, incomplete, or poorly connected, there may be no single matrix that consolidates community waste outputs, no clearly defined sampling point, and no representative sewered catchment signal to analyse (Adhikari & Halden, 2022; Shrestha et al., 2021).

1.2.4.3 WBE Infrastructure Dependency and Exclusion of Non-Sewered Populations

The most recent WHO/UNICEF Joint Monitoring Programme update estimated that in 2024, 3.4 billion people, or 42% of the global population, still lacked safely managed sanitation, underscoring the unevenness of sanitation systems that WBE would need to rely on for representative coverage (WHO/UNICEF, 2025). Importantly, safely managed sanitation does not necessarily imply connection to a sewered wastewater network, as excreta may also be safely treated on-site or transported from non-sewered systems. In many resource-limited and informal settlement contexts, human waste is instead managed through pit latrines, septic tanks, shared sanitation, open defecation, or informal drainage systems, producing waste streams that are spatially variable and often disconnected from centralised collection and treatment infrastructure (Adhikari & Halden, 2022; Holm et al., 2023; Pocock et al., 2023). Under these conditions, wastewater is not consolidated into a single representative sewered catchment but is dispersed across soils, open drains, stormwater channels, and surface water bodies. This

weakens the core sampling logic of conventional WBE and limits its transferability to non-sewered, resource-limited settings (Adhikari & Halden, 2022).

The consequence is the systematic underrepresentation of some of the world's most AMR-vulnerable populations in conventional sewer-based WBE. Communities where resistance is often intensified by poor service delivery are often the same communities where wastewater is not collected through centralised sewerage networks. In such settings, the population-integrating logic of WBE is weakened because human waste is dispersed through means other than being consolidated at a defined sewer sampling point. Recent guidance therefore frames wastewater and environmental surveillance as using sewage when sewer networks are present, but other human-impacted environmental waters where sewer networks are absent, reinforcing the need for alternative surveillance matrices in non-sewered settings (Adhikari & Halden, 2022; Holm et al., 2023). This identifies the surveillance gap that the present study addresses: if conventional WBE cannot adequately represent non-sewered informal settlements, which environmental matrix can serve as its functional equivalent for capturing population-level AMR?

1.2.5 Surface Water as an Alternative Surveillance Matrix in Non-Sewered Settings

1.2.5.1 Conceptual Basis for Surface Water Surveillance in Non-Sewered Settings

Surface water refers to water that occurs on or flows over the Earth's surface, including inland water bodies such as rivers, streams, lakes, ponds, reservoirs, wetlands, bogs, and drainage channels. In water-supply and hydrological contexts, it is commonly distinguished from groundwater because it is exposed to the atmosphere and is directly influenced by runoff, catchment processes, and surrounding land use (Verma et al., 2022). Unlike wastewater, which depends on centralised sewerage infrastructure and therefore mainly represents sewered

populations, surface water is widely present in both sewered and non-sewered environments. In resource-limited settings where sewerage systems are absent or incomplete, surface waters may receive household greywater, faecal-contaminated runoff, overflow or leakage from pit latrines and septic systems, agricultural drainage, stormwater, and untreated or partially treated discharges from healthcare, residential, and informal activities (Edokpayi et al., 2017; Masoner et al., 2019; Ramaite et al., 2022). This makes surface water a potentially useful population-receiving matrix for AMR surveillance, partly analogous to wastewater in sewered systems, although more diffuse and hydrologically complex.This conceptual basis is consistent with Liguori et al. (2022), who suggested that AMR monitoring in water environments can help assess the circulation of resistance genes among human populations, identify dissemination hotspots, and inform epidemiological risk assessment. It is also supported by Bengtsson-Palme et al. (2023), who emphasised the need for environmental AMR monitoring to understand how resistance persists, circulates, and re-enters human and animal populations. This means that if rivers, streams, drainage channels, and other open water bodies receive microbes and resistance genes from surrounding communities, healthcare facilities, agricultural activities, and sanitation-related discharges, then their resistome may contain epidemiologically meaningful information about AMR circulating in the contributing population. However, this proposition depends on two conditions: first, that surface water accumulates AMR signals from nearby human, healthcare-associated, animal, and environmental sources; and second, that these signals correspond sufficiently to AMR patterns in the surrounding community and clinical setting to support its use as a proxy matrix (Karkman et al., 2020). Existing literature supports the first condition (Nnadozie & Odume, 2019), while the second remains less well established (Larsson et al., 2018), particularly in informal settlements where few studies have directly compared surface water isolates with matched human community and clinical isolates using phenotypic and genomic approaches.

1.2.5.2 Surface Waters as Reservoirs of Clinically Relevant AMR

Evidence that surface waters accumulate clinically relevant AMR is now substantial and geographically diverse. A review of freshwater environments reported that ESBL-producing Enterobacterales, particularly *E. coli* and *K. pneumoniae,* are increasingly detected in aquatic systems worldwide, with surface waters affected by domestic, agricultural, and industrial inputs serving as important reservoirs and potential transmission routes (Cho et al., 2023). This pattern is evident across African settings. In Ghana, the implementation of the WHO Tricycle protocol in rivers in Accra and Kasoa showed high levels of *E. coli* and ESBL *E. coli*, with counts increasing as rivers passed through urban areas, indicating contamination from human and animal wastewater (Banu et al., 2021). In Nigeria, Beshiru et al. (2024) detected ESBL-producing *E. coli* in 58.3% of spring water samples and 33.3% of dam water samples; all ESBL-producing isolates were resistant to cefotaxime, ceftriaxone, ceftazidime, and ampicillin, and MDR profiles spanned three to six antimicrobial classes (Beshiru et al., 2024). In Tanzania, environmental sampling along the Msimbazi River basin recovered *K. pneumoniae* and *E. coli* from effluent, river water, sediment, and crop soil, with more than half of the *E. coli* and *K. pneumoniae* isolates classified as multidrug-resistant (Kimera et al., 2021). A consistent pattern has been observed in South Africa, where Nzima et al. (2020) found that wastewater effluent increased downstream *E. coli* counts in recipient surface water and that isolates carried ESBL genes, including bla_{TEM} and $bla_{\text{CTX-M}}$ (Nzima et al., 2020; Mukwevho et al., 2025). These studies show that surface waters can harbour viable, phenotypically resistant Enterobacterales and clinically important resistance determinants across multiple LMIC and African contexts.

1.2.5.3 Genomic Evidence Linking Surface Water and Human-Associated Resistance

Many studies have moved beyond detecting resistant organisms in surface water to examining whether environmental isolates overlap genetically with isolates from human infections. This distinction is important because shared resistance genes alone may indicate common selective pressures or HGT; whereas close phylogenetic relatedness provides stronger evidence that environmental waters and clinical populations are connected through shared resistant lineages. In northern Greece, Kotzamanidis et al. (2024) detected ESBL-producing *E. coli* in river water and identified clinically significant STs, including ST131 and ST10, with phylogenomic analysis revealing that ST131 O25:H4/H30 isolates recovered from surface water were genetically linked to human clinical strains (Kotzamanidis et al., 2024). ST131 is a globally disseminated high-risk extraintestinal pathogenic *E. coli* (ExPEC) lineage associated particularly with urinary tract and bloodstream infections. Its clinical importance is enhanced by its frequent multidrug-resistant phenotype and strong association with ESBL production, particularly $bla_{\text{CTX-M-15}}$ (Nicolas-Chanoine et al., 2014; Pitout & Finn, 2020). In contrast, ST10 is a genetically diverse lineage distributed across human, animal and environmental niches and is capable of carrying a broad range of antimicrobial resistance determinants (Machado et al., 2024). Its clinical relevance is also evident in South Africa, where multidrug-resistant ST10 isolates have been recovered from bloodstream infections (Hetsa et al., 2026). The occurrence of ST10 across clinical and environmental settings therefore makes it relevant to One Health AMR surveillance. Similar evidence was reported in Sweden, where Fagerström et al. (2019) compared ESBL-producing *E. coli* from urine infections and environmental waters and found that five environmental isolates were indistinguishable from, or closely related to, clinical isolates by cgMLST; importantly, environmental ST131 O25:H4/H30 isolates were as closely related to clinical isolates as clinical isolates were to one another (Fagerström et al., 2019). Falgenhauer et al. (2021) further reported a $bla_{\text{CTX-M-15}}$-producing *E. coli* ST949 clone in

German surface waters that was closely related to human clinical isolates . The evidence from these studies shows that surface water can harbour resistant lineages with direct clinical relevance. In sub-Saharan Africa, however, the evidence remains less phylogenetically resolved. For example, studies such as Beshiru et al. (2024) in Nigeria and Moremi et al. (2016) in Tanzania demonstrated that aquatic environments contain clinically important ESBL determinants, although only a few studies have directly compared surface water isolates with matched community and clinical isolates using WGS. This gap is central to the rationale for the present study.

1.2.5.4 South African Evidence for Surface Water as an AMR Reservoir and Community Exposure Interface

South African evidence supports the relevance of surface water and wastewater-impacted aquatic systems as reservoirs of clinically important AMR. Nzima et al. (2020) investigated *E. coli* from wastewater and recipient surface water in South Africa and reported resistance to multiple antibiotics, including β-lactams and tetracycline, as well as ESBL genes such as bla_{TEM} and $bla_{\text{CTX-M}}$ (Nzima et al., 2020). In KwaZulu-Natal, Gumede et al. (2021) characterised ESBL-producing *E. coli* from WWTP influent and effluent in uMgungundlovu District, showing that viable *E.coli* can persist through treatment and remain detectable in the final effluent . Genomic work by Mbanga et al. (2021) further showed that multidrug-resistant *E. coli* from South African wastewater sources carried clinically relevant resistance determinants and could be examined in relation to clinical pathogen populations using WGS and bioinformatic analysis . Adefisoye and Okoh (2017) concluded that treated effluents from Eastern Cape WWTPs could contribute to drug-resistant bacterial loads in receiving waters (Adefisoye & Okoh, 2017). These findings are important because WWTP effluent and poorly managed wastewater can enter receiving river systems. The relevance of exposure to such

polluted waters is heightened in resource-limited communities. In rural Limpopo, Edokpayi et al. (2018) found that all natural water sources tested in their study were positive for faecal contamination at some point, while treated municipal water did not reliably reach all residents (Edokpayi et al., 2017). Thus, it is evident from the cited literature that South African surface waters affected by wastewater, sanitation failures, and intermittent water access may serve as both AMR reservoirs and exposure pathways, particularly for communities with frequent contact with contaminated environmental waters.

1.2.5.5 Technical and Ecological Constraints of Surface Water as an AMR Surveillance Proxy

Despite its perceived relevance as a reservoir and dissemination route for AMR, surface water is technically and ecologically more complex than wastewater as a surveillance matrix. Wastewater generally receives more concentrated faecal inputs from a defined sewered catchment. In contrast, surface water receives mixed, spatially diffuse, and temporally variable inputs from humans, animals, agriculture, healthcare facilities, stormwater runoff, sediments, and natural environmental reservoirs. As a result, AMR signals detected in surface water may be shaped not only by resistance circulating in the surrounding human population, but also by hydrological dilution, rainfall-driven runoff, seasonal flow variation, upstream contamination, sediment reservoirs, biofilm formation, bacterial survival, and non-human faecal sources (Balcázar et al., 2015; Di Cesare et al., 2017; Heß et al., 2018; Jampani et al., 2024; Yu et al., 2023). These factors complicate interpretation because detection of resistant organisms or resistance genes in surface water does not, by itself, establish their origin, relative contribution, or the representativeness of community-level AMR. Comparability across studies is further limited by differences in sampling locations, sampling frequency, sample matrices, culture-based recovery, metagenomic workflows, target organisms, AST, and genomic analysis,

reinforcing the need for standardised methods and clearly defined surveillance objectives (Liguori et al., 2022). Therefore, although surface water may provide a practical surveillance matrix in non-sewered settings, its value as a proxy for human or clinical AMR cannot be inferred solely from detection studies. It requires spatially linked, temporally appropriate, and molecularly resolved comparisons with isolates from the surrounding community and nearby clinical settings.

1.2.6 ESBL-Producing *E. coli* and *K. pneumoniae* as Sentinel Organisms

1.2.6.1 Complementary Sentinel Value of *E. coli* and *K. pneumoniae*

Escherichia coli and *Klebsiella pneumoniae* are members of the order Enterobacterales and the family Enterobacteriaceae, a taxonomically diverse family that includes several medically important Gram-negative pathogens (Janda & Abbott, 2021). Although both organisms are useful for cross-compartment AMR surveillance, they provide distinct but complementary epidemiological signals.

E. coli is a common inhabitant of the human and animal gastrointestinal tract and is also one of the most important human pathogens, causing community-acquired infections (CAIs) and HAIs, particularly urinary tract and bloodstream infections (Vila et al., 2016). Its frequent presence in faeces makes it a widely used indicator of faecal contamination in environmental matrices, although distinguishing human from animal sources requires additional epidemiological, genomic, or microbial source-tracking evidence (Nowicki et al., 2021). Its dual role as a commensal indicator and major pathogen means that resistance detected in environmental *E. coli* can provide insight into both circulating faecal community AMR and clinically relevant resistance.

K. pneumoniae provides a different surveillance signal. It is less useful than *E. coli* as a general faecal indicator, but it is a major opportunistic pathogen associated with healthcare exposure, intensive care settings, and severe infections, including pneumonia, bloodstream infections (BSIs), and urinary tract infections (UTIs) (Caneiras et al., 2019). Its importance is reinforced by its listing as a priority pathogen by the WHO, with carbapenem-resistant *K. pneumoniae* ranked among the most urgent bacterial threats for antibiotic research and public health action (WHO, 2024c). Beyond clinical settings, *K. pneumoniae* has also been recovered from wastewater and environmental waters, and recent genomic studies indicate that wastewater pathways can contribute to the environmental dissemination of clinically relevant *K. pneumoniae* lineages (Davidova-Gerzova et al., 2024; King et al., 2020; Verburg et al., 2024).

The combined use of *E. coli* and *K. pneumoniae* provides a more balanced surveillance approach. *E. coli* captures faecal and community AMR signals, while *K. pneumoniae* captures signals linked to opportunistic infection, healthcare exposure, and high-risk resistance.

1.2.6.2 ESBL-Production as a Clinically Interpretable Resistance Phenotype

Extended-spectrum beta-lactamases are enzymes produced by Gram-negative bacteria, particularly Enterobacterales such as *E. coli* and *K. pneumoniae*, that hydrolyse clinically important β-lactam antibiotics, thereby reducing or stopping their activity (Castanheira et al., 2021; Paterson & Bonomo, 2005). ESBLs characteristically hydrolyse penicillins, aztreonam, and extended-spectrum cephalosporins such as cefotaxime, ceftriaxone, and ceftazidime, antibiotics widely used for severe CAIs and HAIs. ESBLs are typically inhibited by clavulanic acid, a principle used in phenotypic confirmation tests (Drieux et al., 2008; Paterson & Bonomo, 2005).

The ESBL phenotype is useful for surveillance because it is clinically meaningful, microbiologically detectable, and genomically traceable. ESBL production alone does not confer carbapenem resistance, although carbapenem activity may be compromised when ESBL genes co-occur with carbapenemases, porin loss, or other resistance mechanisms (Bialek-Davenet et al., 2017). ESBL genes are often carried on plasmids and other MGEs that may also harbour determinants conferring resistance to fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole, sulfonamides, and tetracyclines. ESBL-producing isolates therefore provide a useful entry point for characterising broader multidrug-resistant plasmid platforms circulating across human, clinical, and environmental compartments (Rozwandowicz et al., 2018).

1.2.6.3 Epidemiological Significance of ESBL Genes

ESBL-producing *E. coli* and *K. pneumoniae* are valuable sentinel organisms because their resistance phenotypes are linked to epidemiologically informative ESBL gene lineages. The major ESBL families include CTX-M enzymes, named for their strong activity against cefotaxime; TEM enzymes, historically derived from early plasmid-mediated β-lactamases; and SHV enzymes, which are particularly associated with *K. pneumoniae* and healthcare-associated Enterobacterales. These families differ in their historical emergence, host associations, geographic distribution, and linkage to MGEs. TEM and SHV enzymes were historically prominent among healthcare-associated Enterobacterales, particularly *K. pneumoniae*, but CTX-M enzymes have become the dominant ESBL family globally, with CTX-M-15 the most widely disseminated variant and CTX-M-14, CTX-M-27, and other variants showing more regionally structured distributions (Bevan et al., 2017; Castanheira et al., 2021; Paterson & Bonomo, 2005). The epidemiological importance of CTX-M-15 is amplified by its frequent association with globally disseminated *E. coli* ST131, a high-risk

extraintestinal pathogenic lineage associated with multidrug resistance, virulence, UTIs, BSIs, and international spread (Nicolas-Chanoine et al., 2014; Pitout & Finn, 2020). In *K. pneumoniae*, ESBL production is also epidemiologically significant because it often occurs in healthcare-associated and high-risk resistant lineages, where ESBL genes may co-occur with additional resistance determinants on MGEs.

Resistance determinants associated with MGEs may appear across different bacterial lineages and ecological compartments, even when isolates are not clonally related. Therefore, comparing ESBL-producing isolates across community, clinical, and surface water sources requires both resistome analysis to identify shared resistance determinants and ST and phylogenetic analysis to determine whether similarities reflect clonal relatedness or broader dissemination of resistance genes across different genomic backgrounds. In this sense, ESBL gene lineages are more than phenotypic resistance markers; they provide molecular signatures for the assessment of cross-compartment overlap in resistance (Bevan et al., 2017; Castanheira et al., 2021).

1.2.6.4 Cross-Compartment Occurrence and Epidemiological Relevance of ESBL-Producing Enterobacterales

ESBL-producing *E. coli* and *K. pneumoniae* are useful candidates for sentinel AMR surveillance because they occur across multiple epidemiological compartments, including asymptomatic human carriage, clinical infection, and environmental contamination. However, the strength of evidence differs between the two organisms and across compartments. Globally representative carriage estimates remain limited, particularly in LMICs, although Woerther et al. (2013) estimated that more than 1.5 billion people may be colonised with ESBL-producing Enterobacteriaceae, largely without symptoms (Woerther et al., 2013). In rural Ghana, Akenten

et al. (2023) reported faecal carriage of ESBL-producing *E. coli* and *K. pneumoniae* in 40.9% of children, with high multidrug resistance and predominance of *bla*$_{\text{CTX-M}}$ variants, illustrating the relevance of community carriage in LMIC settings (Akenten et al., 2023). Their clinical relevance is also well established, as ESBL-producing Enterobacterales are associated with difficult-to-treat infections and reduced effectiveness of commonly used beta-lactam antibiotics (Gutiérrez-Gutiérrez & Rodríguez-Baño, 2019; Paterson & Bonomo, 2005).

Environmental recovery provides an additional basis for their use in cross-compartment surveillance, although the evidence is stronger for ESBL-producing *E. coli* than for *K. pneumoniae* in surface water. ESBL-producing *E. coli* has been repeatedly detected in surface waters, including rivers in Ghana and Greece, where isolates carried clinically important resistance genes and, in some settings, sequence types such as ST131 and ST10 (Banu et al., 2021; Kotzamanidis et al., 2024). Evidence for environmental *K. pneumoniae* is also increasing, particularly in wastewater and wastewater-impacted environments, but direct surface water evidence and matched clinical-environmental genomic comparisons remain less developed. This uneven evidence base is important because cross-compartment similarity is rarely reducible to complete genetic identity between isolates. Studies using One Health genomic designs show that phenotypic resistance, resistance gene content, sequence types, and phylogenetic relatedness may each reveal different degrees of overlap, since resistance genes can disseminate horizontally across unrelated strains while related clones may differ in accessory resistance content (Muloi et al., 2022; Rozwandowicz et al., 2018). For example, Runcharoen et al. (2017) found limited overall genetic similarity between clinical and environmental ESBL-producing *E. coli* in Thailand despite shared resistance determinants. In contrast, Gay et al. (2023) reported extensive cross-compartment overlap among ESBL-producing *E. coli* from humans, animals, and environmental water in Madagascar (Gay et al.,

2023; Runcharoen et al., 2017). Thus, ESBL-producing *E. coli* and *K. pneumoniae* provide a useful framework for assessing whether surface water shares phenotypic resistance patterns, core resistance genes, sequence types, or phylogenetic relatedness with community and clinical isolates, while recognising that surface water may capture partial rather than complete concordance with human AMR patterns.

1.2.7 Molecular Epidemiology Tools for Cross-Compartment AMR Comparison

Establishing whether surface water AMR reflects AMR in co-located human and clinical populations requires more than demonstrating that resistant organisms are present across compartments. It requires determining whether isolates share resistance determinants, sequence types, virulence profiles, or phylogenetic relatedness. Whole-genome sequencing enables high-resolution characterization of bacterial isolates through resistance gene detection, sequence typing, virulence profiling, and phylogenetic reconstruction (Argimón et al., 2020). Culture-based isolation and AST provide the essential phenotypic foundation by confirming ESBL-producing status and defining resistance patterns, but they cannot determine whether isolates are genetically related or whether similar resistance phenotypes arose independently. Molecular epidemiological methods are therefore necessary for cross-compartment AMR comparison because they provide the genotypic resolution needed to assess whether resistance signals are shared across human, environmental, and clinical sources or arise separately within each compartment (Lerminiaux & Cameron, 2019; Köser et al., 2014).

WGS offers comprehensive molecular insights for AMR surveillance and epidemiological investigation by capturing the complete or near-complete genomic sequence of bacterial isolates and multiple outputs from a single analytical platform, including resistance gene detection, virulence gene profiling, sequence type assignment, and phylogenetic comparison (Argimón et al., 2020; Köser et al., 2014; WHO, 2020). This makes WGS particularly suited

to cross-compartment studies such as the present investigation, in which the central question is whether ESBL-producing *E. coli* and *K. pneumoniae* from surface water, healthy residents, and clinical sources share resistome content, virulence profiles, clonal lineages, or phylogenetic relatedness.

The output of WGS analysis is enhanced by different bioinformatic tools that determine the resistome, virulome, mobilome, and phylogenomics of bacterial isolates for comparative genomics.

1.2.7.1 The Resistome

The resistome can be defined broadly as the collection of ARGs and their precursors (Wright, 2007). It can be operationalised in isolate-based WGS as the detectable complement of known ARGs and resistance-associated mutations present in an isolate genome (Kim & Cha, 2021). In practice, this is characterised by querying WGS assemblies against curated resistance databases and tools such as ResFinder, Comprehensive Antibiotic Resistance Database (CARD), and NCBI AMRFinderPlus. ResFinder identifies acquired AMR genes and integrates PointFinder to detect selected chromosomal resistance mutations and support genotype-to-phenotype prediction (Bortolaia et al., 2020; Florensa et al., 2022). CARD provides a curated ontology-based framework for resistance gene annotation and resistome prediction through the Resistance Gene Identifier, linking resistance determinants to antimicrobial classes and mechanisms (Alcock et al., 2019, 2023). NCBI AMRFinderPlus similarly detects acquired AMR genes and resistance-associated point mutations from nucleotide or protein sequences using the curated NCBI Reference Gene Catalog and can also report selected stress response and virulence-associated genes when configured to do so (Feldgarden et al., 2021). These tools enable gene-level comparison of resistance determinants across surface water, community, and

clinical isolates, including *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{TEM}}$ variants and non-β-lactam co-resistance genes.

1.2.7.2 The Virulome

The virulome refers to the detectable repertoire of known virulence-associated genes or loci present in an isolate's genome (Tetzschner et al., 2020). It provides complementary epidemiological information by indicating whether isolates carry determinants associated with pathogenic potential, host adaptation, colonisation, immune evasion, or enhanced transmissibility, although gene presence alone does not necessarily confirm virulence expression or clinical disease severity (Chen, 2004; Liu et al., 2022). These determinants may include adhesins, toxins, siderophores and iron acquisition systems, capsule-associated loci, and other factors involved in colonisation, immune evasion, or host damage (Chen, 2004; Lam et al., 2021; Malberg Tetzschner et al., 2020). In cross-compartment AMR studies, virulome comparison can help determine whether environmental isolates carry virulence profiles similar to those observed in community or clinical isolates. This strengthens the interpretation of public health relevance, while recognising that the presence of virulence genes alone does not prove pathogenicity or clinical risk (Malberg Tetzschner et al., 2020).

1.2.7.3 The Mobilome

The mobilome, comprising inter alia plasmids, integrons, insertion sequences, and transposons, represents an important layer of WGS-based AMR analysis because it helps clarify whether shared resistance determinants reflect clonal spread of resistant organisms or HGT across unrelated bacterial populations (Partridge et al., 2018). In cross-compartment studies, this interpretation should be placed within the broader interaction between humans and the environment. Resistant bacteria and MGEs may enter environmental waters through wastewater, faecal contamination, runoff, manure, and other sanitation-related pathways, while

contaminated environmental waters and reservoirs may create opportunities for subsequent human exposure (Huijbers et al., 2015). Where mobilome analysis is performed, shared plasmid replicon types or mobile genetic element signatures across compartments may suggest that resistance gene transfer, rather than clonal dissemination alone, contributes to cross-compartment AMR overlap. This distinction is particularly relevant in informal settlements, where diverse microbial communities may interact under conditions of high faecal contamination and sustained antimicrobial selection pressure.

1.2.7.4 Multilocus Sequence Typing and Phylogenomics

Multilocus sequence typing (MLST) and phylogenomics enable clonality and phylogenetic structure to be characterised at the isolate level. MLST assigns sequence types based on allelic variation in defined housekeeping gene loci, providing a portable framework for comparing bacterial lineages across studies and surveillance datasets (Maiden et al., 1998). In *E. coli*, the Achtman scheme is commonly used, while the *K. pneumoniae* Pasteur scheme remains widely applied for epidemiological typing (Diancourt et al., 2005; Wirth et al., 2006). These schemes allow identification of globally disseminated high-risk lineages. Core-genome single-nucleotide polymorphism (cgSNP) phylogenetics is the reconstruction of evolutionary relationships among isolates using SNPs identified in genomic regions shared by the isolates under comparison. In AMR surveillance, cgSNP analysis can provide high-resolution evidence of genetic relatedness and may identify closely related isolates across compartments, suggesting recent shared ancestry, common-source exposure, or possible transmission links when supported by epidemiological context (Shakya et al. 2020) . Such analyses are commonly implemented through reference-based SNP calling, SNP alignment extraction, and maximum likelihood phylogenetic inference using tools such as SNP-sites and RAxML (Stamatakis, 2014).

1.2.8 Antimicrobial Resistance and Sanitation Inequality in South Africa

South Africa carries a substantial AMR burden, particularly among Gram-negative pathogens of clinical and public health importance. According to country-level estimates from the Institute for Health Metrics and Evaluation/Global Research on Antimicrobial Resistance (IHME/GRAM), analysis indicate that, in 2019, approximately 9,490 deaths were attributable to bacterial AMR (95% uncertainty interval [UI]: 7,130-13,000), while approximately 39,000 deaths were associated with bacterial AMR (95% UI: 30,000-50,000) in South Africa (Antimicrobial Resistance Collaborators, 2023). These figures are modelled mortality estimates rather than directly observed counts and are derived from the broader GRAM framework, which estimated AMR-attributable and AMR-associated mortality across 204 countries and territories using predictive statistical methods (IHME, 2023; Murray et al., 2022), and should therefore be interpreted within the reported uncertainty intervals.Within the South African AMR surveillance system, BSI data show that *K. pneumoniae* is the most common ESKAPE organism isolated from blood in both public and private sectors (NDoH, 2024). At the same time, the prevalence of ESBL-producing *K. pneumoniae* has remained high, at approximately 66-70% in national surveillance reports. Carbapenem resistance in *K. pneumoniae* has also become a growing concern, with one-third of isolates showing resistance to carbapenems in 2022, while a quarter of *E. coli* bloodstream isolates were reported as ESBL-producers resistant to third-generation cephalosporins (NDoH, 2024). These findings indicate that ESBL-producing Enterobacterales, particularly *E. coli* and *K. pneumoniae*, are central to South Africa's AMR problem and provide a strong organism-level rationale for focusing surveillance on these taxa.

South Africa has made important progress in formal AMR surveillance, including participation in WHO GLASS and the development of a unified national surveillance process coordinated by the NICD through public-sector NHLS laboratories and major private laboratory groups. However, the current human AMR surveillance system remains largely laboratory-based and blood culture-centred. The 2018 national AMR surveillance report described the human AMR system as a public-private laboratory collaboration that provided an almost comprehensive view of AMR in blood cultures for ESKAPE pathogens (NDoH, 2018). The 2021 surveillance report, published in 2022, confirmed the same blood culture-based structure and described two surveillance tiers: laboratory-based AMR surveillance from selected sentinel sites, mainly academic and large referral public-sector laboratories, and electronic surveillance using NHLS and major private laboratory information systems (NDoH, 2022). Although this system provides important data on invasive bacterial infections, its reliance on healthcare access, diagnostic sampling, blood culture submission, and laboratory reporting may underrepresent AMR circulating among asymptomatic community members, unsampled infections, and environmental reservoirs. This limitation aligns with WHO GLASS, which promotes a shift from surveillance based solely on laboratory data toward systems that include epidemiological, clinical, and population-level data (WHO, 2015).

This surveillance limitation is especially relevant in the context of South Africa's structural inequality. South Africa remains one of the most unequal countries globally, with Stats SA reporting a per-capita expenditure Gini coefficient of approximately 0.65 in 2015 (Stats SA, 2019). South Africa's structural inequality is also reflected in uneven access to housing, sanitation, and water infrastructure. Evidence showed that, in 2021, 11.7% of South African households lived in informal dwellings, with informal dwellings particularly common in metropolitan areas, including Johannesburg (17.3%), Cape Town (16.6%) and traditional

dwellings were most common in Eastern Cape (21.6%) and KwaZulu-Natal (9.3%) (Stats SA, 2022). Although national access to improved sanitation had increased from 61.7% in 2002 to 84.1% in 2021, improved sanitation was defined broadly as flush toilets connected to a sewerage system or septic tank, or pit toilets with ventilation pipes. Nationally, only 64.8% of households used flush toilets, while 19.3% used ventilated pit toilets and 13.4% still relied on pit toilets without ventilation pipes. Access also remained uneven across provinces, with improved sanitation most limited in Limpopo (58.5%) and Mpumalanga (63.2%), while KwaZulu-Natal stood at 84.5% (Stats SA, 2022). These figures show that sanitation inequality persists even where broad national access indicators suggest improvement. Importantly, both ventilated and unventilated pit toilets are on-site, non-sewered sanitation systems; therefore, faecal waste from these households is not captured within a centralised sewer catchment for conventional wastewater-based surveillance, reinforcing the surveillance gap in poorly sewered communities.

The relevance of sanitation inequality to AMR lies in the faecal ecology of Enterobacterales. *E. coli* is commonly found in the gut of humans and warm-blooded animals, while *K. pneumoniae* can colonise the gastrointestinal tract and act as a reservoir for subsequent infection. When human waste is inadequately contained, transported, or treated, gut-associated resistant organisms and resistance genes may enter wastewater and surface-water systems. Van der Hoven et al. (2017) showed that land use influenced microbial and physicochemical water quality in the Klein Jukskei catchment, with the informal settlement exerting the greatest negative impact (van der Hoven et al., 2017). Their findings linked increased *E. coli* contamination to inadequate sanitation, refuse removal, and direct disposal of household and excretory waste into the tributary or along its banks. While this study demonstrates faecal contamination rather than AMR specifically, South African studies on WWTPs and their

receiving water bodies show that such environmental compartments can also carry resistant Enterobacterales. For example, Nzima et al. (2020) detected multidrug-resistant *E. coli* carrying ESBL genes, including $bla_{\text{CTX-M}}$ and bla_{TEM}, in wastewater and recipient river water in South Africa (Nzima et al., 2020; Mukwevho et al., 2025). These findings support a two-step rationale, in which sanitation inequality can contribute to faecal contamination of surface water, and wastewater-impacted aquatic systems can harbour clinically relevant resistant Enterobacterales and ESBL genes.

1.3 Study Justification

Antimicrobial resistance surveillance has traditionally relied on clinical, laboratory-based systems. While these approaches remain essential, their effectiveness is constrained in many LMICs, including South Africa, by limited laboratory infrastructure, fragmented data systems, financial constraints, workforce shortages, and uneven access to healthcare. As a result, surveillance data often underrepresents communities that have limited contact with formal healthcare systems, particularly rural and informal settlement populations. These limitations have strengthened the need for integrated One Health surveillance approaches that include environmental monitoring. However, environmental AMR surveillance has largely been advanced through WBE, which depends on centralised sewerage infrastructure and defined wastewater collection points. This creates an important limitation in resource-limited and non-sewered communities, where household waste, sanitation overflows, stormwater runoff, and untreated wastewater often enter open drains and nearby surface water bodies rather than formal sewerage systems. In such settings, surface water may provide a more context-appropriate matrix for capturing community-level AMR signals.

Despite growing evidence that surface waters can harbour resistant bacteria and clinically important resistance genes, it remains unclear whether AMR detected in surface water reflects resistance circulating in surrounding human communities and nearby healthcare facilities. This study therefore investigated ESBL-producing *E. coli* and *K. pneumoniae* recovered from three interconnected sources within a resource-limited South African setting: healthy community residents residing in an informal settlement, surface water flowing through the community, and a proximate healthcare facility. By comparing phenotypic resistance profiles, resistomes, virulomes, mobilomes, clonality, and phylogenetic relatedness using culture-based methods, whole-genome sequencing, and bioinformatics, the study evaluated whether surface water can serve as a proxy matrix for AMR surveillance where conventional clinical surveillance and WBE are limited. The findings will contribute evidence on the feasibility, limits and interpretive value of surface water surveillance in non-sewered communities and may inform context-appropriate One Health AMR surveillance strategies for resource-limited settings.

1.4 Aims and Objectives

1.4.1 Aims of the Study

To assess the potential of surface water as a proxy matrix for AMR surveillance in communities and clinical settings in a resource-limited setting using ESBL-producing *E.coli* and *K. pnemouniae* as indicator organisms.

1.4.2 Study Objectives

Objective 1: To determine the phenotypic AMR profiles of ESBL-producing *E. coli* and *K. pneumoniae* isolates recovered from surface water, healthy community residents, and clinical sources.

Objective 2: To investigate the genomic concordance of AMR profiles among ESBL-producing *E. coli* and *K. pneumoniae* isolates from surface water, healthy community residents,

and clinical sources through comparative analysis of their resistomes, virulomes, clonal relatedness, and phylogenetic relationships.

Objective 3: To determine whether surface water is a feasible AMR monitoring proxy for AMR surveillance in community and clinical sources based on phenotypic and genomic concordance.

1.5 Methodology Synopsis

1.5.1 Ethical Considerations

The protocol was approved by the Biomedical Research Ethics Committee, University of KwaZulu-Natal (BREC/00003640/2021), the KwaZulu-Natal Department of Health Provincial Health Research Ethics Committee (NHRD Ref. KZ_202203_023), and the National Health Laboratory Service (NHLS Ref. PR2225862). Written informed consent was secured from all community volunteers prior to study enrolment. Ethical approval letters are provided in the Appendices.

1.5.2 General Methodology

This study was conducted in the uMgungundlovu District Municipality, KwaZulu-Natal, South Africa. It was a longitudinal cross-compartment investigation designed to evaluate the phenotypic and genomic characteristics of ESBL-producing *E. coli* and *K. pneumoniae* recovered from three epidemiologically connected sources: surface water, healthy community residents residing in an informal settlement, and clinical isolates from a proximate healthcare setting.

Sampling was conducted over eight months, from May to December 2023. Figure 1 summarises the methodology of the study. Surface water samples, stool samples and clinical

bacterial cultures were obtained from the Kwapata River, Ward 22 healthy residents and the Medical Microbiology service of the National Health Laboratory Service serving the district, respectively. Presumptive *E. coli* and *K. pneumoniae* isolates were recovered using selective culture media, purified, and confirmed by PCR. Confirmed isolates were screened for ESBL production, and ESBL-positive isolates were phenotypically confirmed using the combined disc diffusion test with cefotaxime, ceftazidime, and amoxicillin-clavulanate. Antimicrobial susceptibility testing of confirmed ESBL-producing *E. coli* and *K. pneumoniae* was performed using the VITEK 2® system against a panel of 17 antibiotics. Results were interpreted as resistant, intermediate, or susceptible for phenotypic characterisation according to CLSI/FDA guidelines.

For genomic analysis, confirmed ESBL-producing isolates underwent DNA extraction, and purity and quality assessment. Extracted DNA was submitted to the National Institute for Communicable Diseases (NICD) Sequencing Core Facility for WGS using the Illumina NovaSeq platform. Bioinformatics analyses were performed to characterise the resistome, virulome, mobilome, sequence types, and phylogenomic relationships of the recovered *E. coli* and *K. pneumoniae* isolates. Comparative genomic analysis was used to assess cross-source similarity and to determine whether AMR profiles detected in surface water reflected those observed among healthy community residents and clinical isolates. Phenotypic and genomic data were analysed using R in RStudio.

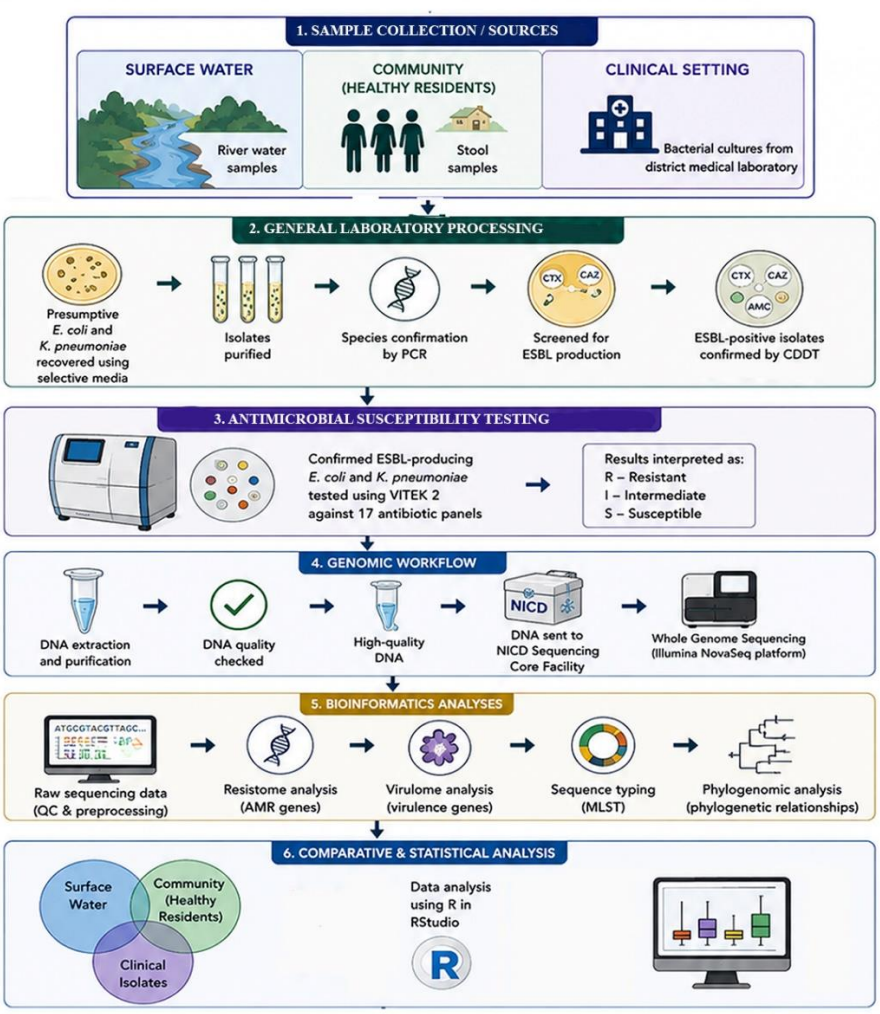

Figure 1: Summary of Study Methodology.

1.6 Outline of the Thesis

This project is presented in the form of submitted manuscripts and consists of the following six chapters:

Chapter One - Introduction and Literature Review: This chapter provides the study background, the rationale for the study, the aim, and the objectives. It also reviews literature on antimicrobial resistance surveillance, its challenges and the need for alternative AMR monitoring approaches.

Chapter Two - Manuscript 1: Can surface water be a good proxy for phenotypic antimicrobial resistance in ESBL-producing *E. coli* from healthcare facilities and healthy informal settlement residents? A triangulation study [Manuscript submitted to "*Environmental Science and Pollution Research (ESPR-D-26-04552)"*].

This chapter answers the ESBL-producing *E. coli* component of objective 1 by comparing the prevalence and phenotypic antimicrobial resistance profiles of ESBL-producing *E. coli* isolates from surface water, with that of isolates from healthy residents in an informal settlement, and clinical samples from proximate hospital.

Chapter Three - Manuscript 2:Monitoring Extended-spectrum β-lactamases-producing *Klebsiella pneumoniae*: Do Surface Water Phenotypic Patterns Reflect Community and Clinical Resistance? [Submitted to "*Archives of Microbiology (Ref: Submission ID a8037649-17ac-4370-999a-cf91defaf71c)"*].

This chapter answers the ESBL-producing *K. pneumoniae* component of objective 1. This study determined the prevalence and AMR profiles of ESBL-producing *K. pneumoniae* isolated

from surface water, apparently healthy residents of an informal settlement and a proximate healthcare facility, to determine similarities and differences.

Chapter Four -Manuscript 3: Does AMR surveillance of surface water reflect AMR in humans?

A genomic comparison of ESBL-producing *Escherichia coli* from surface water, healthy residents, and a clinical facility. [**Published** "*Environmental Research (Manuscript Number: ER-26-9361"*].

This chapter addresses the ESBL-producing *E. coli* component of objective 2. It provides genomic insights into the phenotypic ESBL-producing *E. coli* prevalence in chapter 2 by comparing the resistome, virulome phylogroups, multilocus sequence types and phylogenetic relationships of ESBL-producing *E. coli* from the three sample sources: surface water, healthy community residents, and clinical settings.

Chapter Five: Manuscript 4: Evaluating Surface Water for Genomic Surveillance of Community-Linked Antimicrobial Resistance in ESBL-Producing *Klebsiella pneumoniae [**Published -** Environment International (Manuscript ID: ENVINT-D-26-02964)*].

This chapter answers the ESBL-producing *K. pneumoniae* component of objective 2. It provides genomic insights into the phenotypic ESBL-producing *K. pneumoniae* prevalence in Chapter 3. The study genomically characterised ESBL-producing *K. pneumoniae* isolates from surface water, healthy residents and the clinical setting through analysis of the resistome, virulome, and phylogenetic relationships to ascertain whether genomic AMR profiles in surface water reflect those seen in healthy residents and the clinical setting.

Chapter Six - Conclusion: This chapter gives a summary against each objective including the significance of the work. The limitations and recommendations for future work are also discussed.

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CHAPTER TWO - MANUSCRIPT 1

CAN SURFACE WATER BE A GOOD PROXY FOR PHENOTYPIC ANTIMICROBIAL RESISTANCE IN ESBL-PRODUCING ESCHERICHIA COLI FROM HEALTHCARE FACILITIES AND HEALTHY INFORMAL SETTLEMENT RESIDENTS? A TRIANGULATION STUDY.

Author Contributions

Etando Ayukafangha - as principal investigator, co-conceptualised the study, performed the laboratory work, analysed the data, and drafted the manuscript.

Joshua Mbanga - as co-supervisor, co-conceptualised the study, assisted with sample collection and processing, vetted the results, and critically reviewed the manuscript.

Sabiha Y. Essack - as co-supervisor, co-conceptualised the study, secured funding, guided the literature review and ethical approval application, facilitated data collection, vetted the results, and critically reviewed the manuscript.

Akebe Luther King Abia - as main supervisor, co-conceptualised the study, guided the literature review, data collection, processing and analysis, vetted the results, and critically reviewed the manuscript.

Objectives met - This chapter answers the ESBL-producing *E. coli* component of objective 1

Can surface water be a good proxy for phenotypic antimicrobial resistance in ESBL-producing Escherichia coli from healthcare facilities and healthy informal settlement residents? A triangulation study

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ABSTRACT

Antimicrobial resistance (AMR) has emerged and evolved as one of the greatest societal threats in modern times, with organisms such as extended-spectrum beta-lactamase-producing *E. coli* (ESBL-Ec) posing a major public health threat due to their association with limited therapeutic options and high mortality rates. Despite this recognised burden, the current AMR surveillance has severe limitations, especially in resource-limited settings, making alternative monitoring approaches imperative to better address this growing burden. We assessed whether surface water reflects community and hospital-setting AMR by comparing the prevalence and phenotypic antimicrobial resistance profiles of ESBL-Ec from surface water (SW), healthy residents (HR) in an informal settlement, and clinical (CL) samples from a proximate hospital. Eighty SW, 1,978 HR stool and 2,154 CL samples (N = 4,212) collected over 8 months in South Africa yielded 512, 1,915, and 588 *E. coli* isolates, respectively. Antimicrobial susceptibility testing was conducted using the VITEK 2 system, and data were analysed in R. ESBL-Ec prevalence in SW (16.8%), HR (27.10%) and CL (5.3%) isolates was significantly different ($p < 0.05$). SW and HR isolates showed similar resistance to ampicillin, cephalosporins, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, and piperacillin-tazobactam; SW and CL isolates were limited to ampicillin, cephalosporins, and trimethoprim-sulfamethoxazole. Multidrug resistance (MDR) was 64.5% in CL, 41.6% HR and 5.7% in SW. Antibiogram overlap across sources was limited: one non-MDR pattern was shared between SW and HR isolates, and two patterns (one MDR, one non-MDR) were shared across all three sources. No MDR pattern was shared exclusively between SW and either of the human sources. ESBL-Ec and MDR prevalence differed between sources. SW contained ESBL-Ec and shared some resistance features with HR and CL isolates, but phenotypic AMR profiles alone did not support its use as a reliable proxy for human AMR burden in this setting; hence, the need for high-resolution molecular analysis to ascertain surface as an alternative AMR surveillance system.

Keywords: antimicrobial resistance; WHO priority organisms; slum dwellers; aquatic ecosystems; healthy residents, clinical setting, multidrug resistance; public health

1.0 Introduction

Antimicrobial resistance (AMR) is a major global health threat, driving substantial illness and mortality worldwide (Murray et al. 2022) and complicating the treatment of common infections through prolonged treatment courses and rising medical costs. Left unaddressed, AMR is projected to contribute to over 39 million deaths by 2050 (Wozniak et al. 2019; Naghavi et al. 2024), a trajectory driven largely by the continuous overuse, misuse, and poor regulation of antibiotics in humans and animals (Moyo et al. 2023; Nguyen-Thanh et al. 2024).

Bacterial pathogens account for a disproportionate share of this burden, prompting the World Health Organization (WHO) to issue a priority pathogens list in 2017, updated in 2024 (WHO 2024). *Escherichia coli (E.coli)*, particularly extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant strains, features among the critical-priority organisms, alongside *Klebsiella pneumoniae*, *Enterobacter* spp., *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (WHO 2024); these organisms are frequently multidrug-resistant (MDR) and resistant to beta-lactam therapies (Husna et al. 2023). ESBL-producing *E. coli* is of particular concern given its detection not only in clinical settings (Ibrahim et al. 2023) but also in healthy individuals (Sintondji et al. 2023) and environmental compartments including wastewater (Gumede et al. 2021) and surface water (Erb et al. 2018; Beshiru et al. 2024; Mou et al. 2024), indicating active circulation across human and environmental reservoirs, and making it a useful sentinel organism for tracking AMR across a One Health continuum.

This burden is not evenly distributed: AMR is concentrated in resource-limited low- and middle-income countries (LMICs), and within them, informal settlements are particular hotspots. These unplanned settlements; overcrowded, often in close proximity to livestock, and lacking reliable drinking water, sanitation, and piped sewage infrastructure (Sekhani et al.,

2022; Willis, 2009), combine conditions that both drive AMR emergence and undermine efforts to track it. Overcrowding, informal antimicrobial use, and inadequate waste management (Nadimpalli et al. 2020; Nabirye et al. 2023) elevate transmission risk, while the absence of formal sanitation means household waste is frequently discharged directly into nearby water bodies (Thakur and Onwubu 2024) a pathway reflected in the high prevalence of faecal ESBL-producing *E. coli* reported in urban informal settlements (Erb et al. 2018; Omulo et al. 2021).

These same infrastructural gaps limit conventional AMR surveillance, which in many resource-limited settings is already constrained by limited laboratory capacity, incomplete coverage, and sub-optimal diagnostics (Sulis et al. 2022). This has driven growing interest in complementary One Health surveillance strategies that incorporate environmental compartments (Velazquez-Meza et al. 2022), most commonly wastewater treatment plants (WWTPs) and sewage systems, which can integrate AMR signals from surrounding communities (Hutinel et al. 2019; Schnall et al. 2019; Chau et al. 2022). However, WWTP-based surveillance presumes the existence of formal sanitation infrastructure, an assumption that does not hold in informal settlements and many rural areas, where surface water instead receives community waste directly and may therefore be a more contextually appropriate surveillance target (UNICEF and WHO 2025; Truyens et al. 2025). Drug-resistant bacteria have indeed been documented in such surface water (Gemmell and Schmidt 2013; Beshiru et al. 2024; Mukwevho et al. 2025), yet whether its phenotypic AMR profile actually reflects that of the surrounding community and clinical population, the assumption underpinning its use as a surveillance proxy has not been directly tested in an informal-settlement context..

We therefore aimed to assess whether surface water can serve as a reliable phenotypic proxy for AMR in community and clinical settings, by comparing the prevalence and phenotypic

resistance profiles of ESBL-producing *E. coli* isolated from surface water running through an informal settlement, stool samples from healthy residents of that settlement, and clinical samples from the Regional laboratory serving the same community. We hypothesised that surface-water isolates would more closely resemble community isolates than clinical isolates, given the direct, unmediated exposure of surrounding water to community waste in the absence of formal sewage infrastructure. Findings from this triangulation are intended to inform the design of AMR monitoring strategies using surface water in informal settlements and comparable resource-limited LMIC contexts, where conventional laboratory-based surveillance remains constrained.

2.0 Materials and Methods

2.1 Ethical Considerations

Ethical approval was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Ref: BREC/00003640/2021), the Provincial Health Research Ethics Committee of the KwaZulu-Natal Department of Health (NHRD Ref: KZ_202203_023) for research involving humans, and the National Health Laboratory Service (NHLS Ref: PR2225862) to obtain clinical isolates. All participants from the community were recruited following explicit, voluntary, written informed consent (Appendices 1.2 – 1.4).

2.2 Study Design and Study Area

The KwaZulu-Natal (KZN) Province in South Africa has over 600 informal settlements harbouring over 300,000 households (KZN Human Settlements, 2020). This longitudinal prevalence study was conducted in the uMgungundlovu District Municipality in KZN. The district has several natural water resources, including six significant rivers. Over 63.4% of the population in the district lives below the poverty line; 45.6% have no source of income, and

about 17.8% earn less than 400 ZAR per month and reside in informal settlements (Cooperative Governance and Traditional Affairs 2023). This study involved 8 months of repeated sampling from the same community participants and surface water sites, alongside concurrent collection of clinical isolates from the same diagnostic laboratory over the same period. One of the largest settlements, Ward 22, was used to recruit consenting healthy residents for stool sampling. Stool samples from Ward 22 residents were collected from 6 different strata within the Ward, namely: Seven Ox Emadamini Fedsem, Lay-Centre, Units 3 and 4, and Tehuise.The proximate medical microbiology laboratory, servicing several healthcare facilities in the area, was used to source clinical samples. Surface water samples were collected from the Kwapata River, which discharges into the Msunduzi River, a major tributary of the uMngeni River that flows through the district's capital city, Pietermaritzburg, and several informal settlements. Surface water samples were obtained from Seven Ox and Emadamini (Figure 1).

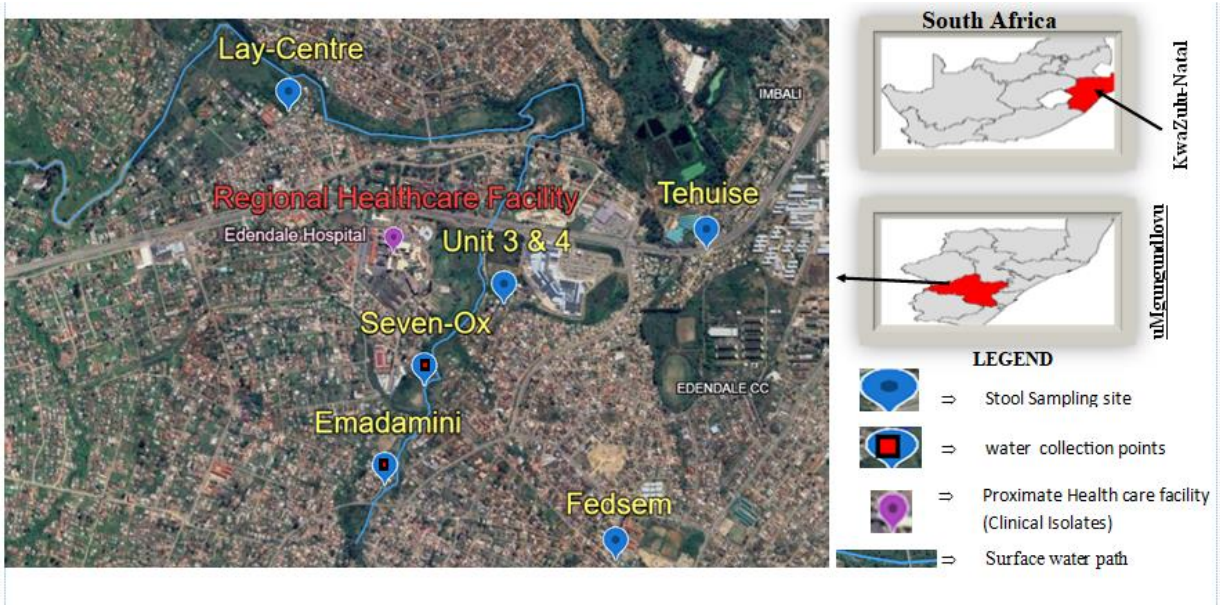

Figure 1: Informal Settlement in Ward 22 in the uMgungundlovu District Municipality, and a Healthcare Facility.

2.3 Sample Collection and Processing

2.3.1 Stool Sampling: Stool samples were collected once a month from each participant over 8 months (May 2023 to December 2023). All participants were given portable camping toilets at the start of the project. Before sampling, consenting participants were educated and trained on the use of the toilets and associated materials to provide a stool sample (Supplementary Material/S1). At the collection site, the samples were checked for consistency, leak-proofness, the absence of urine or other objects, and participant details. Demographic data, including participant name, age, and sex, were also collected. For anonymity, each participant was assigned a unique study code for tracking during subsequent sampling occasions. The stool samples were immediately transported in a cooler box containing ice to a private-sector medical microbiology laboratory, where they were processed for bacterial identification. Downstream analyses were conducted at the isolate level among confirmed ESBL-producing *E. coli*. Participants, therefore, contributed to the analytical dataset only in months when ESBL-producing *E. coli* were detected.

2.3.2 Surface Water Sampling: Surface water samples were collected in duplicate from two sampling sites every two weeks; up to 20 isolates were stored per site per sampling occasion (≤40 isolates fortnightly across both sites) over the 8-month period (May-December 2023). Surface water samples were collected using 500 mL plastic bottles as previously reported (Musselman 2012). During collection, samples were taken near areas of high anthropogenic activity, in the free-flowing, upstream direction, and 10-15 cm below the water surface. Following collection, the samples were transported in an insulated box with ice to the Antimicrobial Research Unit (ARU) at the University of KwaZulu-Natal and processed on the same day.

2.3.3 Clinical Isolate: Positive microbiology cultures (urine, blood cultures, cerebrospinal fluid, pus and other sterile fluids) collected between May and December 2023 from hospitalised adult patients (>18 years) suspected to have a clinical infection were received weekly in temperature-controlled boxes from the public sector medical microbiology unit of the National Health Laboratory Services (NHLS) serving the community. Positive microbiology cultures were received, together with patient demographic and clinical information and diagnostic details (specimen type, culture type, bacteria isolated, ESBL screening, and results of antimicrobial susceptibility testing (AST)), supplied as a Microsoft Excel spreadsheet. The database was filtered for ESBL-producing *E. coli*, and the colonies from the ESBL-positive culture were stored.

The toal number of samples collected and isolate yields are provided in Figure 2.

2.4 Isolation and Molecular Confirmation of Isolates

Putative *E. coli* isolates from surface-water samples were identified, enumerated, and purified using the Colilert®-18/Quanti-Tray® 2000 system (Westbrook, Maine, USA) and Eosin Methylene Blue (EMB) agar (Sigma-Aldrich, Missouri, USA), as previously described (Mbanga et al., 2020). Isolation, purification, and preliminary identification of isolates from stool and clinical samples were performed by private- and public-sector medical microbiology laboratories, respectively. Briefly, stool specimens were cultured on CHROMagar™ ECC (CHROMagar™, Paris, France) under aerobic conditions for 18-24 h, and presumptive *E. coli* colonies were purified. Clinical isolates were received as positive bacteriological culture plates on blood agar or MacConkey agar from the NHLS, and presumptive *E. coli* colonies were subsequently purified on CHROMagar™ ECC under aerobic conditions for 18-24 hour.

DNA was extracted from isolates using the boiling method, and real-time PCR targeting the *uidA* gene was done based on a previously reported protocol (Mbanga et al., 2020). *E. coli* ATCC® 25922 was used as a positive control, and nuclease-free water (ThermoFisher Scientific™) (Woodmead, Johannesburg, South Africa) was used as a template-free negative control (Abrar et al., 2017; Mbanga et al., 2020). All real-time PCR assays were done on an Applied Biosystems QuantStudio 5 Real-time PCR system (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

The pure and molecularly confirmed isolates (water, stool and clinical) were stored in tryptic soy broth (TSB) (Sigma-Aldrich, Missouri, USA) with 20% glycerol (Associated Chemical Enterprises, Johannesburg, South Africa) at -80 °C.

2.5 Determination of ESBL-*E coli* and Antibiotic Susceptibility Testing (AST)

AST was performed using the VITEK® 2 system (bioMérieux, Durham, North Carolina, USA) with the VITEK® 2 AST-N256 card for Gram-negative bacteria, according to the manufacturer's instructions. For clinical isolates, testing was performed by the public medical microbiology laboratory using the VITEK® 2 Advanced Expert System™ (AES), which infers putative ESBL-mediated resistance from the computed third-generation cephalosporin minimum inhibitory concentration (MIC) of the organism. Stool samples from healthy residents and surface water samples were processed by the private medical microbiology laboratory, also using the VITEK® 2 system, with ESBL production additionally confirmed phenotypically using CHROMID® ESBL Agar (bioMérieux, Marcy l'Étoile, France). Both the MIC-based algorithmic approach and the phenotypic agar-based approaches reflects the routine diagnostic workflow of each contributing laboratory, preserving the actual diagnostic pathway through which isolates are characterised for patients and residents in this setting; the

implications of this cross-source methodological difference for comparability are considered in the Limitations. *E. coli* ATCC® 25922 was used as a control throughout.

Seventeen antibiotics from six antibiotic classes were used on all samples (Table 1).

Table 1: List of Antibiotics used on ESBL-producing *E. coli* Isolates.

Antibiotic class		Antibiotics used on Isolates from Surface water & Healthy informal settlement residents	Clinical Isolates
β-lactams	Penicillins	ampicillin (AMP)	ampicillin (AMP)
	β-lactam/β-lactamase inhibitors	amoxicillin/clavulanic acid (AMC)	amoxicillin/clavulanic acid (AMC)
		piperacillin/tazobactam (TZP)	piperacillin/tazobactam (TZP)
	Cephalosporins 2nd Gen	cefuroxime (CXM)	cefuroxime (CXM)
		--	cefoxitin (FOX)
	Cephalosporins 3rd Gen	cefotaxime (CTX)	cefotaxime (CTX)
		ceftazidime (CAZ)	ceftazidime (CAZ)
	Cephalosporins 4th Gen	cefepime (CEP)	cefepime (CEP)
	Carbapenems	doripenem (DRO)	--
		ertapenem (ERT)	ertapenem (ERT)
		imipenem (IMP), meropenem (MEM)	imipenem (IMP), meropenem (MEM)
Aminoglycosides		amikacin (AMK)	amikacin (AMK)
		gentamicin (GEN)	gentamicin (GEN)
		tobramycin (TOB)	--
Fluoroquinolones		ciprofloxacin (CIP)	ciprofloxacin (CIP)
Sulphonamides		trimethoprim-sulfamethoxazole (SXT)	trimethoprim-sulfamethoxazole (SXT).
Glycycycline		tigecycline (TGC)	tigecycline (TGC)
Nitrofurans		--	Nitrofurantoin (NIT)

Total (6)	17	17

Of the 17 antibiotics tested, 15 were common to all three sources; doripenem and tobramycin were tested only on water and stool isolates, and cefoxitin and nitrofurantoin only on clinical isolates, reflecting differences in the standard testing panels of the two laboratories. Comparative analyses across sources were restricted to the [15] shared antibiotics. Antimicrobial susceptibility testing results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints (CLSI 2021), except for tigecycline, which was interpreted using U.S. Food and Drug Administration (FDA) breakpoints (FDA 2023). Tigecycline MICs of ≤2 µg/mL, 4 µg/mL, and ≥8 µg/mL were classified as susceptible, intermediate, and resistant, respectively. Isolates were then classified as MDR (resistance to at least one antibiotic in ≥3 different antibiotic classes (Magiorakos et al. 2012).

2.6 Statistical Analysis

Data were compiled in Microsoft Excel 365 and analysed in R version 4.5.1 using RStudio version 2025.05.1. All analyses were conducted at the isolate level. Because multiple isolates could originate from the same participant or water sampling site over the study period, isolate-level analyses assumed approximate independence of observations. This assumption is revisited as a study limitation, as formal modelling of clustering by participant or sampling site was beyond the scope of the present analysis.

Unless otherwise stated, between-source comparisons of categorical outcomes, including ESBL prevalence, individual antibiotic resistance frequencies, and MDR prevalence, were assessed using overall chi-square tests. Where overall differences were observed, pairwise comparisons were performed using Fisher's exact tests, with Benjamini-Hochberg false discovery rate (FDR) adjustment applied to control for multiple comparisons.

ESBL prevalence was calculated as the proportion of ESBL-positive isolates among all confirmed *E. coli* isolates within each source. Risk ratios (RRs), risk differences (RDs), and corresponding 95% confidence intervals (CIs) were estimated to quantify the magnitude of between-source differences. Antibiotic resistance frequencies were summarised per antibiotic within each source, with comparisons restricted to antibiotics tested across all three sources. Resistance patterns were visualised using hierarchical clustering heatmaps generated with the ggplot2 and pheatmap packages in R.

Pairwise comparisons of MDR prevalence were performed using proportion tests, with FDR adjustment retained for consistency across multiple pairwise analyses. Differences in the mean number of resistant antimicrobial classes per isolate across sources were assessed using the Kruskal-Wallis test.

Overlap in antibiogram patterns between sources was assessed using Venn diagrams generated with the VennDiagram package in R, separately for all antibiograms and MDR antibiograms (Chen and Boutros 2011). Pairwise Jaccard distances were calculated to compare antibiogram-pattern composition between sources, with values closer to 0 indicating greater similarity and values closer to 1 indicating greater dissimilarity. Overall differences in antibiogram-pattern composition were tested using PERMANOVA on a Jaccard distance matrix generated from binary-coded antibiogram-pattern presence/absence data, implemented with the vegan package in R (Oksanen 2025; Oksanen et al. 2025). All statistical analyses were conducted at a 95% confidence level.

3.0 RESULTS

3.1 Prevalence of ESBL-Producing *E coli*.

A total of 4,212 samples were collected across the three sources over the 8-month period: 1,978 stool samples from healthy informal-settlement residents, 2,154 clinical bacteriological

cultures, and 80 surface-water samples from the river adjacent to the settlement. These yielded 1,915, 588, and 512 confirmed *E. coli* isolates, respectively (3,015 isolates in total), of which 636 were ESBL-producing, corresponding to a pooled prevalence of 21.1% across all confirmed isolates. The number of confirmed *E. coli* isolates and the proportion classified as ESBL-producing in each source are shown in Figure 2.

ESBL prevalence differed significantly across the three sources, $\chi^2(2) = 135.64$, $p < 0.001$, and followed a consistent gradient: highest among isolates from healthy residents (27.1%; 519/1,915; 95% CI: 25.2-29.1%), intermediate among surface-water isolates (16.8%; 86/512; 95% CI: 13.8-20.3%), and lowest among clinical isolates (5.3%; 31/588; 95% CI: 3.7-7.4%). All three pairwise comparisons remained significant after FDR correction. Healthy-resident isolates had a higher ESBL prevalence than surface-water isolates (RR = 1.61, RD = 10.3%, adjusted $p < 0.001$), while surface-water isolates had a higher prevalence than clinical isolates (RR = 3.19, RD = 11.5%, adjusted $p < 0.001$). The largest difference was observed between healthy-resident and clinical isolates (RR = 5.14, RD = 21.8%, adjusted $p < 0.001$). This ordered gradient positions surface-water ESBL prevalence as intermediate between the healthy-resident and clinical sources, rather than fully convergent with either source.

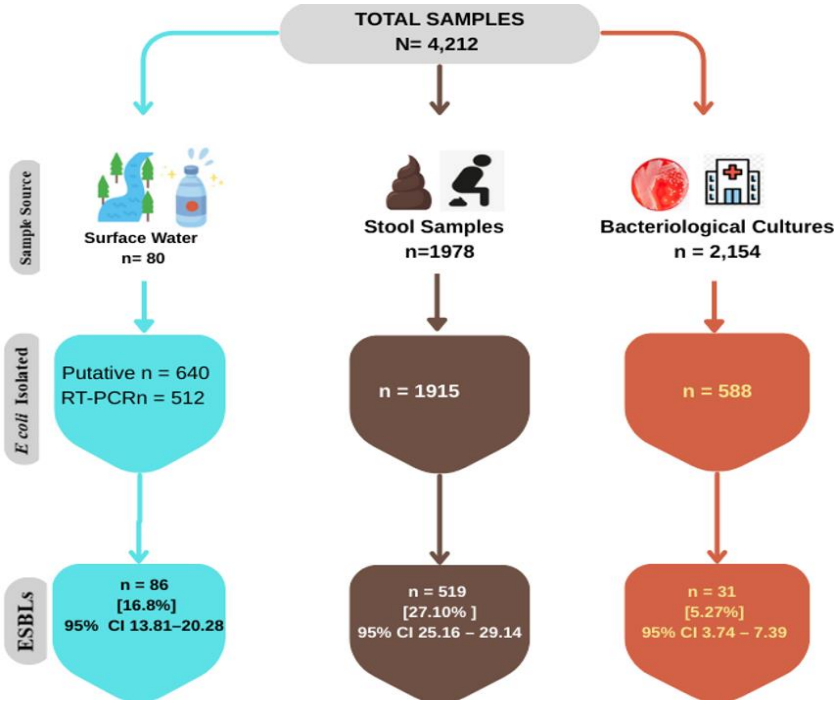

Figure 2: Sampling and ESBL-Producing *E. coli* Prevalence in Surface Water, Healthy Residents, and Clinical Isolates

3.2. Antibiotic susceptibility profiles of ESBL-producing *E coli*

All ESBL-producing *E. coli* isolates were tested against antibiotics representing six antimicrobial classes, with between-source comparisons limited to agents tested across all sources. Overall, resistance was high, particularly to β-lactam antibiotics. Resistance to third- and fourth-generation cephalosporins was consistently high across surface-water, healthy-resident, and clinical isolates, with cefotaxime, ceftazidime, cefuroxime, and cefepime resistance generally exceeding 95%, consistent with the ESBL phenotype.

Surface-water isolates showed high resistance to ampicillin (100.0%), cefotaxime (98.8%), cefuroxime and cefepime (96.5% each), and amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftazidime, and trimethoprim-sulfamethoxazole (95.3% each). In contrast, resistance to ciprofloxacin was low (5.8%), and no resistance was observed to gentamicin or carbapenems.

Healthy-resident isolates exhibited similarly high resistance to β-lactams, including ampicillin (100.0%), cefuroxime (99.8%), cefotaxime (99.6%), cefepime (99.4%), ceftazidime (99.2%), amoxicillin-clavulanic acid (99.0%), and piperacillin-tazobactam (98.8%). Resistance to trimethoprim-sulfamethoxazole was also high (79.8%), while ciprofloxacin resistance was moderate (43.0%) and gentamicin resistance lower (12.7%). Carbapenem and amikacin resistance remained rare.

Clinical isolates showed universal resistance to ampicillin and cephalosporins (100.0%) and high resistance to trimethoprim-sulfamethoxazole (87.1%). However, resistance to amoxicillin-clavulanic acid (29.0%) and piperacillin-tazobactam (19.4%) was lower than in other sources. Ciprofloxacin and gentamicin resistance were higher (48.4% and 32.3%, respectively), while no resistance was observed to carbapenems or amikacin.

Significant between-source differences were observed for several antibiotics, whereas no differences were found for cefotaxime, amikacin, carbapenems, or tigecycline. Ampicillin was not compared due to universal resistance as shown in Table 2.

Table 2 Antibiotic Resistance Profiles of ESBL-producing *E. coli*

Antibiotics	Surface Water Isolates % (N)	Healthy Residents' Isolates % (N)	Clinical Isolates % (N)	X^2 *p*-values	Pairwise SW & Healthy Residents	Pairwise SW & Clinical
Ampicillin	100% (86)	100% (519)	100% (31)	<0.001	N/A	N/A
Amoxicillin/ clavulanic acid	95.3% (82)	99% (514)	29% (9)	<0.001	0.082	<0.001
Piperacillin/ Tazobactam	95.3% (82)	98.8% (513)	19.4% (6)	<0.001	0.041	<0.001
Cefuroxime	96.5% (83)	99.8% (518)	100% (31)	0.0015	0.010	0.564
Cefoxitin	ND	ND	12.9% (4)	N/A	N/A	N/A
Cefotaxime	98.8% (85)	99.6% (517)	100% (31)	0.6373	0.369	1.000
Ceftazidime	95.3%	99.2% (515)	100% (31)	0.0327	0.055	0.970
Cefepime	96.5% (83)	99.4% (516)	100% (31)	0.03024	0.040	0.564

Doripenem	00% (00)	0.8% (4)	ND	N/A	1.000	N/A
Ertapenem,	00% (00)	0.8% (4)	00% (00)	**0.725**	1.000	N/A
Imipenem	00% (00)	0.4% (2)	00% (00)	**0.885**	1.000	N/A
Meropenem	00% (00)	0.4% (2)	00% (00)	**0.797**	1.000	N/A
Amikacin	00% (00)	0.4% (2)	00 % (00)	**0.445**	1.000	N/A
Gentamicin	00% (00)	12.7% (66)	32.3% (10)	<0.001	0.002	< 0.001
Ciprofloxacin	5.8% (5)	43% (223)	48.4% (15)	<0.001	< 0.001	< 0.001
Trimethoprim-Sulfamethoxazole	95.3% (82)	79.8% (414)	87.1% (27)	0.0014	0.002	0.501
nitrofurantoin	ND	ND	00% (00)	N/A	N/A	N/A
Tigecycline	1.2% (1)	3.1% (16)	00% (00)	**0.379**	0.970	1.000

Chi-Square p-values in *Bold correspond to no statistically significant change in resistance between sources. ND = Antibiotic not tested. N/A = Not Applicable. Detailed resistance profiles, including susceptible and intermediate categories, are provided in Supplementary Materials/S2.

Figure 3 presents a heatmap of percentage resistance by isolate source. High resistance to ampicillin, cefuroxime, cefotaxime, ceftazidime, cefepime, and trimethoprim-sulfamethoxazole was observed across all three sources. Surface-water isolates clustered more closely with isolates from healthy residents than with clinical isolates for several β-lactam antibiotics, particularly amoxicillin-clavulanic acid and piperacillin-tazobactam, where resistance exceeded 95% in both surface-water and healthy-resident isolates but was substantially lower among clinical isolates. In contrast, resistance to ciprofloxacin and gentamicin was markedly lower in surface-water isolates than in the two human-associated sources. Overall, hierarchical clustering of percentage resistance profiles suggested tha surface water isolates were more similar to healthy-resident isolates than to clinical isolates for several key resistance patterns.

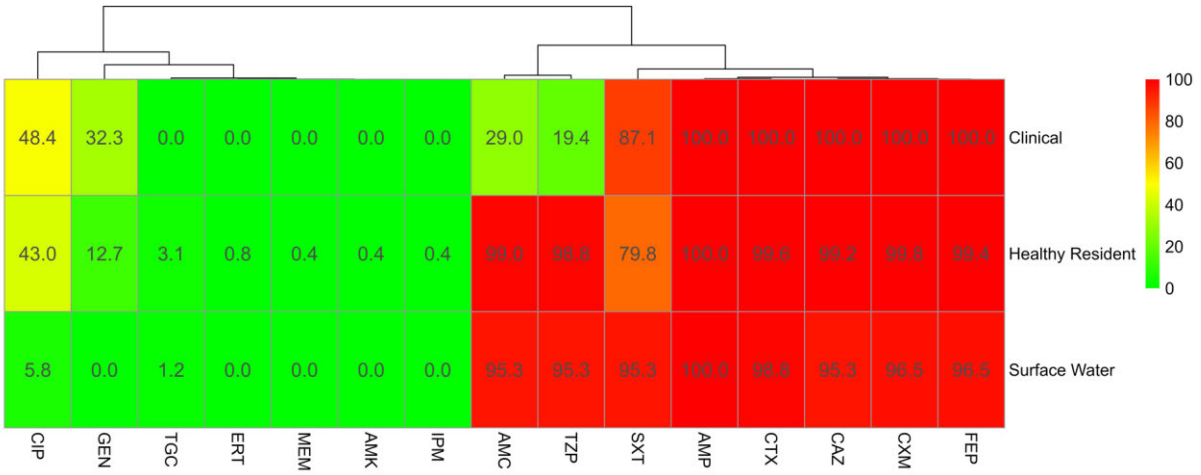

Figure 3:Heatmap of Antibiotic-Resistant Profile of ESBL-Producing *E. coli* by Isolate Source. Rows represent the sources of isolates, and columns represent antibiotics. Red blocks indicate the highest levels of resistance, while green blocks indicate the lowest. The resistance profiles of isolates from surface water closely resembled those from healthy residents than those from clinical sources.

3.3 Antibiograms and Multidrug Resistance of Isolates

MDR prevalence differed across sources, increasing from surface-water isolates to healthy-resident and clinical isolates. Among surface-water isolates, MDR prevalence was low at 5.8% (5/86), with six antibiogram patterns detected. The dominant pattern was non-MDR, AMP-AMC-TZP-CXM-CTX-CAZ-FEP-SXT, observed in 77 isolates (89.5%). The most common MDR pattern was AMP-AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT, observed in four isolates (4.7%) (Figure 4).

In contrast, isolates from healthy residents showed greater antibiogram variation, with 31 distinct patterns, including 20 MDR patterns. MDR prevalence in this group was 41.6% (216/519). The dominant non-MDR pattern was the same as that observed in surface-water isolates, AMP-AMC-TZP-CXM-CTX-CAZ-FEP-SXT, occurring in 197 isolates (38.0%). The most common MDR pattern, AMP-AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT, was also shared with surface-water isolates and was observed in 136 healthy-resident isolates (26.2%).

Clinical isolates had the highest MDR prevalence at 64.5% (20/31), with 14 antibiogram patterns, including nine MDR patterns. The most frequent MDR pattern was AMP-CXM-CTX-CAZ-FEP-CIP-SXT, observed in seven isolates (22.6%), while the dominant non-MDR pattern was AMP-CXM-CTX-CAZ-FEP-SXT, observed in five isolates (16.1%). Compared with surface-water and healthy-resident isolates, the dominant clinical patterns lacked amoxicillin-clavulanic acid and piperacillin-tazobactam resistance, reflecting the lower resistance to these agents among clinical isolates.

The mean number of resistant antimicrobial classes per isolate differed significantly across sources (Kruskal-Wallis test, $p < 0.001$), with the highest average observed among clinical isolates (2.68), followed by healthy-resident isolates (2.39) and surface-water isolates (2.02). Overall, surface-water isolates had the lowest MDR burden but shared dominant antibiogram patterns with healthy-resident isolates, supporting partial phenotypic overlap between these two sourcesFull details of individual resistance patterns and their proportions by source are provided in Supplementary Material/S3

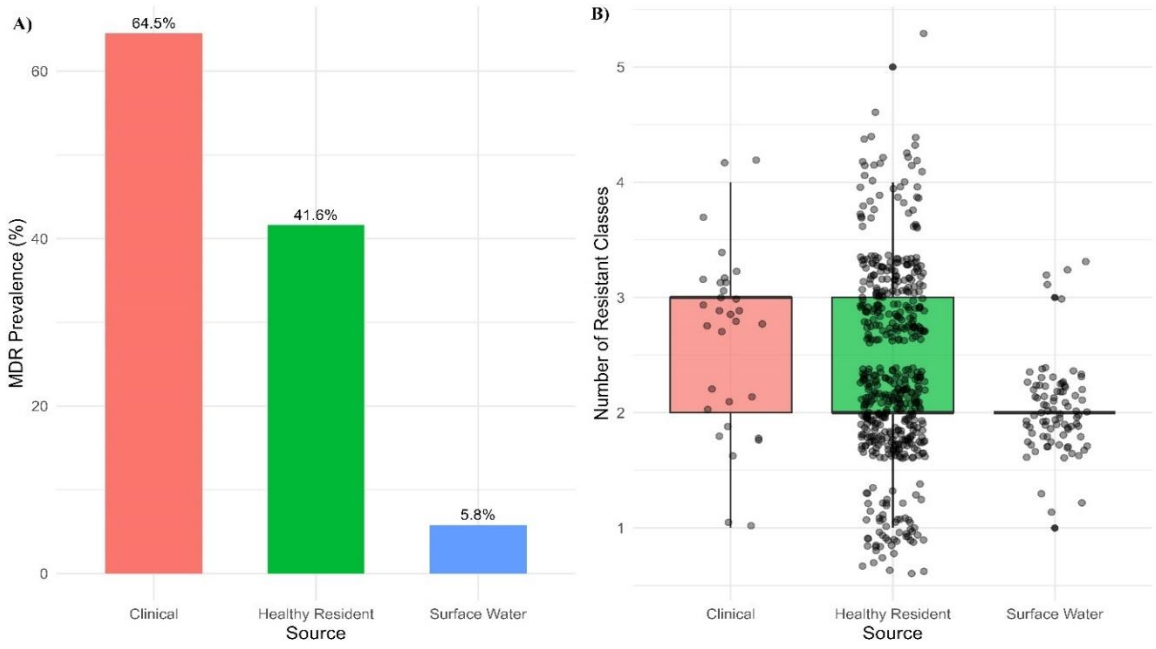

Figure: 4 (a): MDR Prevalence = proportion of isolates per source resistant to ≥3 antibiotic

classes. (b) resistance classes per source. The further the average resistance value is towards ≥3, the more multidrug-resistant the isolates per source were, irrespective of the wide distribution or number of isolates. Clinical isolates were more likely to be MDR than healthy residents or surface water.

3.4 Triangulation of Antibiogram Patterns and MDR between Sources

MDR prevalence differed significantly across the three sources ($p < 0.001$), increasing from surface-water isolates to healthy-resident and clinical isolates. Pairwise comparisons showed that MDR prevalence was significantly lower in surface-water isolates than in clinical isolates, and was also lower than that observed among isolates from healthy residents. Clinical isolates showed the highest MDR prevalence overall.

Across the study, 44 distinct antibiogram patterns were identified, comprising six patterns among surface-water isolates, 31 among healthy-resident isolates, and 14 among clinical isolates. Only two antibiogram patterns were shared across all three sources, including one MDR pattern and one non-MDR pattern. At the non-MDR level, surface-water isolates shared one pattern with healthy-resident isolates, whereas no non-MDR pattern was shared exclusively between surface-water and clinical isolates. The distribution of source-specific and shared antibiogram patterns is shown in Figure 5a, with full details provided in Supplementary Material/S4.

When the analysis was restricted to MDR antibiograms, one MDR pattern was shared across all three sources (Figure 5b). No MDR pattern was shared exclusively between surface-water and healthy-resident isolates or between surface-water and clinical isolates. By contrast, one MDR pattern was shared exclusively between healthy-resident and clinical isolates. Pairwise Jaccard distances based on antibiogram-pattern composition suggested that surface-water isolates were relatively closer to healthy-resident isolates than to clinical isolates, although

between-source dissimilarity remained substantial. The lowest Jaccard distance was observed between surface-water and healthy-resident isolates, while healthy-resident and clinical isolates showed the greatest dissimilarity. PERMANOVA confirmed that overall antibiogram-pattern composition differed significantly among the three sources ($F = 54.586$, $R^2 = 0.1471$, $p = 0.001$).

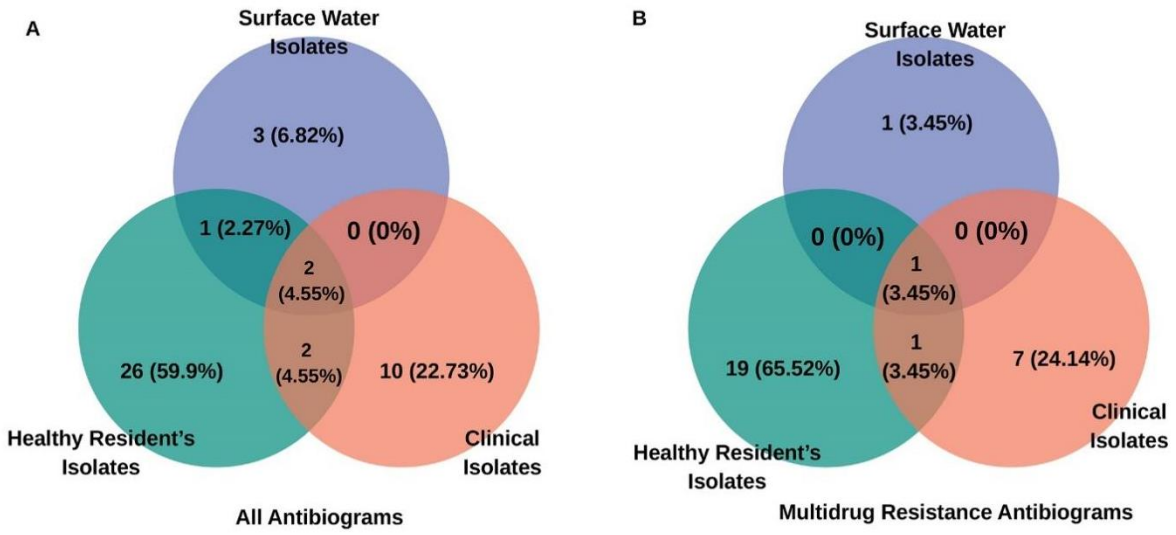

Figure 5: Venn diagrams showing the overlap of ESBL-Producing *E. coli* resistant patterns. One pattern was shared between surface water and healthy residents; no pattern between surface water and clinical; two patterns between healthy residents and clinical; and two patterns shared among all sources (5a). No MDR patterns were shared between surface water and healthy residents, or between surface water and clinical; one MDR pattern was shared between healthy residents and clinical, and one among all sources (5b).

4.0 Discussion

Aquatic environments have increasingly been recognised as reservoirs and possible transmission routes for extended-spectrum beta-lactamase (ESBL)-producing *E. coli*, potentially exposing surrounding communities beyond established healthcare and food-related pathways (Beshiru et al. 2024; Mukwevho et al. 2025). Given the limitations of conventional

AMR surveillance systems, alternative monitoring strategies are needed to better characterise and curb this burden (Schnall et al. 2019). We investigated the prevalence and phenotypic AMR profiles of ESBL-producing *E. coli* isolated from surface water running through an informal settlement, alongside isolates from healthy community residents and clinical samples from the healthcare facilities serving the same settlement, to determine whether resistance levels and patterns in surface water reflect those in the community and in clinical care.

ESBL-producing *E. coli* was recovered from all three sources, and resistance varied significantly between sources for several Access- and Reserve-category antibiotics. Resistance profiles in surface water aligned more closely with those of healthy residents than with clinical isolates for a subset of agents; however, taken as a whole, phenotypic surface-water monitoring did not provide a reliable proxy for AMR in either the community or the clinical setting.

4.1 Prevalence of ESBL-producing *E. coli (*ESBL-Ec)

ESBL-producing ESBL-Ec was recovered from all three sources, supporting its circulation across community, clinical, and environmental compartments in this setting. Prevalence, however, varied markedly by source, highest among healthy residents (27.1%), intermediate in surface water (16.8%), and lowest among clinical isolates (5.3%). This gradient indicates that surface-water prevalence did not directly mirror ESBL-Ec carriage in either human source, but instead represented an intermediate environmental signal.

The higher prevalence among healthy residents than in surface water is consistent with humans representing a more direct reservoir of intestinal ESBL-Ec carriage, whereas environmental detection is shaped by dilution, bacterial survival outside the host, hydrological conditions, and ecological competition. Similar source-dependent differences have been reported elsewhere:

Geuther et al. (2023) found higher community carriage of ESBL-Ec in rural Rwanda than in surrounding aquatic water. Although the aquatic prevalence in that study was lower than observed here, other work has reported surface-water ESBL-Ec contamination comparable to our findings, including 17% and up to 41% in different settings (Azuma et al. 2022; Beshiru et al. 2024). Such variation likely reflects differences in sanitation infrastructure, anthropogenic activity around water bodies, faecal contamination, and sewage or wastewater inputs (Gemmell and Schmidt 2013; Gumede et al. 2021). Because the aquatic environment is rarely the primary source of contamination, prevalence there is typically lower than in humans directly, as observed here, owing to dilution, environmental stress, and ecological competition (Cho et al. 2023).

By contrast, surface-water ESBL-Ec prevalence in this study (16.8%) was notably higher than among clinical isolates (5.3%), the reverse of a previous finding reporting significantly higher prevalence in clinical isolates (34.9%) than in river water (23.7%) (Leopold et al. 2024). While higher ESBL-Ec levels are more commonly observed in clinical settings due to stronger selection pressure, sewage discharge releasing substantial ESBL-Ec loads into aquatic environments cannot be discounted. Fagerström et al. (2019), studying ESBL-producing *E. coli* from clinical urine samples and environmental waters, identified environmental isolates from wastewater, river, and lake water that were indistinguishable or closely related to clinical isolates, supporting a role for wastewater discharge in disseminating ESBL-Ec into aquatic environments. In informal settlements with inadequate sanitation and waste-disposal infrastructure, surrounding surface water may be particularly heavily contaminated with faeces, increasing the environmental burden of resistant organisms (Erb et al. 2018). Earlier work in the same district detected ESBL-producing *E. coli* in the local sewage treatment system (Gumede et al. 2021), supporting the plausibility of environmental contamination in this

setting. However, the lower prevalence observed among clinical isolates should be interpreted cautiously. Clinical isolates were derived from routine diagnostic specimens and therefore primarily represent infection-associated isolates from patients accessing healthcare, whereas stool samples from healthy residents represent asymptomatic faecal carriage. Surface-water isolates, in turn, reflect environmental contamination potentially arising from faecal shedding and other sources. Consequently, prevalence across these compartments should not be interpreted as directly equivalent measures of carriage or infection burden. In addition, methodological differences in isolate detection and classification may have influenced direct prevalence comparisons between sources. Importantly, the lower proportion of ESBL-producing *E. coli* among clinical isolates does not imply a lower overall resistance burden in the clinical setting, as clinical ESBL-producing isolates exhibited the highest MDR prevalence. Rather, the finding reflects differences in the proportion of *E. coli* classified as ESBL-producing within distinct source populations. Although the current study did not assess the plant's contamination risk to surrounding aquatic environment, its presence, alongside poor waste management practice in the informal settlement, might indirectly contribute to the higher surface water ESBL levels found, creating a reservoir that increases the risk of human exposure. Overall, these findings indicate that the prevalence of ESBL-producing *E. coli* in surface water did not reliably reflect the prevalence observed in either the healthy resident or clinical setting. However, the detection of ESBL-producing *E. coli* in all three compartments highlights the relevance of surface water as a potential environmental reservoir within the local AMR landscape. This warrants further investigation using high-resolution molecular approaches to determine whether isolates detected across sources are clonally related or share common ESBL-associated resistance determinants.

4.2 Antimicrobial Susceptibility of ESBL-Producing *E. coli*

Following the observed differences in ESBL-Ec prevalence, resistance profiles also varied significantly by source ($p < 0.05$). As expected, isolates from all three sources showed high resistance to ampicillin and extended-spectrum cephalosporins, consistent with the ESBL phenotype (Husna et al. 2023); high resistance to trimethoprim-sulfamethoxazole was likewise observed across sources, suggesting resistance to commonly used antibiotics is widespread among ESBL-Ec in this setting. The antibiotics evaluated also spanned the WHO Access, Watch and Reserve (AWaRe) categories, a stewardship framework based on resistance potential and recommended use. Among the agents examined, amoxicillin-clavulanic acid, amikacin, gentamicin and trimethoprim-sulfamethoxazole are Access antibiotics; piperacillin-tazobactam, cephalosporins, carbapenems and ciprofloxacin are predominantly Watch antibiotics; while tigecycline is classified as Reserve (WHO, 2021).

This clustering of high resistance to ampicillin, cephalosporins, and trimethoprim-sulfamethoxazole is consistent with previous South African studies reporting high resistance to these agents among *E. coli* from the community (Kubone et al. 2020) wastewater and rivers (Gumede et al. 2021) and hospital settings (Peirano et al. 2011). These older, widely available antibiotics are among the most dispensed or inappropriately prescribed in South Africa (Manderson, 2020; Nsingo et al. 2025), plausibly contributing to elevated resistance across all sources. The prominence of trimethoprim-sulfamethoxazole resistance specifically was expected, given the drug's ready availability at community healthcare centres for HIV/AIDS prophylaxis (Daniels et al. 2019) and its use in animal husbandry (Chipangura et al. 2017), both of which may fuel resistance in humans and their environment.

Despite the observed clustering of ampicillin, cephalosporins and trimethoprim-sulfamethoxazole, the levels were not the same for surface water, healthy residents, or clinical isolates. Variation in resistance levels across sources may reflect differential antibiotic selection pressures linked to the extent of antimicrobial use and discharge (MacFadden et al., 2019). Although this study did not specifically examine the role of drug consumption or discharge, earlier South African studies have reported elevated antibiotic use (Manderson, 2020) and evidence of above-average antibiotic loads discharged from informal settlements (Holton et al., 2023), which can contribute to increased selection pressure.

Interestingly, apart from the clustering of high ampicillin, cephalosporin, and trimethoprim-sulfamethoxazole resistance from all three sources, surface-water isolates showed closer phenotypic clustering with isolates from healthy residents for antibiotics such as amoxicillin-clavulanic acid and piperacillin/tazobactam than with clinical isolates (Figure 3). This aligns with previous findings reporting high levels of antibiotic resistance in both residents of communities and their surrounding water (Jørgensen et al., 2017; Soma et al., 2024). Jorgensen et al., in a tricycle study, sampled humans, wastewater and fresh water in proximity and reported resistance levels to ampicillin, cephalosporins and trimethoprim-sulfamethoxazole all above 50%. Another study (Zarfel et al., 2013) also noted similar drug-specific resistance between human and environmental isolates, with variation to the extent to which isolates from both sources were resistant to antibiotics like ampicillin/clavulanic acid (environment, 54% human; 86%, $p < 0.01$), and trimethoprim-sulfamethoxazole (environment, 44% human; 90%, $p < 0.05$).

Community anthropogenic activities can shape the environmental resistome in water. For example, a study done in Stellenbosch, South Africa, assessing anthropogenic pressures from informal settlements, residential, industrial, agricultural, and pristine sites, found that sites

influenced by informal settlements and residential waste had a higher prevalence of resistance to certain antibiotics (ampicillin/clavulanic acid and piperacillin/ tazobactam) compared to less contaminated sites (Tucker et al., 2022). This evidence highlights the role of community-associated waste in shaping the resistance characteristics of surrounding aquatic environments, reinforcing the need for continuous monitoring of surface water as an environmentally relevant AMR reservoir.

As the clustering noted between surface water and healthy residents isolates for certain antibiotics, we did not observe additional resistance clustering in clinical isolates beyond ampicillin, cephalosporins, and trimethoprim-sulfamethoxazole. This does not exclude the possibility of ecological connectivity between clinical settings and nearby water sources, which may have affected the results if the use of ampicillin/clavulanic acid and piperacillin/tazobactam is more regulated in the hospital setting. Nonetheless, though not specifically examined in the current study, clinical isolates grouped more closely with healthy residents' isolates in terms of resistance to ciprofloxacin and gentamicin.

On the other hand, the level of resistance was not significantly different across ESBL-Ec sources for amikacin, carbapenems, and tigecycline ($p > 0.05$), suggesting their effectiveness against isolates from our study setting. Previous studies have reported zero to very low resistance of *E. coli* to these antibiotics in the community (Kubone et al., 2020), the clinical setting (Peirano et al., 2011), and the environment (Gumede et al., 2021).

Leopold et al. assessed AMR patterns of *E. coli* from surface water and patient in an Austrian study and reported insignificant differences in resistance levels against amikacin, imipenem, meropenem and tigecycline (Leopold et al., 2024). Similarly, another study conducted in Madagascar, aiming to implement WHO’s one health tricycle protocol for ESBL-Ec, reported

insignificant variations in phenotypic resistance against amikacin, imipenem and ertapenem for healthy pregnant women and surface water (Milenkov et al., 2024) These findings highlight the effectiveness of these drugs also known as last-line or reserved and regulated antibiotics under the WHO's AWaRe 2021 list (WHO, 2021) for treatment of MDR *E. coli* strains.

Overall, we observed that ESBL-Ec exhibited considerable variability in resistance to older antibiotics, while resistance to last-line antibiotics remained minimal. Notably, surface water and healthy resident isolates showed similarly high resistance levels to ampicillin, cephalosporins, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, and piperacillin-tazobactam; similarity between surface water and clinical isolates was limited to ampicillin and cephalosporins.

4.3 Antibiotic Resistant Patterns and Multidrug Resistance (MDR) of Isolates

MDR isolates were present across all three sources but were significantly more common among clinical isolates than among healthy-resident or surface-water isolates ($p < 0.05$). Surface-water isolates showed significantly lower MDR proportions than clinical isolates notable given that surface water had a higher overall ESBL prevalence than clinical isolates, indicating that ESBL production and multidrug resistance do not necessarily co-occur at the same intensity across environmental and clinical compartments. This pattern is consistent with a comparison of patient and river-water isolates reporting MDR rates of 7% and 21% in surface water and clinical isolates, respectively Leopold et al. (2024); the higher MDR burden in clinical settings more broadly aligns with literature citing over-reliance on last-line antibiotics, concentration of vulnerable patients, invasive procedures, and nosocomial transmission as drivers of MDR in hospitals (Wang et al. 2018; Cocker et al. 2023; Mustafa et al. 2024).

MDR prevalence among healthy residents was likewise significantly higher than in surface water. High circulation of MDR Enterobacterales has previously been reported in sewage

treatment plants serving this district Gumede et al., (2021) as well as among patients elsewhere (Founou et al. 2019), making the elevated human MDR rates observed here unsurprising. The comparatively lower MDR prevalence in surface water may reflect environmental dilution, microbial competition, and inactivation outside the human host, all of which reduce the concentration and viability of resistant bacteria once released into water (Wellington et al. 2013; Goryluk-Salmonowicz and Popowska 2022).

Despite this variation, cluster analysis of resistance patterns (Figure 5) revealed limited but real overlap across sources, with two shared phenotypic antibiograms present across all three. Notably, several surface-water isolates shared a non-MDR antibiogram with healthy-resident isolates, though no MDR pattern was shared between surface water and either human source. This shared non-MDR antibiogram between surface water and healthy residents suggests some degree of connectivity between the community and surrounding aquatic reservoirs, plausibly via contamination or horizontal gene transfer. PERMANOVA supported this picture ($F = 54.6$, $R^2 = 0.147$, $p = 0.001$): isolate source accounted for approximately 14.7% of total variation in resistance-pattern composition, with the remaining 85.3% likely attributable to unmeasured factors such as environmental exposures and host- or site-specific conditions. Given earlier evidence of ESBL-Ec in wastewater reservoirs in this locality Gumede et al., (2021) and river pollution linked to community activity Gemmell & Schmidt (2013), some ecological connectivity between these compartments whether via direct contamination, shared selection pressures, or circulation of related strains appears plausible, though molecular analysis would be needed to confirm its nature.

Given the source variation observed in our study at all levels, our surface-water isolates did not provide a reliable phenotypic reflection of MDR ESBL-Ec in either the community or clinical

setting. However, the presence of MDR isolates and the shared phenotypic antibiograms observed across sources point to surface water as a potential transmission route and a relevant environmental reservoir, warranting further molecular investigation into shared ESBL-encoding genes or resistance plasmids across these compartments.

Some methodological considerations should be taken into account when interpreting these findings. Differences in ESBL-detection approaches across sources may have influenced direct comparisons, analyses were conducted at the isolate level despite repeated sampling from participants and surface-water sites, and clinical isolates were obtained from a broader routine diagnostic population that may not have fully overlapped with the specific community cohort sampled. In addition, phenotypic data alone cannot establish clonal relatedness, molecular typing would be needed to confirm whether the resistance similarities observed reflect shared strains or shared environmental selection pressures. Nevertheless, the approach used here provides a practical framework for comparing AMR signals across compartment. Given the scarcity of studies directly triangulating phenotypic AMR data across surface aquatic environmental, community, and clinical sources, the approach applied here offers a practical model that could be extended to other informal-settlement and resource-limited settings where conventional wastewater-based surveillance remains infeasible.

5.0 Conclusion

This study identified ESBL-producing *E. coli* at significantly different prevalences across surface water, healthy informal-settlement residents, and proximate clinical isolates, with the highest burden in the community and the lowest in the clinical setting. Surface-water isolates shared phenotypic resistance features with both community and clinical isolates, particularly for older antibiotics, and showed greater overall similarity to community isolates than to

clinical isolates. However, multidrug resistance was concentrated in the human compartments, antibiogram overlap across sources was limited, and surface-water phenotypic profiles did not reliably reproduce the AMR burden observed in either human source. These findings do not support using surface-water phenotypic data alone as a proxy for community or clinical AMR burden in this informal-settlement setting. Nevertheless, the detection of ESBL-producing *E. coli* and shared resistance features across all three compartments highlights the relevance of surface water as an environmental AMR reservoir, and supports its inclusion as a complementary component of One Health surveillance in settings where conventional wastewater-based approaches are not feasible. High-resolution molecular analysis is needed to determine whether the observed phenotypic overlap reflects shared strains, shared resistance determinants, or common environmental selection pressures.

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Declaration of competing interest

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List of Supplementary Materials (Manuscript 1)

- **Supplementary Material/S1** - Ethical Consideration and Informed Consent; Refer to Appendices 1.1 to 1.4 of this Thesis. **Page 284 - 287.**
- **Supplementary Material/S2** - AST of ESBL *E. coli* including Resistant, Intermediate and Sensitive proportions Across Sources. **Page 121.**
- **Supplementary Material/S3** - Antibiograms Patterns, of ESBL *E. coli*, MDR flags and distribution Across Sources. **Page 122**
- **Supplementary Material/S4** - Venn Analysis Antibiograms profiles of ESBL *E. coli* across Sources. **Page 123.**

Supplementary Material/S2 – AST of ESBL *E. coli* including Resistant, Intermediate and Sensitive proportions Across Sources

Antibiotic	Clinical_SIR	Healthy Individuals_SIR	Surface Water_SIR
AMK	I=3.2% (1), S=96.8% (30)	I=3.7% (19), R=0.4% (2), S=96% (498)	S=100% (86)
AMP	R=100% (31)	R=100% (519)	R=100% (86)
AMX	I=22.6% (7), R=29% (9), S=48.4% (15)	R=99% (514), S=1% (5)	R=95.3% (82), S=4.7% (4)
CAZ	R=100% (31)	I=0.4% (2), R=99.2% (515), S=0.4% (2)	I=1.2% (1), R=95.3% (82), S=3.5% (3)
CFX	R=12.9% (4), S=87.1% (27)	NA=100% (519)	NA=100% (86)
CIP	R=48.4% (15), S=51.6% (16)	I=13.1% (68), R=43% (223), S=43.9% (228)	I=3.5% (3), R=5.8% (5), S=90.7% (78)
CTX	R=100% (31)	I=0.2% (1), R=99.6% (517), S=0.2% (1)	R=98.8% (85), S=1.2% (1)
CXM	R=100% (31)	R=99.8% (518), S=0.2% (1)	R=96.5% (83), S=3.5% (3)
DRO	NA=100% (31)	I=1% (5), R=0.8% (4), S=98.3% (510)	S=100% (86)
ERT	S=100% (31)	I=1% (5), R=0.8% (4), S=98.3% (510)	S=100% (86)
FEP	R=100% (31)	R=99.4% (516), S=0.6% (3)	R=96.5% (83), S=3.5% (3)
GEN	R=32.3% (10), S=67.7% (21)	I=0.4% (2), R=12.7% (66), S=86.9% (451)	S=100% (86)
IPM	S=100% (31)	I=0.6% (3), R=0.4% (2), S=99% (514)	S=100% (86)
MEM	S=100% (31)	R=0.4% (2), S=99.6% (517)	S=100% (86)
NIT	I=3.2% (1), S=96.8% (30)	NA=100% (519)	NA=100% (86)
SXT	R=87.1% (27), S=12.9% (4)	R=79.8% (414), S=20.2% (105)	R=95.3% (82), S=4.7% (4)
TGC	S=100% (31)	R=3.1% (16), S=96.9% (503)	R=1.2% (1), S=98.8% (85)
TOB	NA=100% (31)	I=1% (5), R=13.7% (71), S=84% (436), s=1.3% (7)	R=2.3% (2), S=97.7% (84)
TZP	I=12.9% (4), R=19.4% (6), S=67.7% (21)	R=98.8% (513), S=1.2% (6)	R=95.3% (82), S=4.7% (4)

Supplementary Material/S3 – Antibiogram Patterns, of ESBL *E. coli*, MDR flags and distribution Across Sources

Pattern	SurfaceWater	Healthy_Residents	Clinical
AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP (4 isolates, 4.7%, MDR)	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP (136 isolates, 26.2%, MDR)	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP (2 isolates, 6.5%, MDR)
AMC-AMP-CAZ-CTX-CXM-FEP-SXT-TZP	AMC-AMP-CAZ-CTX-CXM-FEP-SXT-TZP (77 isolates, 89.5%, Non-MDR)	AMC-AMP-CAZ-CTX-CXM-FEP-SXT-TZP (196 isolates, 37.8%, Non-MDR)	AMC-AMP-CAZ-CTX-CXM-FEP-SXT-TZP (1 isolates, 3.2%, Non-MDR)
AMC-AMP-CAZ-CTX-CXM-FEP-TGC-TZP	AMC-AMP-CAZ-CTX-CXM-FEP-TGC-TZP (1 isolates, 1.2%, Non-MDR)	AMC-AMP-CAZ-CTX-CXM-FEP-TGC-TZP (2 isolates, 0.4%, Non-MDR)	
AMP	AMP (1 isolates, 1.2%, Non-MDR)		
AMP-CIP-CTX-CXM-FEP-SXT	AMP-CIP-CTX-CXM-FEP-SXT (1 isolates, 1.2%, MDR)		
AMP-CTX	AMP-CTX (2 isolates, 2.3%, Non-MDR)		
AMC-AMK-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP		AMC-AMK-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP (1 isolates, 0.2%, MDR)	
AMC-AMK-AMP-CAZ-CTX-CXM-FEP-SXT-TZP		AMC-AMK-AMP-CAZ-CTX-CXM-FEP-SXT-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-GEN-SXT-TGC-TZP		AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-GEN-SXT-TGC-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-IPM-MEM-SXT-TGC-TZP		AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-IPM-MEM-SXT-TGC-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-SXT-TGC-TZP		AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-SXT-TGC-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT		AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TGC-TZP		AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TGC-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TZP		AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TZP (30 isolates, 5.8%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-TZP		AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-TZP (2 isolates, 0.4%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-FEP-IPM-SXT-TGC-TZP		AMC-AMP-CAZ-CIP-CTX-CXM-FEP-IPM-SXT-TGC-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TGC-TZP		AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TGC-TZP (3 isolates, 0.6%, MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-FEP-TZP		AMC-AMP-CAZ-CIP-CTX-CXM-FEP-TZP (41 isolates, 7.9%, Non-MDR)	
AMC-AMP-CAZ-CTX-CXM-ERT-FEP-SXT-TGC-TZP		AMC-AMP-CAZ-CTX-CXM-ERT-FEP-SXT-TGC-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TGC-TZP		AMC-AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TGC-TZP (2 isolates, 0.4%, MDR)	
AMC-AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TZP		AMC-AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TZP (27 isolates, 5.2%, MDR)	AMC-AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TZP (1 isolates, 3.2%, MDR)
AMC-AMP-CAZ-CTX-CXM-FEP-MEM-SXT-TGC-TZP		AMC-AMP-CAZ-CTX-CXM-FEP-MEM-SXT-TGC-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CTX-CXM-FEP-SXT		AMC-AMP-CAZ-CTX-CXM-FEP-SXT (1 isolates, 0.2%, Non-MDR)	AMC-AMP-CAZ-CTX-CXM-FEP-SXT (2 isolates, 6.5%, Non-MDR)
AMC-AMP-CAZ-CTX-CXM-FEP-SXT-TGC-TZP		AMC-AMP-CAZ-CTX-CXM-FEP-SXT-TGC-TZP (2 isolates, 0.4%, MDR)	
AMC-AMP-CAZ-CTX-CXM-FEP-TZP		AMC-AMP-CAZ-CTX-CXM-FEP-TZP (58 isolates, 11.2%, Non-MDR)	
AMC-AMP-CAZ-CXM-FEP-GEN-SXT-TZP		AMC-AMP-CAZ-CXM-FEP-GEN-SXT-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CAZ-CXM-FEP-SXT-TZP		AMC-AMP-CAZ-CXM-FEP-SXT-TZP (1 isolates, 0.2%, Non-MDR)	
AMC-AMP-CIP-CTX-CXM-FEP-SXT-TZP		AMC-AMP-CIP-CTX-CXM-FEP-SXT-TZP (1 isolates, 0.2%, MDR)	
AMC-AMP-CTX-CXM-FEP-SXT		AMC-AMP-CTX-CXM-FEP-SXT (1 isolates, 0.2%, Non-MDR)	
AMP-CAZ-CIP-CTX-CXM-FEP-TZP		AMP-CAZ-CIP-CTX-CXM-FEP-TZP (1 isolates, 0.2%, Non-MDR)	
AMP-CAZ-CIP-CTX-CXM-SXT		AMP-CAZ-CIP-CTX-CXM-SXT (1 isolates, 0.2%, MDR)	
AMP-CAZ-CTX-CXM-FEP-SXT-TZP		AMP-CAZ-CTX-CXM-FEP-SXT-TZP (1 isolates, 0.2%, Non-MDR)	
AMP-CIP-CTX-CXM-SXT		AMP-CIP-CTX-CXM-SXT (1 isolates, 0.2%, MDR)	
AMP-CTX-GEN		AMP-CTX-GEN (1 isolates, 0.2%, Non-MDR)	
AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT			AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT (1 isolates, 3.2%, MDR)
AMC-AMP-CAZ-CTX-CXM-FEP			AMC-AMP-CAZ-CTX-CXM-FEP (2 isolates, 6.5%, Non-MDR)
AMP-CAZ-CIP-CTX-CXM-FEP			AMP-CAZ-CIP-CTX-CXM-FEP (1 isolates, 3.2%, Non-MDR)
AMP-CAZ-CIP-CTX-CXM-FEP-GEN			AMP-CAZ-CIP-CTX-CXM-FEP-GEN (1 isolates, 3.2%, MDR)
AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT			AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT (2 isolates, 6.5%, MDR)
AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TZP			AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TZP (1 isolates, 3.2%, MDR)
AMP-CAZ-CIP-CTX-CXM-FEP-SXT			AMP-CAZ-CIP-CTX-CXM-FEP-SXT (7 isolates, 22.6%, MDR)
AMP-CAZ-CTX-CXM-FEP-GEN-SXT			AMP-CAZ-CTX-CXM-FEP-GEN-SXT (4 isolates, 12.9%, MDR)
AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TZP			AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TZP (1 isolates, 3.2%, MDR)
AMP-CAZ-CTX-CXM-FEP-SXT			AMP-CAZ-CTX-CXM-FEP-SXT (5 isolates, 16.1%, Non-MDR)

Supplementary Material/S4 – Venn Analysis Antibiograms profiles of ESBL *E. coli* across Sources.

Venn ALL_Patterns_List	
Category	**Pattern**
unique_s1	AMP-CTX
unique_s1	AMP
unique_s1	AMP-CIP-CTX-CXM-FEP-SXT
unique_s2	AMP-CIP-CTX-CXM-SXT
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TZP
unique_s2	AMC-AMP-CAZ-CTX-CXM-FEP-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-TZP
unique_s2	AMP-CTX-GEN
unique_s2	AMC-AMP-CTX-CXM-FEP-SXT
unique_s2	AMC-AMP-CAZ-CXM-FEP-SXT-TZP
unique_s2	AMC-AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CTX-CXM-ERT-FEP-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-GEN-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TGC-TZP
unique_s2	AMC-AMP-CIP-CTX-CXM-FEP-SXT-TZP
unique_s2	AMP-CAZ-CIP-CTX-CXM-FEP-TZP
unique_s2	AMC-AMK-AMP-CAZ-CTX-CXM-FEP-SXT-TZP
unique_s2	AMC-AMP-CAZ-CXM-FEP-GEN-SXT-TZP
unique_s2	AMP-CAZ-CTX-CXM-FEP-SXT-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT
unique_s2	AMP-CAZ-CIP-CTX-CXM-SXT
unique_s2	AMC-AMP-CAZ-CTX-CXM-FEP-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-IPM-MEM-SXT-TGC-TZP
unique_s2	AMC-AMK-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-IPM-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CTX-CXM-FEP-MEM-SXT-TGC-TZP
unique_s3	AMP-CAZ-CTX-CXM-FEP-GEN-SXT
unique_s3	AMP-CAZ-CTX-CXM-FEP-SXT
unique_s3	AMP-CAZ-CIP-CTX-CXM-FEP-SXT
unique_s3	AMP-CAZ-CIP-CTX-CXM-FEP
unique_s3	AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT
unique_s3	AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TZP
unique_s3	AMC-AMP-CAZ-CTX-CXM-FEP
unique_s3	AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TZP
unique_s3	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT
unique_s3	AMP-CAZ-CIP-CTX-CXM-FEP-GEN
shared_s1_s2	AMC-AMP-CAZ-CTX-CXM-FEP-TGC-TZP
shared_s2_s3	AMC-AMP-CAZ-CTX-CXM-FEP-SXT
shared_s2_s3	AMC-AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TZP
shared_all	AMC-AMP-CAZ-CTX-CXM-FEP-SXT-TZP
shared_all	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP

Venn MDR_Patterns_List	
Category	**Pattern**
unique_s1	AMP-CIP-CTX-CXM-FEP-SXT
unique_s2	AMP-CIP-CTX-CXM-SXT
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TZP
unique_s2	AMC-AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CTX-CXM-ERT-FEP-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-GEN-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TGC-TZP
unique_s2	AMC-AMP-CIP-CTX-CXM-FEP-SXT-TZP
unique_s2	AMC-AMK-AMP-CAZ-CTX-CXM-FEP-SXT-TZP
unique_s2	AMC-AMP-CAZ-CXM-FEP-GEN-SXT-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT
unique_s2	AMP-CAZ-CIP-CTX-CXM-SXT
unique_s2	AMC-AMP-CAZ-CTX-CXM-FEP-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-ERT-FEP-IPM-MEM-SXT-TGC-TZP
unique_s2	AMC-AMK-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-IPM-SXT-TGC-TZP
unique_s2	AMC-AMP-CAZ-CTX-CXM-FEP-MEM-SXT-TGC-TZP
unique_s3	AMP-CAZ-CTX-CXM-FEP-GEN-SXT
unique_s3	AMP-CAZ-CIP-CTX-CXM-FEP-SXT
unique_s3	AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT
unique_s3	AMP-CAZ-CIP-CTX-CXM-FEP-GEN-SXT-TZP
unique_s3	AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TZP
unique_s3	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT
unique_s3	AMP-CAZ-CIP-CTX-CXM-FEP-GEN
shared_s2_s3	AMC-AMP-CAZ-CTX-CXM-FEP-GEN-SXT-TZP
shared_all	AMC-AMP-CAZ-CIP-CTX-CXM-FEP-SXT-TZP

Venn ALL_Patterns_Summary		
Region	**Count**	**Percent**
unique_s1	3	6.82
unique_s2	26	59.09
unique_s3	10	22.73
shared_s1_s2	1	2.27
shared_s1_s3	0	0
shared_s2_s3	2	4.55
shared_all	2	4.55

Veen MDR_Patterns_Summary		
Region	**Count**	**Percent**
unique_s1	1	3.45
unique_s2	19	65.52
unique_s3	7	24.14
shared_s1_s2	0	0
shared_s1_s3	0	0
shared_s2_s3	1	3.45
shared_all	1	3.45

Legend
S1 = Surface water
S2 = Healhty Residents
S3= Clinical

CHAPTER THREE - MANUSCRIPT 2

MONITORING EXTENDED SPECTRUM β-LACTAMASES-PRODUCING KLEBSIELLA PNEUMONIAE: DO SURFACE WATER PHENOTYPIC PATTERNS REFLECT COMMUNITY AND CLINICAL RESISTANCE?

Author Contributions

Etando Ayukafangha - as principal investigator, co-conceptualised the study, performed the laboratory work, analysed the data, and drafted the manuscript.

Joshua Mbanga - as co-supervisor, co-conceptualised the study, assisted with sample collection and processing, vetted the results, and critically reviewed the manuscript.

Sabiha Y. Essack - as co-supervisor, co-conceptualised the study, secured funding, guided the literature review and ethical approval application, facilitated data collection, vetted the results, and critically reviewed the manuscript.

Akebe Luther King Abia - as main supervisor, co-conceptualised the study, guided the literature review, data collection, processing and analysis, vetted the results, and critically reviewed the manuscript.

Objectives met - This chapter answers the ESBL-producing *K. pneumoniae* component of objective 1.

Monitoring Extended-spectrum-β-lactamases-producing *Klebsiella pneumoniae*: Do Surface Water Phenotypic Patterns Reflect Community and Clinical Resistance?

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ABSTRACT

Purpose: Purpose: ESBL-producing *Klebsiella pneumoniae* (ESBL-Kp) prevalence and resistance profiles were evaluated to determine whether surface water (SW) could serve as an Alternative Antimicrobial-resistance Monitoring System (AlARMS) for nearby community healthy residents (HR) and clinical (CL) settings. Method: Over eight months, 462 SW, 1,167 HR, and 244 CL *K. pneumoniae* isolates were screened for ESBL production; antimicrobial susceptibility testing was conducted using VITEK 2. **Results**: ESBL-Kp prevalence varied significantly ($p < 0.05$) among SW (13.0%), HR (6.3%), and CL (15.2%). Multidrug resistance (MDR) was highest in CL (94.6%) and lowest in SW (25.0%). SW and HR isolates showed similar resistance ($p > 0.05$) to amoxicillin-clavulanic acid, piperacillin-tazobactam, cephalosporins, carbapenems, gentamicin, and ciprofloxacin, but differed significantly ($p < 0.05$) for tobramycin, trimethoprim-sulfamethoxazole, and tigecycline. SW and CL isolates showed similar resistance ($p > 0.05$) mainly to cephalosporins, carbapenems, amikacin, and tigecycline, but differed significantly ($p < 0.05$) for amoxicillin-clavulanic acid, piperacillin-tazobactam, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole. A non-MDR pattern was common to all sources, with greater SW-HR than SW-CL overlap. MDR pattern overlap was observed between SW and HR but not between SW and CL. **Conclusion:** Surface-water isolates did not fully mirror clinical or community phenotypes; nevertheless, shared clinically relevant resistance patterns, particularly with healthy residents, suggest potential epidemiological linkages, necessitating refined molecular insights for AlARMS evaluation.

Keywords: One-Health; Early warning systems; aquatic ecosystems; antimicrobial resistance; public health

1.0 INTRODUCTION

The World Health Organisation (WHO) considers infections caused by multidrug-resistant (MDR) bacteria as a significant public health concern. MDR is a type of antimicrobial resistance (AMR) in which microbes, such as bacteria, are resistant to multiple antibiotics from three or more classes (WHO, 2023b). The AMR crisis has intensified dramatically in recent decades, fuelled primarily by the misuse and overuse of antibiotics. This resistance surge has significantly broadened the range and number of bacterial pathogens that defy treatment, accounting for up to 4.71 million deaths associated with bacterial AMR, including 1.14 million attributable deaths as of 2021 (Naghavi et al., 2024). As a result, regulatory bodies such as the WHO published a list of priority bacterial pathogens for research and development of new antibiotics in 2017 and later updated it in 2024. Extended-spectrum β-lactamase-producing and carbapenem-resistant Enterobacterales, including *Klebsiella pneumoniae* (*K. pneumoniae*), are among the critical pathogens on the list (WHO 2017, WHO 2024).

K. pneumoniae is a Gram-negative bacterium of the Enterobacterales family, found in the environment (soil, surface water), on mammalian mucus membranes and in humans, where it colonises the upper part of the nasopharynx and gastrointestinal tract (Choby et al., 2020). *K. pneumoniae* possesses intrinsic resistance to ampicillin via the SHV-1 β-lactamase; however, it has acquired multidrug resistance through various mechanisms. In addition to producing extended-spectrum β-lactamases (ESBLs) and carbapenemases that degrade β-lactams, it employs aminoglycoside-modifying enzymes, mutations in DNA gyrase genes conferring fluoroquinolone resistance, and plasmid-mediated mcr genes conferring colistin resistance. Furthermore, active efflux pumps and decreased membrane permeability often render these strains resistant to nearly all available antibiotic classes (Han et al., 2025; Li et al., 2023).

The emergence of ESBL-Kp resistance represents a serious public health concern in both healthcare (Founou et al., 2019) and community settings (Olaitan et al., 2025). Due to its opportunistic nature, current evidence suggests it has a wider ecological distribution and greater AMR diversity than other Gram-negative opportunists, suggesting its role in disseminating resistance between humans and their surrounding environments (Hooban et al., 2020; Wyres & Holt, 2018). The presence of ESBL-Kp has been reported across various niches, including the environment (Kagambèga et al., 2024), community residents (Mahomed et al., 2014) and clinical settings (Founou et al., 2019; Starzyk-Łuszcz et al., 2017). These studies highlighted the need for integrated surveillance that links human, animal, and environmental health to effectively manage the risks of exposure and transmission.

AMR surveillance in bacteria, including *K. pneumoniae*, has relied on conventional laboratory detection methods, which have been noted to underestimate or overestimate AMR depending on the representativeness and robustness of the surveillance programme (Sugianli et al., 2020). In Low- and Middle-Income Countries (LMICs), the underutilisation of microbiological testing for both patient management and surveillance is largely driven by high costs, limited laboratory infrastructure, and a lack of standardised reporting(Lim et al., 2021b) This surveillance gap is particularly pronounced in environmental and community settings, where the absence of routine monitoring obscures the transmission pathways of resistant strains. With the existence of resistant bacteria in the environment and the potential usefulness of the environment in monitoring AMR (Kagambèga et al., 2024; Rolbiecki et al., 2025), there has been a surge in exploring environmental elements like wastewater treatment plants (WWTPs) and hospital influent and effluent, and surface water for monitoring of AMR.

The presence of resistant bacteria and genes in the environment could result from inappropriate disposal of faecal waste, community-level animal and agricultural activities, and sewage system effluents (Mills & Lee, 2019; Nashwan et al., 2024). These contamination sources may differ by community type.

Informal settlements, also called slums, are unplanned, improvised human settlements that are not surveyed as residential areas and are characterised primarily by informal structures (Ooi & Phua, 2007). These settlements are usually overcrowded, with infrastructure deficits, including inadequate access to clean water, sanitation (including toilet facilities), and poor drainage systems, thereby increasing residents' vulnerability to health risks and making the settlements prime hotspots for AMR (Nadimpalli et al., 2020; Nkonki-Mandleni et al., 2021). In addition to agricultural activities and animal husbandry, challenges such as poor drainage outlets significantly contribute to the discharge of human and household waste, including drug-resistant bacteria, into rivers (Thakur & Onwubu, 2024). Up to 4000 informal settlements exist in South Africa, with over 2 million households, and over 400 of these settlements are in the province of KwaZulu-Natal (Gamede, 2024; Housing Development Agency, 2021).

This study determined the prevalence and AMR profiles of ESBL-producing *K. pneumoniae* isolated from surface water, apparently healthy residents of an informal settlement and a proximate healthcare facility, to determine similarities and differences, and to ascertain whether AMR surveillance in surface water in proximity to informal settlements is a potential proxy for AMR circulating in the community.

2.0 METHODS

2.1 Ethical Approval and Consent to Participate

This study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Ref: BREC/00003640/2021), the Provincial Health Research Ethics Committee of the KwaZulu-Natal Department of Health Ref: KZ_202203_023), and the National Health Laboratory Service (NHLS) Ref: PR2225862). All informal settlement residents were recruited after providing explicit, written, and voluntary informed consent (Supplementary Material/S1)

2.2 Study Design and Study Area

This study was a longitudinal prevalence study undertaken in the uMgungundlovu District Municipality (UMDM). UMDM is a Category C municipality with its seat in Pietermaritzburg, in the KwaZulu-Natal Province of South Africa, and comprises seven local municipalities, including Msunduzi, where the sampling was conducted. UMDM provides water and wastewater services to six municipalities, serving over 1.2 million inhabitants (UMDM, 2023)Despite several environmental water resources, including six significant rivers, 19.6% of the population is not included in the “regional/local water scheme”, 5.3% still obtain water directly from rivers, 2.5% from springs and 1.4% from water vendors (uMgungundlovu District Municipality, 2023). Three per cent of the population has no form of toilet facilities (Development Bank of Southern Africa, 2025). About 63.4% of the district’s population lives below the poverty line; 45.6% have no source of income, and 17.8% earn less than R400 per month and reside in informal settlements (Cooperative Governance and Traditional Affairs, 2023).

Consenting participants for this study were recruited from Ward 22, one of the largest settlements in the Msunduzi municipality, to provide stool samples. Residents of the Ward 22

informal settlement residing in six different areas, namely: Seven Ox, Emadamini, Fedsem, Lay-Centre, Units 3, and Tehuise, provided stool samples. Surface water samples were collected from the Kwapata River, which discharges into the Msunduzi River, a major tributary of the uMngeni River, which travels across the district. The Medical Microbiology service of the NHLS in UMDM provided the clinical isolates from proximate healthcare facilities, primarily the regional and tertiary hospitals Fig1.

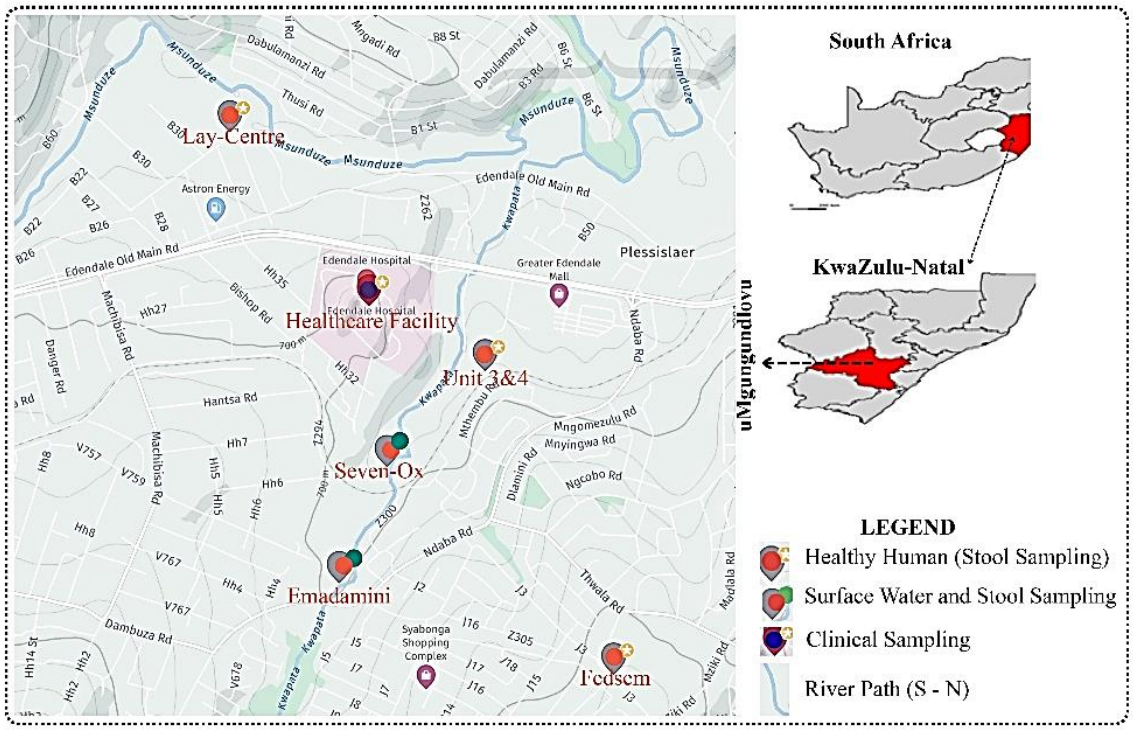


Figure 1: Informal Settlement Ward 22 in the umgungundlovu District Municipality and Healthcare Facility

2.3 Sample Collection and Processing

2.3.1 Stool Sampling from Healthy Residents

We collected stool samples monthly for eight months (May 2023 to Dec 2023) after obtaining voluntary, written, informed consent. Participants were trained on the proper use of camping toilets and other materials, as well as the faeces collection procedure (Appendices 3.1 and 3.2). Demographic information, such as age and gender, was collected, and participants were

resupplied with personal protective equipment (PPE) and stool sampling containers for each sampling date. The received samples were immediately transported in a cooler box at 4 °C–10 °C to the laboratory and processed for bacterial identification.

2.3.2 Surface Water Sampling

Within the same period of stool sampling, surface water samples were collected from the Kwapata River, which drains into the Msunduzi River, a major tributary of the uMngeni River flowing across the district. Duplicate water samples were collected every 2 weeks from the two sampling points mentioned above near areas of high anthropogenic activity, for a total of 80 samples in the study. Grab water sampling was used to collect 500 mL of water using a plastic bottle placed in a free-flowing, upstream direction, 10–5 cm below the water surface (Musselman, 2012). The collected samples were placed in a cooler box (4 °C–10 °C) and transported to the Antimicrobial Research Unit (ARU) at the University of KwaZulu-Natal for same-day processing.

2.3.3 Clinical Isolate Sampling

Positive bacteriological culture plates of specimen types**:** stool, urine, blood cultures, cerebrospinal fluid, pus and other sterile fluids from hospitalised adult patients (>18 years) suspected to have a clinical infection were received weekly in cooler boxes (4 °C–10 °C) from the NHLS. A Microsoft Excel spreadsheet listed the samples, with patient demographic information and diagnostic details (clinical diagnosis, specimen type, bacteria isolated, ESBL screening, and antimicrobial susceptibility testing results). The database was sorted for putative *Klebsiella pneumoniae* and ESBL+ *K. pneumoniae* positive plates using ID codes from the database, and the colonies from the positive culture were purified and molecularly confirmed.

2.4 Bacteria Isolation and Phenotypic Identification.

The duplicate surface water samples per collection point were mixed, diluted (10^{-1}, 10^{-2}, 10^{-3}) and plated on Simmons Citrate Agar supplemented with 1% (w/v) myo-inositol (SCAI) (Oxoid™, Basingstoke, United Kingdom; Sigma-Aldrich, St. Louis, Missouri, USA) for 44 hours at 42°C (Bobis Camacho et al., 2024). The SCAI medium is not commercially available and was prepared in-house using previously described protocols (Rodrigues, 2020). The medium's pH was sometimes below 7.2 after preparation and was therefore adjusted to 7.2 using 30% NaOH before pouring into petri dishes. Putative *K. pneumoniae* that appeared as large, yellow, and round colonies were further sub-cultured onto SCAI agar plates to obtain pure, distinct colonies after 42-44 hours of incubation.

K. pneumoniae from the faecal samples from healthy residents were isolated at a private pathology laboratory in Durban, South Africa, using the SCAI media following protocols for *Klebsiella* isolation from human and animal faecal material as described previously (Brisse et al., 2019). Clinical isolates, initially grown on MacConkey agar, were obtained in accordance with the standard operating protocol of the microbiology unit at the NHLS, uMgungundlovu.

All pure isolates were stored in tryptic soy broth (TSB) (Sigma-Aldrich, Missouri, United States of America (USA) in cryobank tubes with 20% glycerol (Associated Chemical Enterprises, Johannesburg, South Africa) for molecular confirmation.

2.5 DNA Extraction and Molecular Confirmation of *Klebsiella pneumoniae*

Pure colonies of presumptive *K. pneumoniae* isolates were subjected to DNA extraction using the standard boiling method, as previously described (Tiemtoré et al., 2022). Molecular confirmation of *K. pneumoniae* was performed by real-time PCR (qPCR) targeting the *phoE* housekeeping gene, a highly conserved, species-specific marker previously validated for discriminating *K. pneumoniae* from closely related Enterobacteriaceae (Sun et al., 2010).

Briefly, the 10-µL reaction contained 5 µL Luna® Universal qPCR Master Mix (New England Biolabs, Ipswich, MA, USA), 1 µL nuclease-free water (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL each of the forward and reverse primers, and 3 µL of template DNA. The *phoE*-specific primers, phoE-F (5′-TGCCCAGACCGATAACTTTA-3′) and phoE-R (5′-CTGTTTCTTCGCTTCACGG-3′), amplified a 142-bp product (Sun et al., 2010). Amplification comprised an initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, followed by melt-curve analysis from 65 °C to 95 °C with continuous fluorescence acquisition. Isolates showing amplification with a single melt peak corresponding to that of the *K. pneumoniae* ATCC 35657 positive control were considered *phoE*-positive. A no-template control was included in each run to detect possible contamination.

2.6 ESBL-producing *Klebsiella pneumoniae* Detection and Antibiotic Susceptibility Testing (AST)

Surface water and human stool isolates were first screened and selected for presumptive ESBL producers using CHROMID® ESBL Agar (bioMérieux, Marcy l'étoile, France). Subsequent confirmation of ESBL production was performed by a private pathology laboratory in Durban, South Africa, using the combination disk method. The Isolates were tested with cefotaxime (30 µg) and ceftazidime (30 µg) disks, both alone and in combination with clavulanic acid (10 µg). A ≥5 mm increase in the zone of inhibition diameter for either antimicrobial agent tested in combination with clavulanic acid, compared to the agent tested alone, was interpreted as a positive result for ESBL production(CLSI, 2021). Confirmed ESBL-producing *K pneumoniae* isolates were subjected to AST on the VITEK® 2 system (bioMérieux, North Carolina, USA) using the VITEK® 2 AST-N256 card for Gram-negative bacteria, according to the manufacturer's instructions.

Clinical isolates underwent AST at the NHLS using the VITEK® 2 Compact System. To detect ESBL production, the system utilised its Advanced Expert System™ (AES), which analyses the computed Minimum Inhibitory Concentration (MIC) of third-generation cephalosporins to detect putative ESBL-producers (Young et al., 2019). *K. pneumoniae* (ATCC-700603) was used as a control for all samples.

AST results were interpreted as susceptible, intermediate, or resistant according to the standards and breakpoints defined by the Clinical and Laboratory Standards Institute (CLSI) CLSI, 2021). Seventeen (17) antibiotics representing seven antibiotic classes were tested on isolates recovered from stool and surface water samples. Clinical isolates were tested against antibiotics from eight antibiotic classes by the NHLS. The antibiotics assessed included β-lactams: amoxicillin/clavulanic acid (AMC), piperacillin/tazobactam (TZP), cefoxitin (FOX), cefuroxime (CXM), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), imipenem (IMP), doripenem (DOR), meropenem (MEM), and ertapenem (ERT); aminoglycosides: amikacin (AMK), gentamicin (GEN), and tobramycin (TOB); fluoroquinolone: ciprofloxacin (CIP); sulfonamide: trimethoprim-sulphamethoxazole (SXT); and glycylcycline: tigecycline (TGC). Notably, cefoxitin (FOX) and nitrofurantoin were excluded from testing on surface water and healthy resident (stool) isolates, whereas tobramycin (TOB) and doripenem (DOR) were not included in the clinical isolate testing panel based on NHLS and private laboratory testing protocols. Isolates were classified as MDR if they demonstrated resistance to at least one antibiotic in three or more distinct antibiotic classes (Magiorakos et al. 2012).

2.7 Statistical Analysis

Data were compiled in Microsoft Excel 365 and analysed using R version 4.5.1 within RStudio 2025.05.1 (https://cran.rstudio.com/). The prevalence of ESBL-producing *K. pneumoniae* was

calculated as the proportion of positive isolates within each sampling source. Differences in prevalence and resistance profiles among sources were evaluated using chi-squared tests, while pairwise Fisher's exact tests with Benjamini-Hochberg false discovery rate (FDR) correction were applied for within-source comparisons. Effect sizes, including risk ratios and risk differences, with 95% confidence intervals (CIs), were estimated. Antimicrobial resistance profiles were visualised using hierarchical clustering heatmaps (Ain et al., 2025) generated with the ggplot2 and pheatmap packages(Wickham, 2011). Variations in MDR proportions were assessed using chi-squared tests. Overlap and similarity between antibiogram profiles were explored using Venn diagrams (Khan et al., 2019) constructed with the VennDiagram package, while pattern variation was assessed using permutational multivariate analysis of variance (PERMANOVA) from the vegan package in R (Oksanen et al., 2025). The Shannon diversity index was employed to quantify the complexity and evenness of resistance profiles across sources. All statistical analyses were performed at a 95% confidence level.

3.0 RESULTS

3.1 Detection of ESBL-producing *K. pneumoniae* (ESBL-Kp)

The study analysed 4,212 samples for *K. pneumoniae* over eight months (1,978 stool samples from healthy informal residents, 2,154 clinical cultures from healthcare settings, and 80 surface water samples) (Figure 2). The overall chi-square test confirmed significant variation in ESBL prevalence between the three sources ($\chi^2 = 29.9$, df = 2, $p < 0.00001$. ESBL-Kp prevalence was lowest among healthy residents (6.3%, 95% CI 5.1- 7.9), higher in surface water (13.0%, 95% CI 10.2-16.4), and highest in clinical isolates (15.2%, 95% CI 11.2–20.2). Pairwise comparisons showed that ESBL-Kp in healthy informal residents differed significantly from both surface water (RR 0.45, RD −8.2%, $p < 0.001$) and clinical isolates (RR 0.38, RD −11.1%, $p < 0.001$).

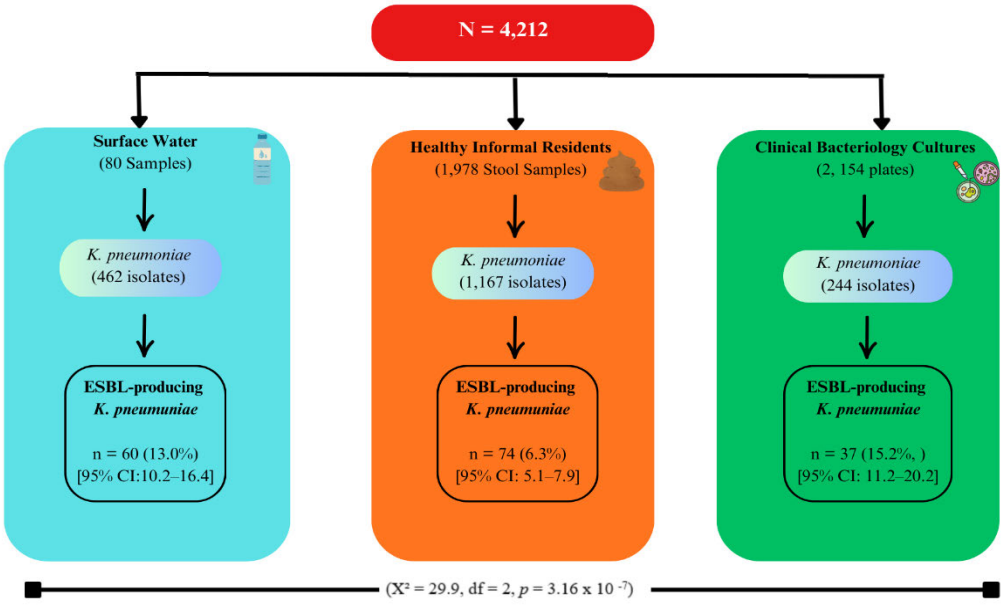


Figure 2: Samples and Isolates Processed and Prevalence of ESBL-producing *K. pneumoniae.*

3.2 Antibiotic Susceptibility Profiles of ESBL-producing *K. pneumoniae*

There was noticeable variability in the percentage resistance to some antibiotics across sources. The last-line antibiotics (amikacin, ertapenem, imipenem, and meropenem) consistently showed low or absent resistance rates (0-4.1%) across all three sources.

Percentage resistance varied significantly across the three sources ($p < 0.001$) for amoxicillin/clavulanic acid, piperacillin/tazobactam, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, and tigecycline. For amoxicillin/clavulanic acid and piperacillin/tazobactam, resistance was notably high in isolates from both surface water and healthy residents. Amoxicillin/clavulanic acid resistance was 100% in surface-water isolates and 98.6% among healthy residents but was substantially lower among clinical isolates at 45.9%. Similarly, piperacillin/tazobactam resistance was 96.7% in surface-water isolates and 97.3% among healthy residents, compared with 48.6% among clinical isolates. Conversely, clinical isolates displayed significantly higher resistance to gentamicin, ciprofloxacin, and

trimethoprim-sulfamethoxazole (GEN: 89.2%, CIP: 56.8%, SXT: 97.3%) than healthy residents (GEN: 28.4%, CIP: 21.6%, SXT: 52.7%) and surface water (GEN: 25.0%, CIP: 10.0%, SXT: 26.7%), respectively.

Consistent with the presence of ESBL phenotypes, resistance was consistently high across all sources to β-lactams, specifically second-, third-, and fourth-generation cephalosporins (cefuroxime, cefotaxime, ceftazidime, cefepime). Conversely, resistance to carbapenems (ertapenem, imipenem, and meropenem) was low or absent across all sources ($p > 0.05$), as shown in Table 1.

Pairwise comparisons between surface-water and healthy-resident isolates showed similar resistance profiles ($p > 0.05$) for β-lactams (amoxicillin/clavulanic acid, piperacillin/tazobactam, cephalosporins, and carbapenems), gentamicin, and ciprofloxacin. No resistance was detected to amikacin in either source. Significant differences were observed only for tobramycin, trimethoprim-sulfamethoxazole, and tigecycline ($p < 0.05$). For surface-water and clinical isolates, resistance to cephalosporins, carbapenems, amikacin, and tigecycline did not differ significantly ($p > 0.05$). However, resistance differed significantly ($p < 0.05$) for amoxicillin/clavulanic acid, piperacillin/tazobactam, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole.

Overall, surface-water ESBL-producing *K. pneumoniae* isolates showed greater similarity to healthy-resident isolates than to clinical isolates, particularly for AMC, TZP, cephalosporins, carbapenems, GEN, and CIP. However, significant differences remained for TOB, SXT, and TGC, indicating that surface water only partially reflected the phenotypic resistance profiles observed among healthy-resident isolates and did not fully mirror clinical resistance profiles.

Table 1: **Antibiotic Resistance Profiles of ESBL-producing *K. pneumoniae***

Antibiotics	**Isolate source**			**All-source analysis**	**Pairwise Analysis**	
	Surface Water	**Healthy Residents**	**Clinical**	**X^2 – *p*-value R (all 3 sources)**	**Pairwise Surface Water and Healthy Residents**	**Pairwise Surface Water & Clinical**
Amoxicillin clavulanic acid	100% (60)	98.6% (73),	45.9% (17)	***p* < 0.001**	*p*>0.05*	***p* < 0.001**
piperacillin/ tazobactam	96.7% (58)	97.3% (72)	48.6% (18),	***p* < 0.001**	*p*>0.05	***p* < 0.001**
cefoxitin	NT	NT	10.8% (4)	---	N/A[b]	N/A[b]
Cefuroxime	95% (57)	98.6% (73),	100% (37)	*p* = 0.216	*p* = 0.469	*p* > 0.436
Cefotaxime	100% (60)	98.6% (73),	100% (37)	*p* = 0.517	*p* = 0.999	N/A[a]
Ceftazidime	95% (57)	97.3% (72)	100% (37)	*p* = 0.359	*p* = 0.315	*P*=0.436
Cefepime	100% (60)	93.2% (69)	97.3% (36)	*p* = 0.102	*p* = 0.111	*P*=0.806
Doripenem	0% (0)	1.4% (1)	NT	--	***p* > 0.05**	N/A[b]
Ertapenem	1.7% (1)	4.1% (3)	0% (0)	*p* = 0.375	***p*>0.05**	*p*>0.05
Imipenem	0% (0)	0% (0)	0% (0)	--	N/A[a]	N/A[a]
Meropenem	1.7% (1)	0% (0)	0% (0)	*p* = 0.394	*p* > 0.05	p > 0.05
Amikacin	0% (0)	0% (0)	0% (0)	--	N/A[a]	N/A[a]
Gentamicin	25% (15)	28.4% (21)	89.2% (33)	***p* < 0.001**	*p* = 0.212	*p* < 0.001
Tobramycin	78.3% (47)	28.4% (21),	NT	--	***p* < 0.001**	N/A[b]
Ciprofloxacin	10% (6)	21.6% (16)	56.8% (21)	***p* < 0.001**	***p* > 0.05**	***p* = 0.001**
trimethoprim-sulfamethoxazole	26.7% (16)	52.7% (39)	97.3% (36)	***p* < 0.001**	***p* = 0.003**	***p* < *0.001***
nitrofurantoin	NT	NT	40.5% (15)	---	N/A[b]	N/A[b]
Tigecycline	15% (9)	39.2% (29)	2.7% (1)	***p* < 0.001**	*p* = **0.004**	***p* > 0.05**

NT = not tested. N/A[a] = statistical comparison not performed because no resistance variation was observed between compared groups. N/A[b] = statistical comparison not performed because the antibiotic was not tested in one or more compared sources. Pairwise comparisons were performed using Fisher's exact test or chi-square test, as appropriate. p-values < 0.05 were considered statistically significant.

We performed hierarchical clustering of antibiotic resistance percentages using a heatmap (Figure 3) on antibiotics common to all sources to visualise similarities and differences in resistance profiles across isolate sources. β-lactams, excluding carbapenems, generally clustered closely across the three sources. In particular, surface water isolates and isolates from healthy residents clustered at higher resistance (>95%) for piperacillin/tazobactam, amoxicillin/clavulanic acid, cefuroxime, cefotaxime, ceftazidime, and cefepime compared to clinical isolates, which differed (resistance <50%) for piperacillin/tazobactam and amoxicillin/clavulanic acid. None of the three sources showed variation at low levels of carbapenem and amikacin resistance (dark-green rows, Figure 3). Resistance to ciprofloxacin and gentamicin showed a close resemblance at low to moderate levels in both surface water and healthy residents' isolates whereas SXT surface water resistance differed significantly from either sources. In general, surface water isolates displayed a better mirroring of resistance to community isolates than to clinical isolates.

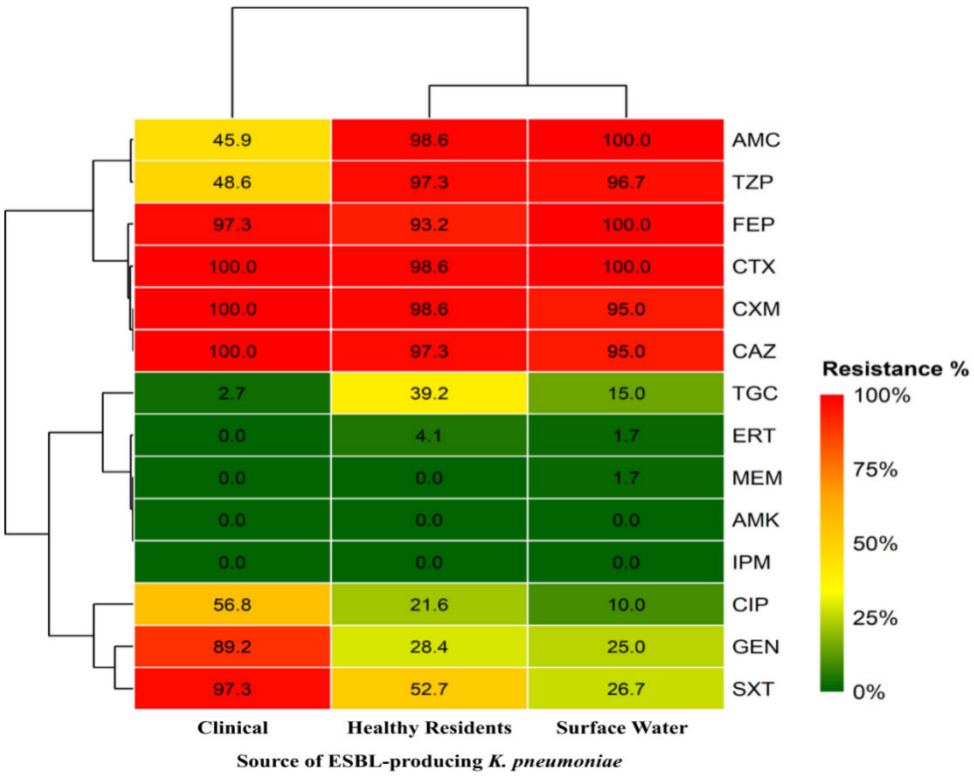


Figure 3: Heatmap of Antibiotic-Resistant Profile of *K. pneumoniae* by Isolate Sources. Columns represent the sources of isolates, and rows represent antibiotics. Red blocks indicate the highest percentage of resistance, while green blocks indicate the lowest. The percentage resistance of isolates from surface water more closely resembles that of healthy residents than that of isolates from clinical sources.

3.3 Antibiograms and Multidrug Resistance (MDR) of Isolates

There were 31 antibiograms (Figure 4) from all isolates across the three sources; 18 of the patterns were MDR. A complete list of all MDR and non-MDR patterns per source is available (Supplementary Material/S2). While some antibiogram patterns were shared between sources, the majority of the patterns were seen in healthy residents' isolates (total =18, MDR;10, non-MDR;8), followed by clinical isolates (total =11, MDR; 5, non-MDR; 6), and surface water isolates (total =11, MDR; 5, non-MDR; 6). We used the Shannon Diversity test to assess the complexity and evenness of patterns across sources (Supplementary Material/S3). Healthy residents exhibited the highest overall antibiogram pattern diversity (H′ = 2.4916, 95% CI: 2.1367-2.5675; Evenness J = 0.8620), followed by clinical isolates (H′ = 1.7729, 95% CI: 1.2356-1.9453; Evenness J = 0.7394), then surface water (H′ = 1.5067, 95% CI: 1.0752-1.7334; Evenness J = 0.6283).

The most common MDR antibiogram for surface water was AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT (6 isolates, 10.0%, MDR); for clinical isolates, it was AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT (11 isolates, 29.7%, MDR); and for healthy residents, it was AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT (9 isolates, 12.2%, MDR). The most common non-MDR antibiogram was AMC-TZP-CXM-CTX-CAZ-FEP (surface water; 36 isolates, 60.0%, non-MDR), AMC-TZP-CXM-CTX-CAZ-FEP-SXT (clinical; 1 isolate, 2.7%, non-

MDR) and AMC-TZP-CXM-CTX-CAZ-FEP-TGC (healthy residents; 10 isolates, 13.5%, non-MDR).

Among these patterns, MDR was evident in 94.59% of clinical isolates, 41.89% of healthy residents isolates, and 25% of surface water isolates ($p < 0.001$). Pairwise analysis of MDR prevalence showed that clinical isolates had higher MDR prevalence than surface water and healthy resident isolates ($p < 0.001$).

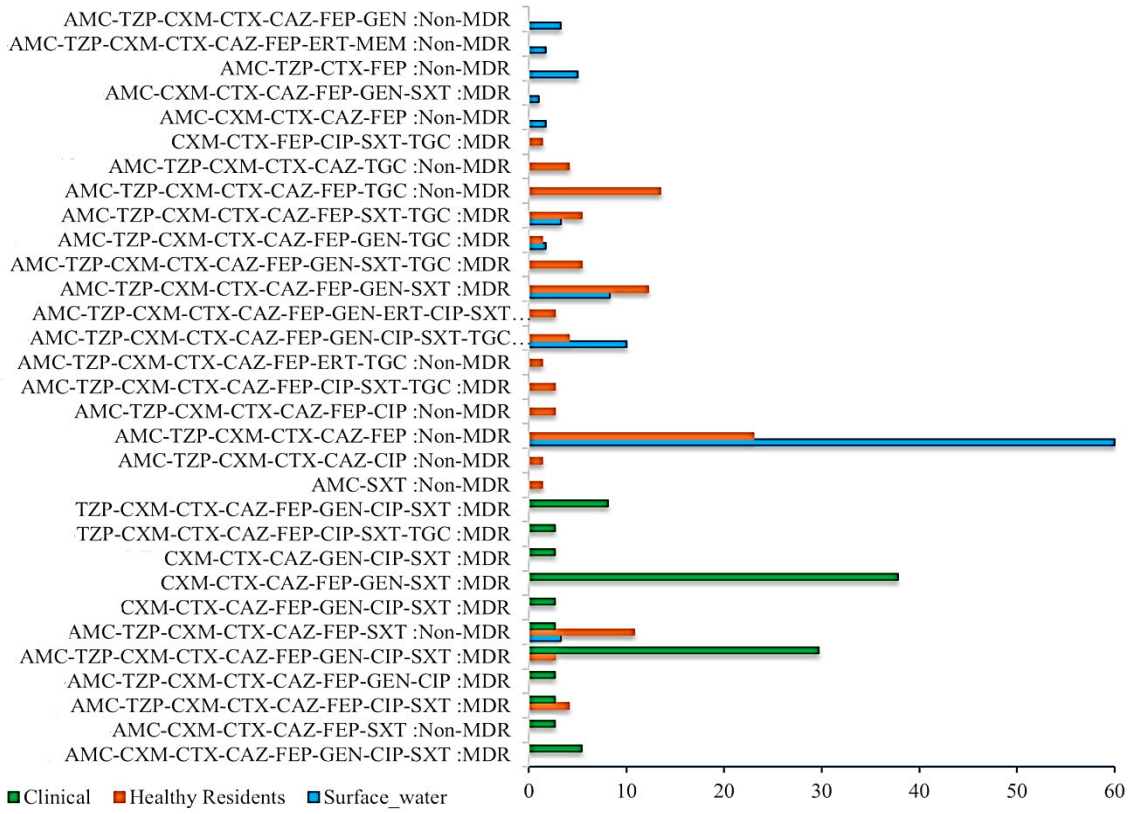


Figure 4: Antibiograms of ESBL-producing *K. pneumoniae* across Sources.

3.4 Triangulation of Antibiogram Patterns and MDR Phenotypes.

Venn diagram analysis revealed that resistance patterns were largely source-specific (Figure 5). Clinical isolates harboured the most unique MDR patterns (n=7), while healthy residents possessed the highest number of unique non-MDR profiles (n=6). Overall, MDR antibiograms were more prevalent in clinical isolates, whereas non-MDR patterns were more prevalent in healthy residents. The overall composition of resistance patterns varied significantly by source

(R^2 = 0.1223, F = 11.7088, p = 0.001). Stratification by MDR status revealed source-specific differences remained significant for both MDR (R^2 = 0.1332, F = 5.9907, p = 0.001) and non-MDR patterns (R^2 = 0.1270, F = 6.329, p = 0.001), In both groups, the source accounted for approximately 12-13% of the observed compositional variance.

Four MDR patterns were shared by isolates from surface water and healthy residents (Jaccard index = 0.3636, p < 0.001), two from healthy resident and clinical isolates (Jaccard index = 0.1176, p < 0.001) and none by surface water and clinical isolates. No unique MDR pattern was shared across all three sources. For non-MDR patterns, one pattern was shared by all three sources: one by surface water and healthy resident isolates (Jaccard index = 0.1667, p < 0.001), and none between surface water and the clinical isolates (Jaccard index = 0.1429, p < 0.001). Details of patterns unique to and shared by sources, as per the Venn analysis, are available in Supplementary Material/S4.

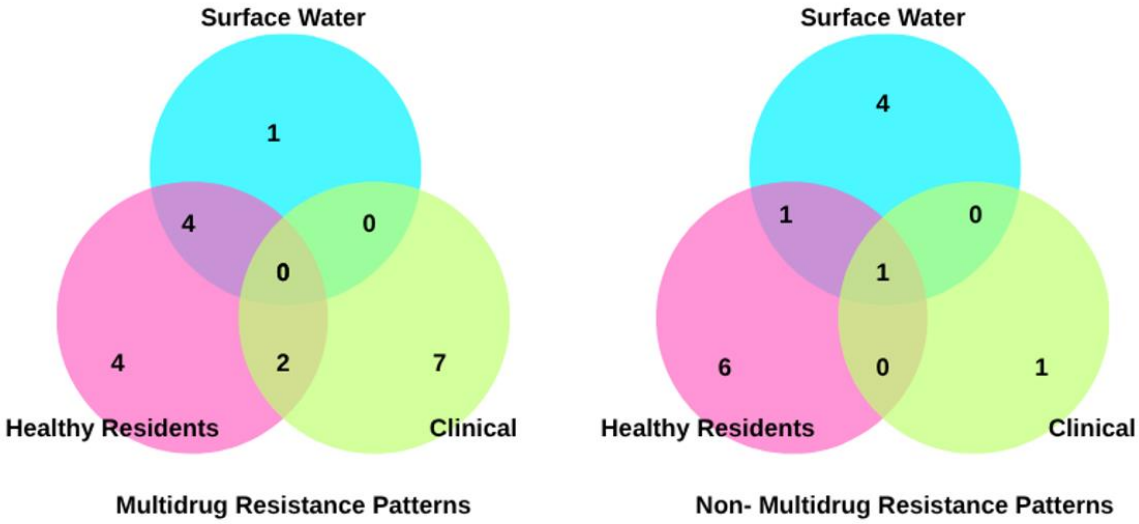


Figure 5: Venn Diagram Showing Overlap of MDR (Left) and Non-MDR (Right) Patterns of ESBL *K. pneumoniae* Isolates across Sources: The central intersection of the three circles represents the antibiotic resistance patterns that are common to all three sources: Clinical (light-green), Healthy Residents (Pink), and Surface Water (sky-blue). The side intersections represent patterns shared by any two sources, and the larger, non-overlapping parts of each circle represent the antibiotic resistance patterns that are unique to each source.

4.0 Discussion

Klebsiella pneumoniae remains one of the common ESBL-producing Enterobacterales that cause drug-resistant infections in both hospital and community settings, resulting in high morbidity, mortality and medical expenses (Olaitan et al., 2025). Traditionally, AMR surveillance relies heavily on clinical and hospital-based sampling and data, which provide critical insights into resistance trends in patient populations. However, these systems are often limited by underreporting, unequal laboratory capacity, and their inability to capture resistance circulating silently in the broader community or environmental reservoirs (Frost et al., 2021; Kusuma et al., 2025). With the potential seen in recent studies (Kagambèga et al., 2024; Rolbiecki et al., 2025) on the role of environment in AMR surveillance, this study explored phenotypic AMR profiles of ESBL-Kp isolated from surface water running through an informal settlement to see if resistance reflects what is seen among healthy community residents and the proximate clinical setting.

We detected ESBL-Kp with significantly different resistance prevalence and profiles in the three sources. Isolates were multidrug resistant, peaking in clinical isolates and least evident in surface water. Resistance levels differed per antibiotic per source. Surface water isolates shared MDR and non-MDR antibiograms more frequently with healthy residents isolates than with

clinical ones. Patterns were more unique to respective sources, and surface water did not generally reflect phenotypic resistance in human sources

4.1 Detection of ESBL-producing *K. pneumoniae*

ESBL-Kp was detected in all three isolate sources, with the lowest prevalence among healthy residents (6.3%), through surface water (13.0%), and the highest prevalence in clinical isolates (15.2%). According to a 2025 meta-analysis including 119 eligible studies from 25 countries from the Sub-Saharan African subregions (Olaitan et al., 2025), the pooled prevalence of ESBL-Kp in South Africa was reported to be 23.3% (95% CI: 6.5–45.8), which is comparable to the ESBL-Kp prevalence observed in our study (ranging from 6.3% to 15.3%) across all three settings. Although our findings are slightly lower than the point estimate, they fall entirely within the wide confidence interval, indicating that they are not statistically distinct from the national average.

Compared with clinical isolates, the prevalence of ESBL-producers in surface water isolates in our study was significantly lower, consistent with a study in Ethiopia (Abera et al., 2016) that compared clinical and water isolates and found significantly higher ESBL-Kp in clinical isolates (69.94%) than in water isolates (33.3%). Clinical environments concentrate vulnerable and infected patient populations and are sites of high antimicrobial selective pressure, leading to the persistence and dissemination of resistant bacteria. Conversely, aquatic environments are typically more open, subject to dilution, and exposed to environmental factors that generally limit bacterial survival (Ferreira et al., 2023; Freeman et al., 2018).Thus, the prevalence of bacteria such as ESBL-Kp is likely to be higher in clinical settings than in aquatic environments, as seen in our study.

Interestingly, we detected higher ESBL-Kp in surface (13.0%) water than among healthy informal settlement residents (6.3%). Similar findings have been reported in a Malawian study, which sampled human stool from residents of a rural community and their surrounding river water and reported up to 50% prevalence of ESBL-Kp in river water compared to 17% in residents (Cocker et al., 2023). Another study by Yasmeen and colleagues (2023) in Punjab, Pakistan, sampled ESBL-Kp from several niches, including healthy residents and water samples and observed higher rates of resistant isolates in water (6.7%) than among healthy residents (3.0%). These findings may suggest that the surrounding aquatic environment is being contaminated with ESBL-Kp from unidentified sources, or that apparently healthy people are less colonised by the bacteria. Although we initially assumed that surface water contamination in the informal settlement was primarily of human origin, as per literature (Cocker et al., 2023b; Martak et al., 2022), other studies have highlighted that the presence of ESBL-Kp is not uncommon in surface waters near sources like hospital wastewater treatment plants and sites where there are animal husbandry and agricultural activities (Cocker et al., 2023; Lepuschitz et al., 2019; Yasmeen et al., 2023) For instance, previous studies conducted within the same locality identified *Klebsiella* spp. and other ESBL-producing Enterobacterales in hospital effluents and wastewater treatment plants (Gumede et al., 2021; King et al., 2020). Although our study did not explore the potential links between sewage systems and the release of bacteria into surrounding aquatic environments, studies worldwide have documented that poorly maintained wastewater treatment plants can disseminate resistant bacteria, contributing to higher levels of contamination in surface waters than carriage among community residents (Buriánková et al., 2021; Freeman et al., 2018; Ojer-Usoz et al., 2014). These studies confirmed the dissemination of resistant bacteria, directly or indirectly, to surrounding aquatic environments, including surface water, making surface water a potential reservoir for ESBL-

Kp and other organisms, thereby necessitating further studies to elucidate bacterial transmission routes into the aquatic environments proximate to this community.

Overall, we confirmed the presence of ESBL-Kp across all three sources and observed that the prevalence in surface water isolates differed significantly ($p < 0.05$) from that in community healthy resident and clinical isolates These prevalence comparisons should, however, be interpreted contiously. Clinical isolates originated from routine diagnostic specimens from hospitalised patients and therefore primarily represent infection-associated isolates, whereas stool samples from healthy residents reflect asymptomatic faecal carriage. Surface-water isolates represent environmental occurrence influenced by faecal inputs, dilution and other environmental sources. Therefore, differences in prevalence across the three compartments should not be interpreted as directly equivalent measures of human carriage or clinical infection burden. Notwithstanding its relatively low prevalence, this finding highlights its potential as a epidemiologically connected reservoir for community residents of the settlement.

4.2 Antibiotic Susceptibility Profiles of ESBL-producing *K. pneumoniae*

The percentage of isolates resistant to an antibiotic may vary widely depending on the source of isolates and local selection pressure. In clinical settings, hospitals drive and maintain high-level resistance through sustained antibiotic use and dense human contact; communities maintain moderate, transient resistance through limited exposure; and surface waters dilute bacterial populations, showing varying phenotypic resistance at different levels for different sources (Husna et al., 2023). However, due to interactions among sources, isolates may exhibit similar resistance patterns to some antibiotics.

We observed that resistance to amoxicillin-clavulanic acid and piperacillin/tazobactam in surface water isolates was similar to community isolates but differed from clinical isolates, consistent with the report by Yasmeen et al. (2023), who observed a 100% resistance rate of

ESBL-Kp against amoxicillin-clavulanic acid and piperacillin/ tazobactam in both surface water isolates and community isolates. In South Africa, penicillin-inhibitor combinations such as amoxicillin-clavulanic acid are among the most frequently prescribed antibiotics in outpatient and primary healthcare settings (Alabi & Essack, 2022). While cephalosporins exert direct selective pressure on ESBL phenotypes, widespread community use of amoxicillin-clavulanate likely contributes through co-selection. Because ESBL genes are frequently co-located on multidrug-resistance plasmids alongside genes conferring resistance to inhibitor combinations, frequent AMC exposure can indirectly maintain and propagate ESBL-Kp lineages within the population. The extensive use of antibiotics leads to the discharge of significant quantities of parent compounds and metabolites into sewage systems via human excretion. Because conventional wastewater treatment is often inadequate, these antibiotic residues frequently reach surface waters (Ncube et al., 2021). Consequently, both the community gut microbiota and environmental niches may be exposed to comparable selective pressures, which may result in similar resistance profile.

Tigecycline resistance was higher among healthy residents than in surface-water isolates, 39.2% compared with 15.0%, respectively, and this difference was statistically significant. This finding suggests that surface-water isolates did not fully reflect community tigecycline resistance. Our findings also differ from those of Yasmeen et al. (2023), a One Health study in Pakistan, which did not detect tigecycline resistance among water and human isolates, likely due to differential antibiotic selection pressure, as tigecycline use and prescribing policies in human and animal health differ between South Africa and Pakistan. An in vitro study conducted at a tertiary care hospital in Pakistan reported universal susceptibility to tigecycline among nosocomial isolates and explicitly noted minimal prior clinical exposure to the drug at the institution at the time of sampling (Shakoor et al., 2010). Tigecycline in our study area

remains reserved as a parenteral, reserve antibiotic for severe, complicated infections (Brink et al., 2010; Cassir et al., 2014). Resistance (>10%) in *K. pneumoniae* has, however, been recently documented in inpatients and hospital effluent samples (Masalane et al., 2025a). While tigecycline usage is restricted to hospital settings (Cassir et al., 2014), first-generation glycylcyclines such as tetracyclines are widely used and less controlled in non-clinical settings, including food animal production (Mupfunya et al., 2021). Evidence suggests decreased susceptibility to tigecycline following overexposure of bacteria to tetracyclines, mediated through efflux pumps (Nasralddin et al., 2025). Also, the dissemination of tetracycline-resistant bacteria into the environment following animal husbandry introduces both resistant bacteria and tetracycline antibiotic residues (Ramessar et al., 2025). These residues may exert selective pressure in the surrounding aquatic environment and may contribute to co-selection of tigecycline resistance through shared resistance mechanisms (Mupfunya et al., 2021; Ramessar et al., 2025).

Clinical isolates showed significantly higher resistance to gentamicin and trimethoprim-sulfamethoxazole than surface-water isolates, indicating that surface water did not reflect clinical resistance for these antibiotics. This observation is contrast with a One Health study by Craddock et al. (2025), which investigated ESBL-Kp among Bedouin communities in Israel. Craddock's study found that resistance to these specific drugs was also statistically similar ($p > 0.05$) between clinical urine isolates (GEN: 27.3%, SXT: 77.3%) and environmental water isolates (GEN: 35.3%, SXT: 88.2%). In South Africa, gentamicin and trimethoprim-sulfamethoxazole are common antibiotics used to treat uncomplicated UTIs, with the latter further used to prevent opportunistic infections in HIV-infected persons (Fourie et al., 2021; Goodlet et al., 2018). Recent local studies have reported high gentamicin and trimethoprim-sulfamethoxazole resistance in patients and hospital effluent (Bassetti et al., 2023; Masalane et

al., 2025), as well as lower resistance levels (0-3%) for gentamicin and 0-30% for sulfamethoxazole in urban water influenced by anthropogenic activities (Tucker et al., 2022). In South Africa, Masalane and colleagues observed that *K. pneumoniae* clinical isolates were fully resistant to gentamicin and trimethoprim-sulphamethoxazole, whereas isolates recovered from hospital effluent remained completely susceptible. Within the environmental context, Tucker's study of the urban water cycle in Stellenbosch reported gentamicin resistance reaching up to 3% and sulfamethoxazole resistance up to 30% in river water and wastewater treatment plants situated near informal settlements. These findings suggest that clinical settings may maintain stronger selective pressure for these antibiotics than surrounding surface waters. Although the healthcare facilities and surface-water sampling sites were located within the same broader geographical area, hospital effluent was not sampled and direct hydrological connectivity between the facilities and the sampled river sites was not assessed. Therefore, the resistance detected in surface water cannot be specifically attributed to healthcare-associated discharge and may reflect multiple anthropogenic sources. Nevertheless, the detection of resistance in surface-water isolates is consistent with contributions from multiple anthropogenic sources to the surrounding aquatic environment, although the specific sources could not be determined in this study. However, high-resolution genomic analyses to detect shared antimicrobial resistance genes are required to establish whether ESBL-*K. pneumoniae* and related ESBL determinants are exchanged between clinical settings and the aquatic environment.

Overall, surface-water isolates more closely resembled healthy-resident isolates than clinical isolates, particularly amoxicillin-clavulanic acid, piperacillin-tazobactam, cephalosporins, carbapenems, gentamicin, and ciprofloxacin, while among clinical isolates and surface water, there was resemblance in cephalosporins, carbapenems, amikacin, and tigecycline. Resistance

to amikacin, imipenem, meropenem, and ertapenem was generally absent or very low across all sources.

4.3 Antibiograms and Multidrug Resistance (MDR) of ESBL *K. pneumoniae* isolates

Antibiogram patterns were used as a phenotypic screening tool to assess overlap in resistance profiles across sources. Shared patterns between clinical, community, and environmental isolates suggest phenotypic convergence, which may reflect common selective pressures or potential inter-source dissemination, distinctions that require molecular characterisation to resolve. MDR was significantly lower in surface water (25%) compared to healthy residents (41.89%) and clinical isolates (94.59%). While integrated studies evaluating all three reservoirs simultaneously are scarce, our findings align with individual reports from around the world. In Thailand, Chaisaeng et al., (2024) assessed 507 non-duplicate clinical isolates from a clinical setting and reported an ESBL-Kp MDR rate of 100%. Similarly, healthy students at Dilla University student cafeteria in Ethiopia revealed faecal carriage ESBL-Kp MDR rate of 37.5% (Diriba et al., 2020) and in the environment, a study profiling antibiotic resistance in *K. pneumoniae* isolates from Mula-Mutha River (Pune, India) (Kaur et al., 2025) showed that 15.3% of the MDR isolates harboured ESBL activity. A study of the peri-urban area of Burkina Faso assessed ESBL-producing Enterobacterales, including ESBL-Kp, using a One Health approach in healthy cattle farmers and their environments, and reported higher MDR rates in farmers' stools (36.2%) than in water (27.8%) (Soma et al., 2024b).

Although specific percentages vary by studies and regions, these data reinforce a consistent global trend: MDR burden peaks in clinical settings and remains comparatively lower in aquatic environments.

Notably, our observations and those of other authors projected lower MDR in the surface water environment than in human populations. Although some authors have reported MDR rates of up to 95% in water sources (Davidova-Gerzova et al., 2024), MDR strains exhibit lower persistence in aquatic environments. This has previously been attributed to factors such as dilution effects, environmental stressors, and other ecological variables that limit bacterial persistence in aquatic systems (Larsson & Flach, 2022), further emphasising the differences in MDR rates between sources. The consistent detection of MDR ESBL-Kp in surface water indicates that these aquatic systems may act as reservoirs of antibiotic-resistant bacteria. The overlapping resistance profiles among healthy residents, clinical isolates and the environment suggest potential epidemiological connectivity between human populations and surrounding surface waters; however, the direction and extent of transmission cannot be established from phenotypic resistance profiles alone. Within informal settlements characterised by poor infrastructure and insufficient water, sanitation, and hygiene services, direct interaction between residents and polluted surface water increases exposure risks. Such environmental exposure may facilitate the circulation of ESBL-resistant bacteria (Erb et al., 2018; Kim et al., 2025; Nadimpalli et al., 2020) and remains a significant public health threat.

Our results further showed that, among the circulating MDR isolates, several antibiogram patterns overlapped between sources. These shared phenotypic profiles highlight a degree of resistance similarity between clinical, community, and environmental ESBL-Kp populations. Although there were significant differences in non-MDR pattern composition across sources ($p < 0.001$), surface water isolates shared more patterns with healthy residents (n=38, 63.3%) than they did with clinical isolates (n=2, 3.3%; Jaccard index = 0.1667). Similarly, surface water isolates (14 isolates, 23.3%) shared four MDR patterns with community isolates, while no overlap was observed with clinical isolates (Jaccard index = 0.3636, $p < 0.001$). There was

significant correlation in resistance patterns between sources ($p < 0.001$); despite modest overlap in specific profiles. However, the Jaccard similarity coefficients for non-MDR (0.1667) and MDR strains (0.3636) indicate that, while the distribution of resistance is not random, the majority of resistance phenotypes remain source-specific, suggesting that, while common selective pressures may exist, the community and surface water reservoirs maintain distinct resistance signatures. Previous research has demonstrated that human interaction with the environment, whether through direct use of surface waters for domestic activities, animal watering, and irrigation, or indirectly through household wastewater discharge and natural hydrological processes, can facilitate the exchange of antibiotic-resistant bacteria and their associated genetic elements (Craddock et al., 2025; Kim et al., 2025). Similarly, inadequate maintenance and inefficiencies in community and hospital wastewater treatment systems (Department of Water and Sanitation, 2023; Hounmanou et al., 2024; Zagui et al., 2025) have been linked to the persistence and spread of resistant ESBL-producing Enterobacterales within interconnected ecosystems (Gumede et al., 2021; Zagui et al., 2025). The presence of resistant bacteria, including MDR strains observed in this study, represents a critical public health concern. Therefore, high-resolution genomic insight on isolates displaying comparable resistance profiles is warranted to confirm the presence of shared resistance genes and mobile genetic elements within these settings, thereby elucidating the potential of surface water as a tool for AMR surveillance in the community and clinical setting while exploring routes and mechanisms driving resistance dissemination across environmental and human interfaces.

5.0 Conclusion

Our study confirmed the presence of ESBL-Kp in surface water, healthy community residents, and clinical isolates, with statistically significant differences in prevalence among these sources. Surface-water isolates showed resistance rates similar to community isolates for

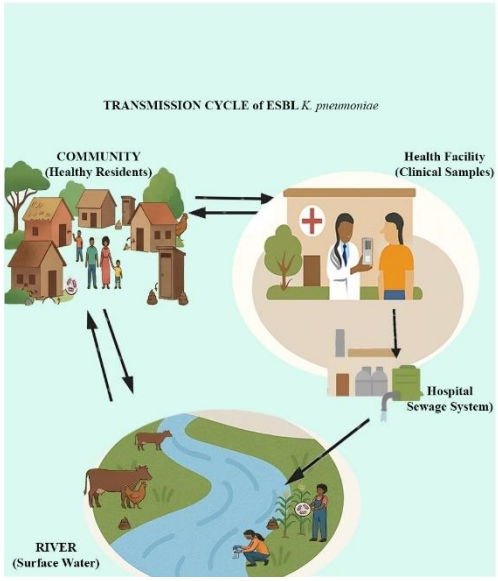
amoxicillin-clavulanic acid, piperacillin-tazobactam, cephalosporins, carbapenems, gentamicin, and ciprofloxacin, but differed for tobramycin, trimethoprim sulfamethoxazole, and tigecycline. Compared with clinical isolates, surface-water isolates showed similarity mainly for cephalosporins, carbapenems, amikacin, and tigecycline, but differed significantly for amoxicillin-clavulanic acid, piperacillin-tazobactam, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole.

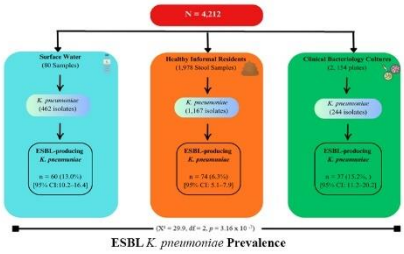
Multidrug resistance profiles varied between sources, with the highest MDR burden observed among clinical isolates and the lowest among surface water isolates. Surface-water isolates shared MDR and non-MDR antibiograms more frequently with community isolates than with clinical isolates. Although surface-water isolates exhibited resistance to clinically important antibiotics, as observed in both community and clinical isolates, their overall prevalence, MDR burden, and antibiogram composition did not fully reflect the resistance profiles observed in human sources. These findings indicate that surface water captures selected community-associated ESBL-Kp resistance signals but remains a complementary surveillance matrix rather than a standalone proxy for community or clinical AMR burden.

Study Highlights

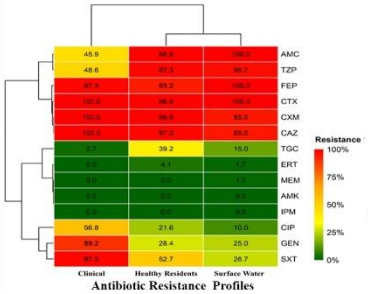
- Samples from surface water, community residents, and the hospital all carried ESBL-Kp.
- Resistance patterns varied across antibiotics within the WHO AWaRe categories and between isolate sources.
- Multidrug resistance is widespread, peaking in clinics and lowest in surface water.
- Surface water antibiogram overlapped more with community than clinical isolates.
- Phenotypic AMR in surface water did not mirror community- or clinical-level AMR.

Graphical Abstract

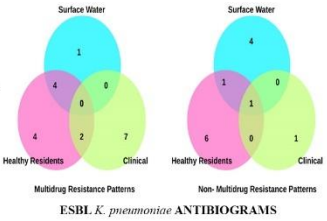




ESBL *K. pneumoniae* Prevalence



Antibiotic Resistance Profiles



ESBL *K. pneumoniae* ANTIBIOGRAMS

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List of Supplementary Materials (Manuscript 2)

- **Supplementary Material/S1** - Ethical Consideration and Informed Consent; Refer to Appendices 1.1 to 1.4 of this Thesis. **Pages 284 - 287.**
- **Supplementary Material/S2** - MDR and Non-MDR Antibiogram Patterns of ESBL-*producing K. pneumoniae* isolates across Sources. **Page 169**.
- **Supplementary Material/S3** - Distribution and Diversity Metrices of Antibiogram patterns of ESBL-*producing K. pneumoniae* across sources. **Page 170**.
- **Supplementary Material/S4** - MDR and Venn Analysis of Antibiograms profiles of ESBL-*producing K. pneumoniae* across Sources. **Page 171-172**.

Supplementary Material/S2 – MDR and Non-MDR Antibiogram Patterns of ESBL-*producing K. pneumoniae* isolates across Sources.

Pattern	MDR_Flag	Clinical	Healthy_Individual	Surface_water
AMC-CXM-CTX-CAZ-FEP-GEN-CIP-SXT	MDR	AMC-CXM-CTX-CAZ-FEP-GEN-CIP-SXT (2 isolates, 5.4%, MDR)		
AMC-CXM-CTX-CAZ-FEP-SXT	Non-MDR	AMC-CXM-CTX-CAZ-FEP-SXT (1 isolates, 2.7%, Non-MDR)		
AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT	MDR	AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT (1 isolates, 2.7%, MDR)	AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT (3 isolates, 4.1%, MDR)	
AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP	MDR	AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP (1 isolates, 2.7%, MDR)		
AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT	MDR	AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT (11 isolates, 29.7%, MDR)	AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT (2 isolates, 2.7%, MDR)	
AMC-TZP-CXM-CTX-CAZ-FEP-SXT	Non-MDR	AMC-TZP-CXM-CTX-CAZ-FEP-SXT (1 isolates, 2.7%, Non-MDR)	AMC-TZP-CXM-CTX-CAZ-FEP-SXT (8 isolates, 10.8%, Non-MDR)	AMC-TZP-CXM-CTX-CAZ-FEP-SXT (2 isolates, 3.3%, Non-MDR)
CXM-CTX-CAZ-FEP-GEN-CIP-SXT	MDR	CXM-CTX-CAZ-FEP-GEN-CIP-SXT (1 isolates, 2.7%, MDR)		
CXM-CTX-CAZ-FEP-GEN-SXT	MDR	CXM-CTX-CAZ-FEP-GEN-SXT (14 isolates, 37.8%, MDR)		
CXM-CTX-CAZ-GEN-CIP-SXT	MDR	CXM-CTX-CAZ-GEN-CIP-SXT (1 isolates, 2.7%, MDR)		
TZP-CXM-CTX-CAZ-FEP-CIP-SXT-TGC	MDR	TZP-CXM-CTX-CAZ-FEP-CIP-SXT-TGC (1 isolates, 2.7%, MDR)		
TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT	MDR	TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT (3 isolates, 8.1%, MDR)		
AMC-SXT	Non-MDR		AMC-SXT (1 isolates, 1.4%, Non-MDR)	
AMC-TZP-CXM-CTX-CAZ-CIP	Non-MDR		AMC-TZP-CXM-CTX-CAZ-CIP (1 isolates, 1.4%, Non-MDR)	
AMC-TZP-CXM-CTX-CAZ-FEP	Non-MDR		AMC-TZP-CXM-CTX-CAZ-FEP (17 isolates, 23.0%, Non-MDR)	AMC-TZP-CXM-CTX-CAZ-FEP (36 isolates, 60.0%, Non-MDR)
AMC-TZP-CXM-CTX-CAZ-FEP-CIP	Non-MDR		AMC-TZP-CXM-CTX-CAZ-FEP-CIP (2 isolates, 2.7%, Non-MDR)	
AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT-TGC	MDR		AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT-TGC (2 isolates, 2.7%, MDR)	
AMC-TZP-CXM-CTX-CAZ-FEP-ERT-TGC	Non-MDR		AMC-TZP-CXM-CTX-CAZ-FEP-ERT-TGC (1 isolates, 1.4%, Non-MDR)	
AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT-TGC	MDR		AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT-TGC (3 isolates, 4.1%, MDR)	AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT-TGC (6 isolates, 10.0%, MDR)
AMC-TZP-CXM-CTX-CAZ-FEP-GEN-ERT-CIP-SXT	MDR		AMC-TZP-CXM-CTX-CAZ-FEP-GEN-ERT-CIP-SXT (2 isolates, 2.7%, MDR)	
AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT	MDR		AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT (9 isolates, 12.2%, MDR)	AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT (5 isolates, 8.3%, MDR)
AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT-TGC	MDR		AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT-TGC (4 isolates, 5.4%, MDR)	
AMC-TZP-CXM-CTX-CAZ-FEP-GEN-TGC	MDR		AMC-TZP-CXM-CTX-CAZ-FEP-GEN-TGC (1 isolates, 1.4%, MDR)	AMC-TZP-CXM-CTX-CAZ-FEP-GEN-TGC (1 isolates, 1.7%, MDR)
AMC-TZP-CXM-CTX-CAZ-FEP-SXT-TGC	MDR		AMC-TZP-CXM-CTX-CAZ-FEP-SXT-TGC (4 isolates, 5.4%, MDR)	AMC-TZP-CXM-CTX-CAZ-FEP-SXT-TGC (2 isolates, 3.3%, MDR)
AMC-TZP-CXM-CTX-CAZ-FEP-TGC	Non-MDR		AMC-TZP-CXM-CTX-CAZ-FEP-TGC (10 isolates, 13.5%, Non-MDR)	
AMC-TZP-CXM-CTX-CAZ-TGC	Non-MDR		AMC-TZP-CXM-CTX-CAZ-TGC (3 isolates, 4.1%, Non-MDR)	
CXM-CTX-FEP-CIP-SXT-TGC	MDR		CXM-CTX-FEP-CIP-SXT-TGC (1 isolates, 1.4%, MDR)	
AMC-CXM-CTX-CAZ-FEP	Non-MDR			AMC-CXM-CTX-CAZ-FEP (1 isolates, 1.7%, Non-MDR)
AMC-CXM-CTX-CAZ-FEP-GEN-SXT	MDR			AMC-CXM-CTX-CAZ-FEP-GEN-SXT (1 isolates, 1.7%, MDR)
AMC-TZP-CTX-FEP	Non-MDR			AMC-TZP-CTX-FEP (3 isolates, 5.0%, Non-MDR)
AMC-TZP-CXM-CTX-CAZ-FEP-ERT-MEM	Non-MDR			AMC-TZP-CXM-CTX-CAZ-FEP-ERT-MEM (1 isolates, 1.7%, Non-MDR)
AMC-TZP-CXM-CTX-CAZ-FEP-GEN	Non-MDR			AMC-TZP-CXM-CTX-CAZ-FEP-GEN (2 isolates, 3.3%, Non-MDR)

Supplementary Material/S3 – Distribution and Diversity Metrices of Antibiogram patterns of ESBL-*producing K. pneumoniae* across sources

Source	MDR_Status	Number_of_Patterns	Total_Isolates	Shannon_Index	Hill_D1	Shannon_CI_Lower	Shannon_CI_Upper	Shannon_95CI	Evenness_J
Clinical	Overall	11	37	1.7729	5.8880	1.2356	1.9453	1.7729 (1.2356 - 1.9453)	0.7394
Clinical	Non-MDR	2	2	0.6931	2.0000	-0.0000	0.6931	0.6931 (-0.0000 - 0.6931)	1.0000
Clinical	MDR	9	35	1.6123	5.0145	1.1236	1.8102	1.6123 (1.1236 - 1.8102)	0.7338
Healthy_Residents	Overall	18	74	2.4916	12.0803	2.1367	2.5675	2.4916 (2.1367 - 2.5675)	0.8620
Healthy_Residents	Non-MDR	8	43	1.6098	5.0021	1.2346	1.7477	1.6098 (1.2346 - 1.7477)	0.7742
Healthy_Residents	MDR	10	31	2.0915	8.0974	1.6066	2.1646	2.0915 (1.6066 - 2.1646)	0.9083
Surface_water	Overall	11	60	1.5067	4.5118	1.0752	1.7334	1.5067 (1.0752 - 1.7334)	0.6283
Surface_water	Non-MDR	6	45	0.8050	2.2367	0.3924	1.0777	0.8050 (0.3924 - 1.0777)	0.4493
Surface_water	MDR	5	15	1.3624	3.9057	0.7299	1.5066	1.3624 (0.7299 - 1.5066)	0.8465

Supplementary Material/S4 – MDR and Venn Analysis of Antibiograms profiles of ESBL-*producing K. pneumoniae* across Sources

Table S4/A - MDR Prevalence					
Source	**MDR_Status**	**Number_of_Isolate**	**Total_Source_Isolates**	**Prevalence**	**Patterns_Included**
Clinical	Non-MDR	2	37	5.405405405	AMC-CXM-CTX-CAZ-FEP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-SXT
Clinical	MDR	35	37	94.59459459	AMC-CXM-CTX-CAZ-FEP-GEN-CIP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT | CXM-CTX-CAZ-FEP-GEN-CIP-SXT | CXM-CTX-CAZ-FEP-GEN-SXT | CXM-CTX-CAZ-GEN-CIP-SXT | TZP-CXM-CTX-CAZ-FEP-CIP-SXT-TGC | TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT
Healthy_Residents	Non-MDR	43	74	58.10810811	AMC-SXT | AMC-TZP-CXM-CTX-CAZ-CIP | AMC-TZP-CXM-CTX-CAZ-FEP | AMC-TZP-CXM-CTX-CAZ-FEP-CIP | AMC-TZP-CXM-CTX-CAZ-FEP-ERT-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-TGC | AMC-TZP-CXM-CTX-CAZ-TGC
Healthy_Residents	MDR	31	74	41.89189189	AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-ERT-CIP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-SXT-TGC | CXM-CTX-FEP-CIP-SXT-TGC
Surface_water	Non-MDR	45	60	75	AMC-CXM-CTX-CAZ-FEP | AMC-TZP-CTX-FEP | AMC-TZP-CXM-CTX-CAZ-FEP | AMC-TZP-CXM-CTX-CAZ-FEP-ERT-MEM | AMC-TZP-CXM-CTX-CAZ-FEP-GEN | AMC-TZP-CXM-CTX-CAZ-FEP-SXT
Surface_water	MDR	15	60	25	AMC-CXM-CTX-CAZ-FEP-GEN-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-SXT-TGC
Table S4/B - MDR Chi-Square Test					
Test	**Chi_Square_Statistic**	**Degrees_of_Freede**	**P_Value**	**Significance**	**Interpretation**
Chi-Square Test of Independence	46.03220721	2	1.00979E-10	Significant	MDR prevalence differs significantly across sources
MDR - Pairwie Comparison					
Clinical vs Healthy_Residents	Clinical	26.278	0.0000	Yes	Clinical has higher MDR prevalence
Clinical vs Surface_water	Clinical	41.639	0.0000	Yes	Clinical has higher MDR prevalence
Healthy_Residents vs Surface_wat	Healthy_Residents	3.478	0.0622	No	No significant difference
Table S4/C - Venn Analysis					
MDR_Status	**Region**	**Count**	**Patterns**		
MDR	All three sources	0			
MDR	Clinical and Healthy_Residents only	2	AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT		
MDR	Clinical and Surface_water only	0			

MDR	Healthy_Residents and Surface_wat	4	AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-SXT-TGC		
MDR	Clinical only	7	AMC-CXM-CTX-CAZ-FEP-GEN-CIP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-CIP | CXM-CTX-CAZ-FEP-GEN-CIP-SXT | CXM-CTX-CAZ-FEP-GEN-SXT | CXM-CTX-CAZ-GEN-CIP-SXT | TZP-CXM-CTX-CAZ-FEP-CIP-SXT-TGC | TZP-CXM-CTX-CAZ-FEP-GEN-CIP-SXT		
MDR	Healthy_Residents only	4	AMC-TZP-CXM-CTX-CAZ-FEP-CIP-SXT-TGC | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-ERT-CIP-SXT | AMC-TZP-CXM-CTX-CAZ-FEP-GEN-SXT-TGC | CXM-CTX-FEP-CIP-SXT-TGC		
MDR	Surface_water only	1	AMC-CXM-CTX-CAZ-FEP-GEN-SXT		
Non-MDR	All three sources	1	AMC-TZP-CXM-CTX-CAZ-FEP-SXT		
Non-MDR	Clinical and Healthy_Residents only	0			
Non-MDR	Clinical and Surface_water only	0			
Non-MDR	Healthy_Residents and Surface_wat	1	AMC-TZP-CXM-CTX-CAZ-FEP		
Non-MDR	Clinical only	1	AMC-CXM-CTX-CAZ-FEP-SXT		
Non-MDR	Healthy_Residents only	6	AMC-SXT | AMC-TZP-CXM-CTX-CAZ-CIP | AMC-TZP-CXM-CTX-CAZ-FEP-CIP | AMC-TZP-CXM-CTX-CAZ-FEP-ERT-TG		
Non-MDR	Surface_water only	4	AMC-CXM-CTX-CAZ-FEP | AMC-TZP-CTX-FEP | AMC-TZP-CXM-CTX-CAZ-FEP-ERT-MEM | AMC-TZP-CXM-CTX-CAZ-F		
Table S4/D- PERMANOVA_antibiogram patterns					
Analysis	**R_squared**	**F_statistic**	**P_Value**	**Significance**	**Interpretation**
Overall Patterns	0.1223	11.7088	0.0001	Significant	Resistance pattern composition differs significantly among sources
MDR Patterns Only	0.1332	5.9907	0.0001	Significant	MDR pattern composition differs significantly among sources
Non-MDR Patterns Only	0.1270	6.3259	0.0001	Significant	Non-MDR pattern composition differs significantly among sources

CHAPTER FOUR - MANUSCRIPT 3

DOES AMR SURVEILLANCE OF SURFACE WATER REFLECT AMR IN HUMANS? A GENOMIC COMPARISON OF ESBL-PRODUCING ESCHERICHIA COLI FROM SURFACE WATER, HEALTHY RESIDENTS, AND A CLINICAL FACILITY.

Author Contributions

Etando Ayukafangha - as principal investigator, co-conceptualised the study, performed the laboratory work, analysed the data, and drafted the manuscript.

Joshua Mbanga - as co-supervisor, co-conceptualised the study, assisted with sample collection and processing, vetted the results, and critically reviewed the manuscript.

Arshad Ismail - conducted data analysis, vetted the results and critically reviewed manuscript.

Sabiha Y. Essack - as co-supervisor, co-conceptualised the study, secured funding, guided the literature review and ethical approval application, facilitated data collection, vetted the results, and critically reviewed the manuscript.

Akebe Luther King Abia - as main supervisor, co-conceptualised the study, guided the literature review, data collection, processing and analysis, vetted the results, and critically reviewed the manuscript.

Objectives met – This chapter addresses the ESBL-producing *E. coli* component of objective 2

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Does AMR surveillance of surface water reflect AMR in humans? A genomic comparison of ESBL-producing *Escherichia coli* from surface water, healthy residents, and a clinical facility

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ABSTRACT

Wastewater antimicrobial resistance (AMR) surveillance is limited in many low- and middle-income countries where wastewater infrastructure is absent or inadequate. Surface water may provide an alternative for AMR surveillance in such settings. We investigated the resistome, virulome, and phylogenetic relationships of ESBL-producing *Escherichia coli* (ESBL-Ec) from surface water (SW), healthy residents (HR), and a clinical facility (CL) to determine whether SW isolates reflect human-associated AMR. During an eight-month longitudinal study in South Africa, 95 ESBL-Ec isolates (SW = 32, HR = 32, CL = 31) underwent whole-genome sequencing. Bioinformatics was used to characterise antimicrobial resistance genes (ARGs), virulence genes, sequence types (STs), phylogroups, and phylogenetic relationships. Seven phylogroups and 21 STs were identified. Phylogroups A, B1, and D and STs 10 and 38 occurred across all sources. Several clinically important lineages, including ST131, were shared between SW and HR isolates; no ST was unique to clinical isolates. Phylogenetic analysis revealed predominantly ST-driven clustering, with SW isolates showing greater similarity to HR isolates than to clinical isolates. Forty-five ARGs were detected, including 12 β-lactamase genes. $bla_{CTX\text{-}M\text{-}15}$ was the dominant ESBL gene in all clinical isolates and in approximately half of SW and HR isolates. Resistance and virulence gene profiles were more frequently shared between SW and HR than between SW and CL isolates. Surface water did not fully reflect community and clinical ESBL-Ec populations but captured clinically relevant lineages and resistance determinants. These findings support SW monitoring as a complementary AMR surveillance approach in resource-limited settings lacking wastewater infrastructure.

1. Introduction

Antimicrobial resistance (AMR) is increasingly undermining the effectiveness of standard therapies, resulting in prolonged infections, higher healthcare costs, and rising treatment failure in both community and hospital settings worldwide (Murray et al., 2022; Naghavi et al., 2024). Despite this growing burden, surveillance capacity remains highly unequal, particularly across many low- and middle-income countries (LMICs), where limited diagnostic infrastructure and fragmented reporting systems constrain both clinical and community-level monitoring and contribute to substantial underestimation of AMR burden (Abia et al., 2023; Seale et al., 2017; Tornimbene et al., 2022). These surveillance limitations hinder timely detection of emerging or escalating AMR as well as transmission trends and resistance dissemination. This is especially true for high-burden pathogens circulating between community, healthcare, and environmental interfaces, notably extended-spectrum β-lactamase–producing *Escherichia coli* (ESBL-Ec), which has become a dominant drug-resistant Enterobacterales species globally (Gay et al., 2023; Shamsrizi et al., 2020).

The spread of ESBL-Ec is driven by the interaction of antimicrobial

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selection and environmental transmission. While antibiotic misuse selects for resistance, sub-optimal infection prevention in hospitals, inadequate water, sanitation, and hygiene infrastructure, overcrowding, and poor waste management at the community level facilitate its dissemination. As a genetically plastic commensal and opportunistic pathogen, ESBL-Ec is a good indicator organism for tracking AMR transmission between human and environmental reservoirs (Nguyen et al., 2024). Drug-resistant bacteria, antibiotic residues, and ARGs may enter the environment through human waste streams, where mobile genetic elements can further promote dissemination across bacterial populations (Bengtsson-Palme et al., 2018). Consistent with this, ESBL-associated genes from the bla_{TEM}, bla_{CTX}, bla_{SHV} families have been reported in healthy community residents (Sintondji et al., 2023), clinical settings (Lesani et al., 2020) and environments such as wastewater treatment plants (Gumede et al., 2021) and surface water (Mou et al., 2024), highlighting the multi-reservoir nature of the problem and the potential of surface water as a reflection of circulating AMR in humans.

Because conventional laboratory-based surveillance is often resource-intensive, aquatic environments are increasingly being explored as a complementary approach for monitoring AMR circulation in human populations. Most such work has focused on wastewater and sewage systems, where community and healthcare effluent can be sampled through established sewerage networks. In high-income settings, wastewater-based surveillance has provided useful population-level insights into the burden of AMR (Chau et al., 2022; Hutinel et al., 2019; Schnall et al., 2019; WHO, 2025). However, this model is less easily transferred to many resource-limited settings, where sewerage infrastructure is fragmented and environmental monitoring is incomplete. In these contexts, human-associated waste may instead enter streams, drainage channels, and other surface waters through informal sanitation, runoff, or direct discharge. Surface water may therefore represent a more accessible receiving matrix for resistant bacteria and resistance genes originating from both community and healthcare-associated activities. Although ESBL-producing Enterobacterales have been described in wastewater and downstream aquatic environments (Gumede et al., 2021; Mbanga et al., 2021; Mukwevho et al., 2025), relatively few studies have tested whether surface water reflects the genomic characteristics of human-associated ESBL-Ec in spatially linked community and clinical settings.

In South Africa, β-lactamase genes have been documented in healthy communities (Msolo et al., 2020), clinical settings (Founou et al., 2019; Peirano et al., 2011), and the environment (Gumede et al., 2021), and some studies have shown overlap in resistance determinants between clinical and environmental compartments, with evidence that mobile genetic elements may contribute to their spread (Ekwanzala et al., 2018; Mbanga et al., 2021). This interface may be particularly important in and around informal settlements, where inadequate sanitation, overcrowding, informal latrines, and poorly regulated drainage increase exposure to contaminated surface waters and create favourable conditions for the persistence and transmission of AMR. Given that South Africa is home to up to 4000 informal settlements that house more than 2 million households (Gamede, 2024; Housing Development Agency, 2021), understanding whether surface water captures human-associated AMR signals is both epidemiologically and operationally important.

This study, therefore, compared the resistome, virulome, and phylogenomic structure of ESBL-Ec from surface water, healthy community residents, and clinical settings to determine whether AMR in surface water reflects AMR in community and clinical populations, and to assess the potential of surface water as a proxy matrix for human-associated AMR surveillance in resource-limited settings.

2. Materials and methods

2.1. Ethical considerations

The protocol was reviewed and approved by the Biomedical Research Ethics Committee, University of KwaZulu-Natal (Ref. BREC/00003640/2021), the KwaZulu-Natal Department of Health Provincial Health Research Ethics Committee (NHRD Ref. KZ_202203_023), and the National Health Laboratory Service (NHLS Ref. PR2225862). All community volunteers provided written informed consent prior to enrolment (Supplementary Material/S1).

2.2. Study design, setting and sample collection

This study was nested within a larger eight-month (May - December 2023) longitudinal investigation assessing phenotypic and genomic profiles of ESBL-producing *E. coli* across environmental, community, and clinical sources in uMgungundlovu District Municipality (UMDM), KwaZulu-Natal, South Africa. This study investigated isolates from surface water, healthy community residents, and clinical specimens at the genomic level to determine whether surface water reflects genomic features observed in healthy community residents and clinical sources.

Community samples comprised stool specimens collected from healthy residents of an informal settlement. Clinical samples were obtained from bacteriological cultures of stool, urine, blood, cerebrospinal fluid, pus, and other body fluids from hospitalised adults (>18 years). Surface water samples were collected from the Kwapata River, which drains into the Msunduzi River, a tributary of the uMngeni River, as previously described (Ayukafangha et al., 2026).

2.3. E. coli identification, ESBL confirmation and phenotypic antimicrobial susceptibility testing (AST)

The samples comprising 80 surface water, 1978 stool, and 2154 clinical culture specimens were processed and yielded 512, 1,915, and 588 *E. coli* isolates, respectively. These confirmed *E. coli* isolates were subsequently screened for ESBL production and subjected to AST, resulting in 86, 519, and 31 ESBL-producing isolates, as reported elsewhere (Ayukafangha et al., 2026). Briefly, Putative *E. coli* were identified and isolated using chromogenic agar as previously described (Mbanga et al., 2020). Molecular confirmation of *E. coli* was performed by real-time PCR targeting the uidA gene, with *E. coli* ATCC® 25922 used as a positive control and nuclease-free water as a template-free negative control.

ESBL screening was performed using CHROMID® ESBL agar (bioMérieux, Marcy l'Etoile, France), and ESBL confirmation was performed using the double disk synergy test (DDST) with cefotaxime (30 μg) and ceftazidime (30 μg) disks, placed 20 mm away from the centrally placed amoxicillin clavulanic acid (10 μg). A ≥5 mm increase in inhibition zone diameter for either antimicrobial agent was interpreted as ESBL-positive. AST was conducted using the VITEK® 2 system with the VITEK® 2 AST-N256 card for Gram-negative bacteria, according to the manufacturer's instructions and interpretation done according to CLSI guidelines (CLSI, 2021). Confirmed ESBL-producing *E. coli* from each source then underwent genomic DNA extraction using the GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St Louis, Missouri, USA). DNA concentration and purity were measured with a NanoDrop microvolume spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) by assessing the 260/280 nm ratio according to the manufacturer's instructions.

2.4. Whole-genome sequencing (WGS) and selection of study isolates

The extracted DNA from confirmed ESBL-producing *E. coli* isolates underwent whole-genome sequencing (WGS) at the National Institute for Communicable Diseases (NICD, Johannesburg, South Africa).

Sequenced isolates were quality-checked for non-*E. coli* contamination, after which 31 clinical isolates remained, constituting the smallest group. This number was used as the baseline for comparative analyses across all sources. To achieve a balanced design, 32 isolates were randomly sampled from both the community and surface water groups, with monthly stratification of four isolates per source. The resulting dataset included 96 ESBL-producing *E. coli* isolates: 31 clinical, 32 community, and 32 surface water. This balanced, month-stratified selection was used to reduce over-representation of the larger surface water and healthy resident isolate pools while preserving temporal coverage.

2.5. Bioinformatic analyses

Multiplexed paired-end libraries were prepared using the Illumina DNA Prep Kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions. Library quality and fragment size distribution were assessed using an Agilent TapeStation (Agilent Technologies, Santa Clara, CA, USA), and library quantification was performed using a Qubit 4.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Standardised libraries were pooled at 4 nM to target approximately 100× coverage per sample and sequenced at the National Institute for Communicable Diseases (NICD, Johannesburg, South Africa) on the Element Biosciences AVITI and Illumina NextSeq 1000/2000 platforms to generate 2 × 150 bp paired-end reads. Raw reads were quality checked with FASTQC (v0.12.0) (Andrews, 2010), followed by trimming using Sickle (v1.33; https://github.com/najoshi/sickle) and assembled *de novo* using SKESA (v2.4.0; https://github.com/ncbi/SKESA). A minimum read depth of 30×, at least 95% coverage, and Phred scores of 30 or higher were applied as quality criteria. Short contigs (<200 bp) and assemblies outside 4.4 - 6.5 Mb were excluded. Genomes were submitted to GenBank for annotation under the bioproject number PRJNA1253337.

Multi-locus sequence typing (MLST) was performed using MLST v2.0 (https://bitbucket.org/genomicepidemiology/mlst/) to assign and reconfirm sequence types. *E. coli* phylogroups were determined using EzClermont v0.7.0 (https://github.com/nickp60/ezclermont), implementing the Clermont scheme. Serotype prediction was done using SerotypeFinder 2.0 to detect lipopolysaccharide (O-antigen) and capsular flagella (protein; H-antigen). ABRicate (v1.0.1) (https://github.com/tseemann/abricate) was used to blast assemblies against ResFinder and AMRFinderPlus (Feldgarden et al., 2019) for resistome analysis, and the results were further confirmed using the Comprehensive Antimicrobial Resistance Database (CARD), which also identified resistance mechanisms (https://card.mcmaster.ca/) (Alcock et al., 2023). Virulence factors were determined using the Virulence Factor Database (VFDB), and virulence genes were classified into functional classes (Liu et al., 2022) and pathotypes based on criteria previously described (Johnson et al., 2005, 2019; Naidoo and Zishiri, 2025). Isolates were classified as single pathotype when they carried virulence markers corresponding to one pathotype only (e.g., EPEC), and as hybrid pathotype when they carried virulence markers associated with two or more pathotypes (e.g., EPEC + UPEC or EPEC + UPEC + NMEC).

Phylogenomic analysis was performed on isolates across sources. All genomes were retrieved, annotated, and processed using the Bacterial and Viral Bioinformatics Resource Center (BV-BRCB; https://www.bv-brc.org/). Maximum-likelihood phylogenetic reconstruction was carried out within BV-BRC, and resulting trees were visualised in Interactive Tree of Life (iTOL; https://itol.embl.de). Annotation, customisation, and incorporation of associated metadata were performed using the itol.toolkit R package (Zhou et al., 2023).

2.6. Statistical analysis

Statistical analyses were conducted in R (version 4.5.0; 2025-04-11 ucrt) using RStudio (version 2025.9.0.387). Associations between categorical variables were assessed using the chi-square test, with statistical significance defined at $p \leq 0.05$. These variables included source-wise comparisons of ARG classes, resistance mechanisms, and virulence/pathotype categories. Where relevant, standardised residuals were examined to identify the categories that contributed most to significant associations. For analyses involving multiple comparisons, *p*-values were adjusted using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). Diversity was quantified using the Shannon diversity index and Pielou's evenness metric (Oksanen et al., 2025). Phylogroup and sequence type overlap between sources was summarised descriptively as proportions of isolates sharing the same lineage assignments across compartments. Close phylogenetic clustering between sources was assessed qualitatively from the maximum-likelihood tree based on nearest-neighbour relationships and short-branch clustering patterns, and was interpreted as supportive of lineage-latedness rather than definitive transmission (Valiente, 2006). Data visualisations, including plots and heatmaps, were generated in R using ggplot2 and ComplexHeatmap (Wickham, 2011).

3. Results

3.1. Genomic features of ESBL-Ec isolates

Assembly metrics for the ESBL-producing *E. coli* isolates are summarised in Supplementary Material/S2. Genome sizes ranged from 4.66 to 5.61 Mb, with GC content ranging from 50.32% to 50.89%. Draft assemblies comprised 59 to 256 contigs, with N50 values ranging from 60.9 kb to 263.9 kb and L50 values ranging from 6 to 53. Gene annotation identified 4637 to 5869 coding sequences (CDS), 71 to 90 tRNA genes, and 2 to 13 rRNA genes per genome. Assembly quality scores ranged from 99.1% to 99.9% (coarse consistency) and 89.6% to 99.4% (fine consistency).

3.2. Detected AMR genes of ESBL-E. coli isolates across sources

A total of 45 ARGs were detected across 95 ESBL-producing *E. coli* isolates, of which β-lactamases accounted for 26.6% (12/45) and aminoglycoside genes 20.0% (9/45). The resistome comprised a shared core across all three sources, but surface water consistently showed greater overlap with isolates from healthy residents than with clinical isolates.

Among β-lactamase genes, $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1B}}$ were detected in isolates from all sources. $bla_{\text{CTX-M-15}}$ was the dominant ESBL gene, occurring in 100% (31/31) of clinical isolates, 50.0% (16/32) of surface-water isolates, and 43.7% (14/32) of isolates from healthy-residents, while $bla_{\text{TEM-1B}}$ was concentrated in isolates from healthy residents (37.5%; 12/32) and surface water (28.1%; 9/32) and was rare in clinical isolates (3.2%; 1/31). Notably, $bla_{\text{CTX-M-27}}$ and $bla_{\text{OXA-1}}$ were detected only in isolates from surface water and healthy residents, and no β-lactamase gene was exclusively shared between surface water and clinical isolates. Eight β-lactamase genes were detected only in isolates from healthy residents, while no β-lactamase gene was unique to clinical or surface water isolates (Fig. 1a).

A similar pattern was observed for non-β-lactam resistance genes: 10 genes were evident in isolates from all sources, 12 were common in isolates from both surface water and healthy residents, and none were common to both surface water and clinical isolates, as shown in Fig. 1b.

Isolates from healthy residents had the greatest overall ARG richness and diversity, while clinical isolates had the least, and surface water showed an intermediate profile. For β-lactamase ARGs, richness and diversity were highest in isolates from healthy residents ($S = 12$; $H = 2.06$), intermediate in surface water isolates ($S = 4$; $H = 1.16$), and lowest in clinical isolates ($S = 2$; $H = 0.14$). Significant source-associated differences were observed for tetracycline, macrolide, fluoroquinolone, and amphenicol ARGs ($p < 0.05$), whereas β-lactamase, aminoglycoside, sulphonamide, and trimethoprim ARGs did not differ significantly across sources ($p > 0.05$) (Supplementary Material/S3)

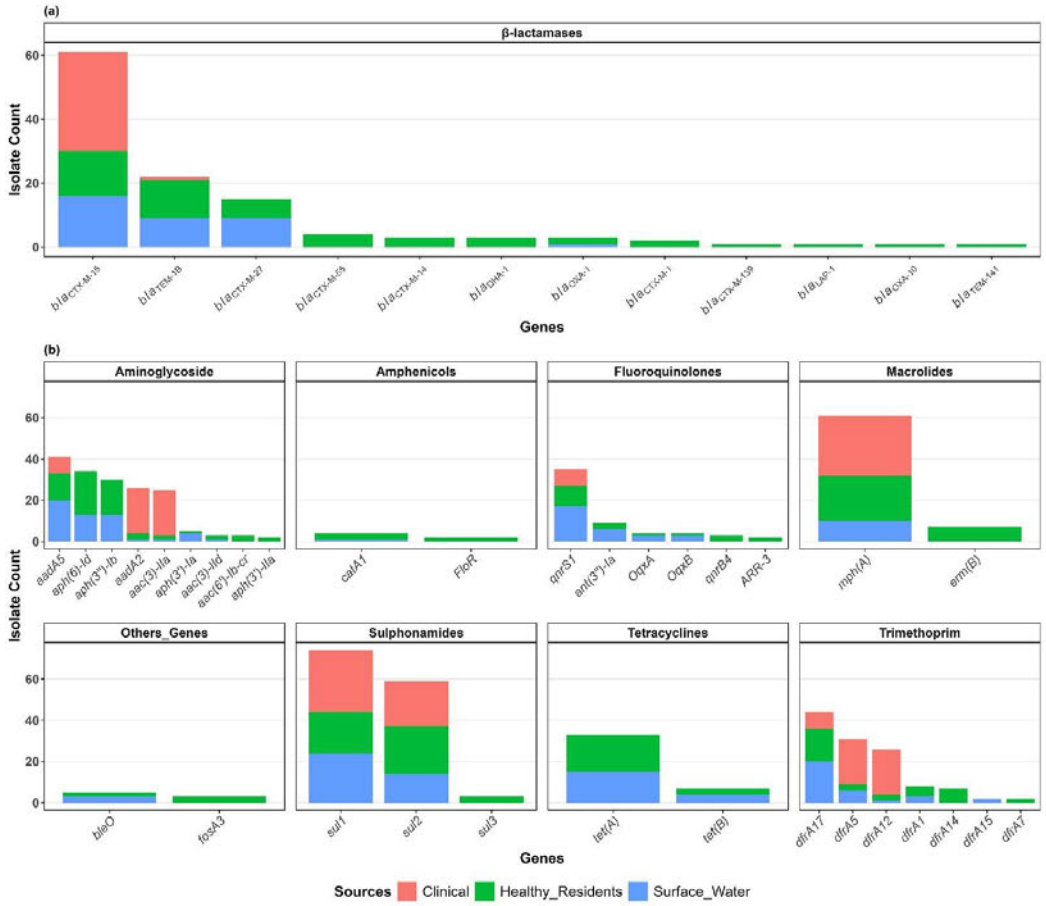

Fig. 1. Distribution of (a) β-lactamases and (b) non-β-lactamases ARGs in isolates across sources.

3.3. Resistome and associated resistance mechanisms across sources

CARD analysis identified 112 ARGs spanning six broad resistance mechanisms. All resistance mechanisms were detected across clinical, healthy resident, and surface water isolates, with distributions highly comparable across sources. Efflux-associated determinants dominated in all three compartments, particularly RND efflux systems (41.6% in clinical, 40.5% in healthy resident, and 42.2% in surface water isolates), followed by MFS efflux systems (21.0%, 22.6%, and 23.7%, respectively). Other mechanism categories, including target alteration, β-lactamases, and aminoglycoside-modifying enzymes, showed only minor variation across sources (Supplementary Material/S4).

The top 60 ARGs per source are represented in Fig. 2. Shannon diversity and Pielou evenness indicated highly comparable resistance-mechanism profiles with minimal divergence across sources. Richness was identical ($S = 6$), with Shannon indices ranging narrowly from 1.25 to 1.31 and evenness from 0.695 to 0.731. Resistance-mechanism distributions were statistically indistinguishable across sources ($p = 0.595$), consistent with comparable Shannon diversity and evenness.

3.4. Virulence determinants of ESBL-E. coli isolates across sources

Virulome analysis identified 285 distinct virulence genes across all isolates, with the highest proportion detected in isolates from healthy residents (36.3%), followed by surface water (33.4%) and clinical isolates (29.7%) ($p < 0.05$; all standardised residuals $|z| < 2$). Fifty-four virulence genes were unique to isolates from healthy residents, five were unique to surface-water isolates, and none were exclusive to clinical isolates. Virulence genes were assigned to 11 functional categories, dominated by nutritional/metabolic factors, followed by effector delivery system-associated genes, which were detected across all sources. Exotoxin genes were the least frequent and showed significant source-specific variation, occurring in 22 clinical, 9 healthy-resident, and 1 surface-water isolate ($p = 0.004$).

Both single and hybrid pathotypes were observed across sources, with their distributions differing significantly ($p < 0.0001$). Hybrid pathotypes predominated overall (46 isolates; 48.4%). The EPEC + UPEC + NMEC hybrid was most frequent among clinical isolates (n = 22), UPEC + NMEC predominated among isolates from healthy residents (n = 16), and EPEC + UPEC + NMEC was the most frequent hybrid

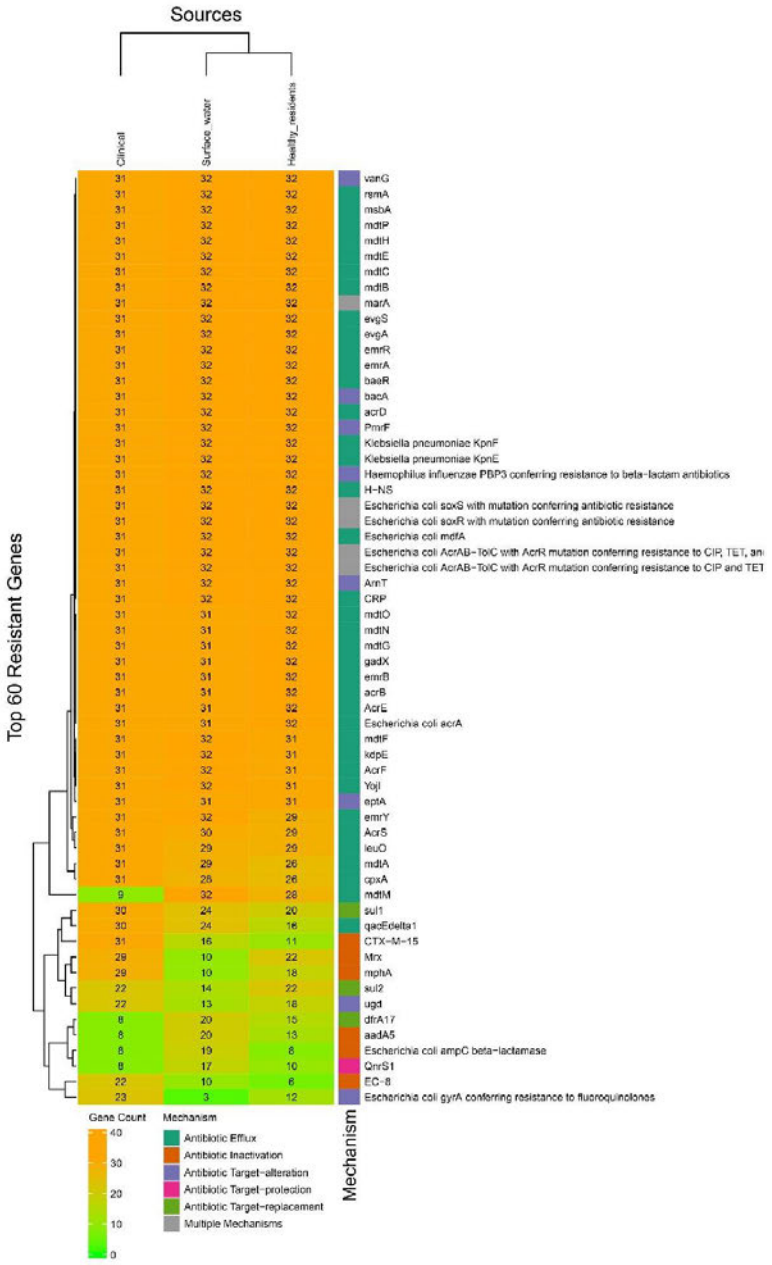

Fig. 2. Heatmap of resistance genes associated with MDR mechanisms across sources. ARGs and corresponding isolate counts are colour-coded from green (low) to orange (high), with each row representing a gene. Genes are annotated by resistance mechanism, indicated by colour-coded bars on the right. Efflux, inactivation, and target alteration mechanisms were dominant across all sources, whereas target replacement and reduced permeability were less frequently observed.

combination in surface-water isolates (n = 8). By contrast, single pathotypes, mainly EPEC, were most common in surface water (n = 21) and least common in clinical isolates (n = 9). At the broader pathotype-group level, InPEC predominated among isolates across all sources, whereas single ExPEC pathotypes were detected only in surface-water isolates ($p < 0.0001$).

Lineage-specific patterns were also evident within the major pathotype groups. Among clinical isolates, the EPEC + UPEC + NMEC hybrid predominated, with most (n = 21) belonging to phylogroup D and ST131 or ST10. In isolates from healthy residents, the UPEC + NMEC pathotype combination was most commonly associated with phylogroup B2, including ST131 and ST1193 (n = 9 isolates). In surface water isolates,

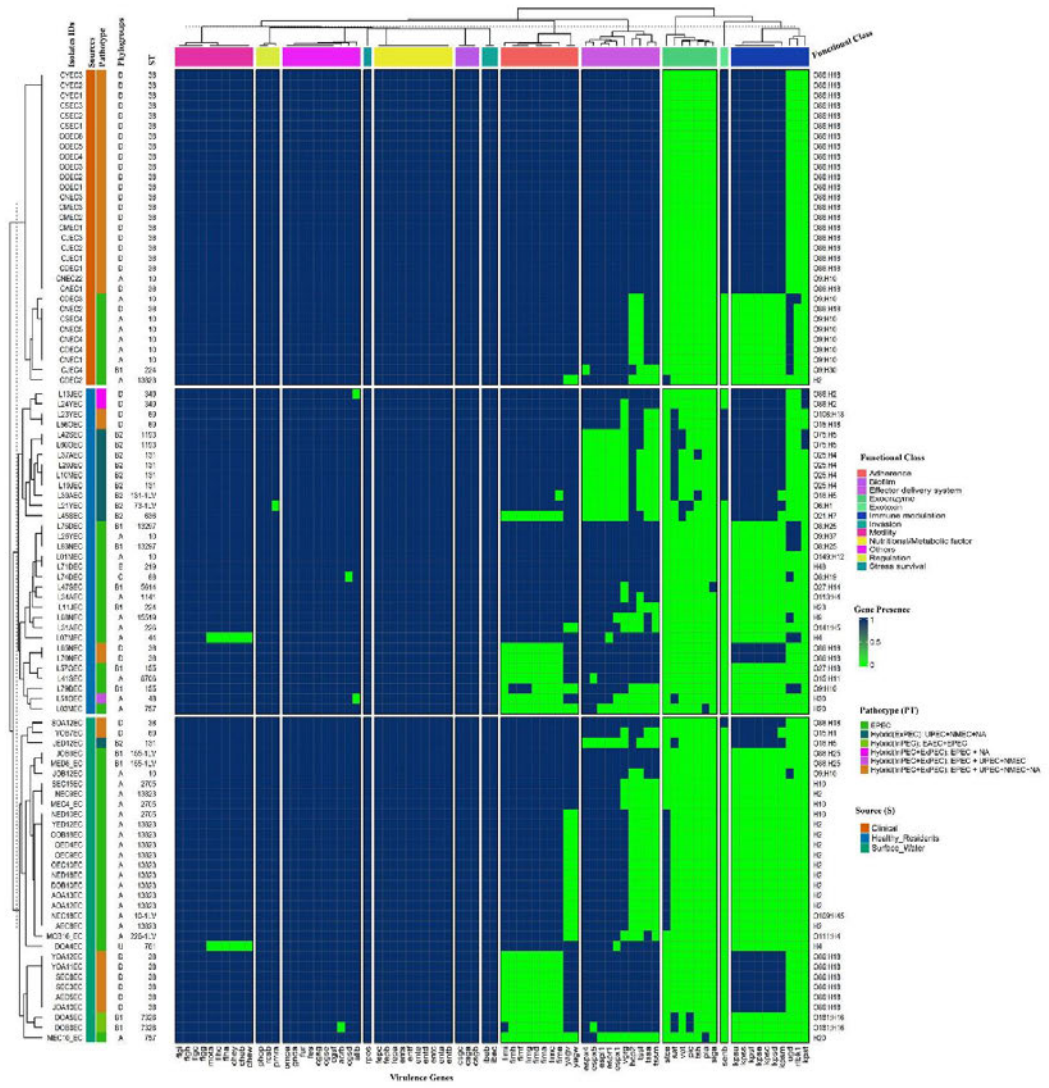

Fig. 3. Hierarchically clustered presence/absence matrix of virulence-associated genes of ESBL-producing *E. coli* across sources. Rows represent individual isolates and columns represent virulence genes, grouped by functional categories (top annotation bar). Gene presence is indicated in green (1) and absence in dark blue (0). Dendrograms depict hierarchical clustering of both isolates and genes based on similarity in virulence gene profiles. Additional annotations include isolate metadata such as phylogroup, sequence type (ST), serotype, pathotype, and source of isolation. Virulence profiles clustered mainly by lineage-related features rather than source, although source-associated differences in pathotype composition and selected functional classes were evident. EPEC = enteropathogenic *E. coli*; EAEC = enteroaggregative *E. coli*; InPEC = intestinal pathogenic *E. coli*; ExPEC = extraintestinal pathogenic *E. coli*; UPEC = uropathogenic *E. coli*; NMEC = neonatal meningitis-associated *E. coli*; NA = not assigned or not further classified.

the EPEC pathotype was predominantly associated with phylogroup A, including ST10, ST13828, and ST2705 (n = 18 isolates). All reported *p*-values remained significant after Benjamini–Hochberg adjustment (adjusted $p < 0.05$). Fig. 3 presents the top 10 virulence genes per functional class, stratified by source, sequence type, phylogroup, and serotype.

3.5. MLST and phylogenomic analysis

Twenty-one sequence types were detected in the 95 isolates. ST10 and ST38 were found in isolates across all three sources. ST131, ST69, and ST757 were detected in surface water and in isolates from healthy residents. ST13823 was detected in surface water and in clinical isolates, and ST224 was detected in isolates from healthy residents and in clinical isolates. While several STs were restricted to isolates from surface water or HR, none were exclusive to the clinical isolates, as detailed in Supplementary Material/S5. The isolates belonged to seven phylogroups (A, B1, B2, C, D, E, U), with three phylogroups (A, B1, and D) common to isolates from all sources, B2 common to isolates from surface water and healthy residents, and none to surface water and clinical isolates. In contrast, phylogroups C and E were exclusive to isolates from healthy residents, while U was exclusive to surface water isolates. Clinical isolates had no exclusive phylogroup.

Phylogenetic analysis included all 95 ESBL-Ec isolates and one reference genome (*E. coli* str. K-12 substr. MG1655, ST10). The codon-based maximum-likelihood tree revealed clonal lineage-driven population structure rather than the source of isolation. Isolates were distributed across a mixture of shared major lineages, source-enriched subclades, and tight cross-source terminal groupings. At the broadest level, the tree was dominated by a single large, well-supported core lineage containing 93 of the 96 taxa (95 study isolates plus the ST10 reference), while two healthy-resident ST131 isolates, L10MEC and L20JEC (dark dotted nodes), branched outside this strongly supported core as solitary divergent lineages (Fig. 4).

Within the major core lineage, several internal structures were evident. The most prominent was a large phylogroup D/ST38-centered assemblage that contained isolates from all three sources but was heavily weighted towards clinical isolates. A strongly supported 31-isolate clade (BS = 100) grouped 22 clinical isolates, 7 SW isolates, and 2 isolates from healthy residents. A second major clade was a surface-water-dominant lineage centered on ST13823. A strongly supported 15-isolate clade (BS = 100) contained 13 SW isolates, together

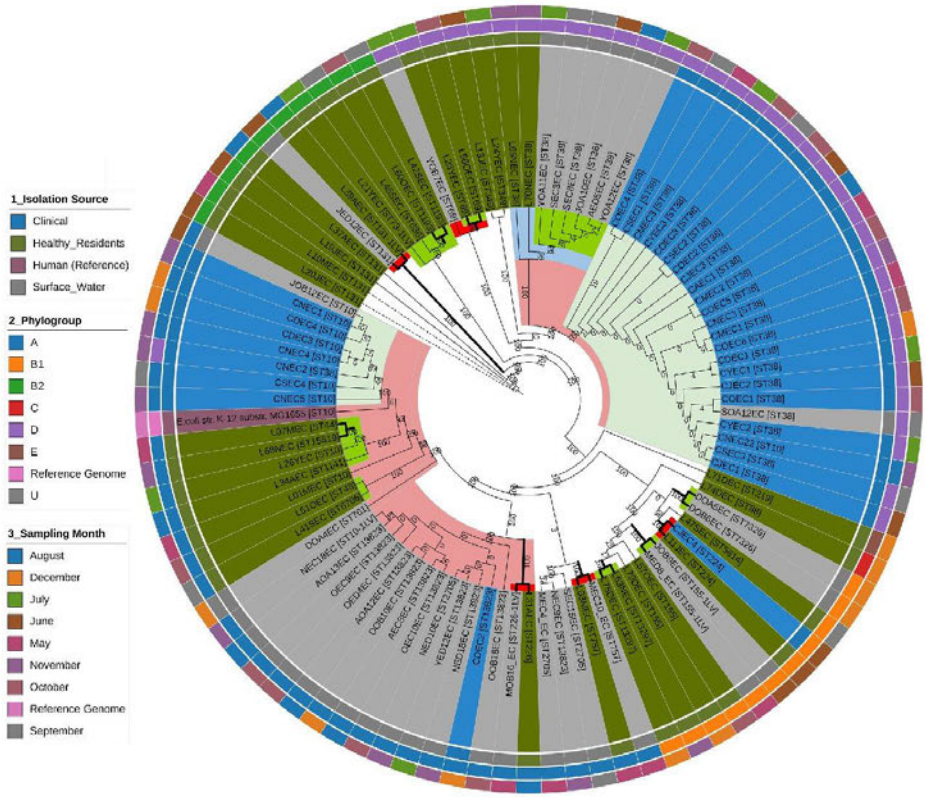

Fig. 4. Phylogenetic relationship of ESBL-Ec isolates between sources. The circular phylogenetic tree shows the relationships among study isolates based on a maximum-likelihood analysis. Isolate IDs and sequence types (STs) are displayed at the tips. Tip background colours indicate source of isolation: clinical (blue), healthy residents (dark green), and surface water (grey). Concentric metadata rings, shown from inner to outer, represent isolation source, phylogroup, and sampling month. The three major clades are shaded (red) to aid visualisation of broad lineage structure, and selected inter-source subclades are additionally highlighted. Red markers denote tight or sister cross-source clustering, whereas green markers denote tight within-source clustering. Internal node values indicate bootstrap support (0-100).

with one isolate from healthy residents (L31AEC [ST226]) and one clinical isolate. The third major clade was a mixed ST10-associated lineage, which provided one of the clearest overlaps across all three sources. A broad 17-member clade (BS = 100) and its corresponding 16-member internal subclade (BS = 98) grouped the ST10 reference isolate with isolates from healthy residents, clinical samples, and surface water.

At finer phylogenetic resolution, direct clustering analysis, defined here as high-confidence sister-taxon pairings or terminal isolate pairs connected by very short branch lengths (red-coloured clade, Fig. 4), revealed cross-source relationships. Three of the strongest involved isolates from surface water and healthy residents. First, L03MEC [ST757] (healthy residents) formed a direct sister pair with MEC10_EC [ST757] (surface water) with BS = 100. Second, L39AEC [ST131-1LV] (healthy residents) paired directly with JED12EC [ST131] (surface water), also with BS = 100. Third, MOB16_EC [ST226-1LV] (surface water) clustered directly with L31AEC [ST226] (healthy residents), again with BS = 100. Only one equally strong, healthy resident-clinical direct cross-source sister pair was observed: L11JEC [ST224] (healthy residents) clustered directly with CJEC4 [ST224] (clinical) at BS = 100. A few apparent surface water-clinical isolate terminal associations were present within ST38 and ST10, but these were supported by very low bootstrap values (BS = 0-4). Thus, the strongest terminal evidence in the tree supports the proximity of surface water to healthy residents.

Overall, both major-clade structure and terminal isolate clustering indicate that the phylogenetic relationships among the isolates are shaped more strongly by clonal lineage than by isolation source, with isolates from healthy residents occupying the central bridging position between surface-water and clinical isolates, and with surface water isolates showing their strongest fine-scale relatedness to the isolates from healthy residents.

4. Discussion

Conventional antimicrobial resistance surveillance is constrained by clinical and laboratory infrastructure and resources, limiting its reach in resource-limited settings. Environmental surveillance has emerged as a complementary approach, with wastewater and sewage systems serving as the most widely studied matrices for community-level AMR monitoring (Gumede et al., 2021; Jørgensen et al., 2017; Mbanga et al., 2021). However, the utility of wastewater-based surveillance depends on the presence of functional sewage infrastructure, an assumption that cannot be made in informal settlements, where domestic and community waste frequently drains directly into surrounding surface water bodies rather than into centralised collection systems. In such settings, surface water may potentially represent a more ecologically appropriate and accessible surveillance matrix than wastewater. To evaluate this proposition, we investigated the genomic relationships among ESBL-producing *E. coli* from surface water, healthy community residents, and clinical isolates from a spatially connected informal settlement in South Africa, using whole-genome sequencing to compare their resistomes, virulomes, and phylogenetic relationships. The following discussion integrates these analytical dimensions to evaluate the extent to which the genomic profile of surface water ESBL-*E. coli* reflects what circulates in the community and clinical setting, and considers the implications for water-based AMR surveillance in resource-limited environments.

In this study, ESBL-producing *E. coli* from surface water showed genomic overlap represented by a small subset of shared sequence types with both isolates from healthy community residents and clinical isolates, indicating partial genomic overlap across environmental and human-associated compartments. At the broader population level, phylogroups A, B1, and D were shared across isolates from all sources. Shared core sequence types included ST10 and ST38, which were detected across isolates from all three sources, while additional pairwise overlaps were observed for ST131, ST69, ST757, and ST13823. The presence of ST38 and ST10 in all three settings is consistent with previous reports describing ST38 as a widely disseminated extra-intestinal pathogenic *E. coli* (ExPEC) lineage associated with urinary tract and bloodstream infections (Musicha et al., 2026; Ogundare et al., 2024), and ST10 as a successful environmental lineage commonly found in aquatic systems (Kamal et al., 2021; Machado et al., 2024). Consistent with this, ST38 and ST10 in our dataset were recovered from multiple clinical specimen types, including blood and urine, although they were also observed in other sources, such as sterile sites and pus, indicating a broader clinical niche. In South Africa, ST10 has been reported in clinical isolates, while both ST10 and ST38 have been identified in community- and water-associated One Health studies (Hetsa et al., 2026; Mbelle et al., 2019; Ogundare et al., 2024). Similar to ST10 and ST38, though less documented locally, ST38 harbours a broad resistome across diverse plasmid replicons, a well-characterised virulome, and a demonstrated capacity to persist across human, animal, and environmental niches globally (Kamal et al., 2021; Roy Chowdhury et al., 2023). These shared genomic attributes likely underpin the cross-ecological circulation of both lineages, consistent with their co-detection across all three sources in this study.

Among these shared lineages, we observed greater similarity between surface water isolates and isolates from healthy residents than between surface water isolates and clinical isolates. Notably, the sequence types (ST131, ST69, ST155, and ST757) co-occurred in isolates from surface water and healthy residents, but not in clinical isolates (ST13823). This observation was further reinforced via phylogenetic analysis, which refined this pattern of overlap by showing that, although surface-water isolates occurred within mixed-source clades containing both healthy-resident and clinical isolates, their closest fine-scale relationships were more often with healthy residents than with clinical isolates. This was most evident in the terminal clustering patterns, where the strongest cross-source sister pairings involving surface water were consistently observed with healthy-resident isolates, including the well-known ST131, the ST757 pair L03MEC and MEC10_EC, the ST131/ST131-1LV pair L39AEC and JED12EC, and the ST226/ST226-1LV pair MOB16_EC and L31AEC, all supported by BS = 100. By contrast, equally strong direct clustering between surface-water and clinical isolates was not observed, with apparent surface water-clinical terminal associations within ST38 and ST10 showing weak bootstrap support. While there is a paucity of literature on ST131 in isolates from healthy community residents in South Africa, the strain has since been reported in the clinical setting (Hetsa et al., 2026; Peirano et al., 2011). Similarly, ST131 and ST69 were previously reported in river and wastewater treatment plant effluents within the same district (Mbanga et al., 2021; Ndlazi et al., 2025), suggesting their established circulation in the local environment. The study setting, characterised by informal infrastructure, open drainage systems, and close geographical proximity between residential areas and surrounding waterways and streams, likely facilitates the exchange of circulating strains between the aquatic environment and community residents. This ecological intimacy may explain why surface water more closely reflects strains carried in the community than those captured in the clinical setting at a genomic level. While our findings do not exclude the presence of these shared lineages in clinical infections, the passive clinical sampling in this study may have reduced the probability of detecting strains that circulate at lower frequencies in our study setting.

Most importantly, the overlap observed in our study was limited to a small set of isolates, whereas a larger proportion showed source exclusiveness and within-source phylogenetic clustering. Notably, sixteen source-exclusive sequence types were observed among isolates from healthy residents, six in surface water and none in the clinical setting. This was also evident in the phylogenetic clustering, where a few isolates showed cross-source clustering, while most showed within-source clustering. This observation reinforces that, although some overlap exists across compartments, the different sources may still preserve source-associated strains and phylogenetic structure (Touchon et al., 2020). Although no sequence types were exclusive to clinical isolates, the

strong within-source clustering, dominated by ST38, suggests clonal enrichment of human-adapted ExPEC lineages, a pattern expected in infection-derived samples and not indicative of a distinct clinical-only reservoir (Fonseca et al., 2022; Nguyen et al., 2024). The high variability in community-exclusive sequence types may reflect the well-recognised diversity of intestinal *E. coli* carriage among healthy individuals, where multiple resident and transient lineages can coexist, generating a broader pool of low-frequency or singleton sequence types than is often seen in clinically sourced enriched by infection-driven sequence types (Martinson and Walk, 2020; Stoesser et al., 2015).

This source exclusiveness in surface water isolates was most clearly driven by Achtman MLST ST13823, a lineage that also dominated within-source clustering yet remains poorly documented both locally and internationally. In the phylogeny, most ST13823 isolates clustered together, although some occurred near ST2705 isolates with only moderate bootstrap support. Notably, ST2705 has previously been reported in healthy Australians (Moran and Hall, 2018), retail aquatic products and fish (Zhang et al., 2024), and chilled chicken and chicken faecal samples (Chang et al., 2020), suggesting a possible animal-environmental transmission interface. ST13823 in the current study also formed weak clustering with a locally documented ST10 strain, further suggesting that, despite being sources restricted in our study, it may have ecological overlap with established environmental lineages in this setting. To further contextualise ST13823, we searched EnteroBase and BV-BRC and identified a small number of publicly available ST13823-associated records, predominantly from EnteroBase, assigned to ST165 complex and lineage A. These records were geographically distributed across the United Kingdom, France, Spain, Malawi, Tanzania and Kenya, with available metadata indicating mainly human/Homo sapien-associated sources, although source information was incomplete for some entries (Supplementary Material/S6), suggesting a broader but under-characterised geographic distribution. This broader but sparse public-database distribution indicates that ST13823 has been detected in other studiesand in human-associated contexts. Therefore, its apparent restriction to surface water in our dataset may partly reflect the limited number of sequenced healthy-resident and clinical isolates, rather than a true absence from human-associated compartments. We therefore interpret ST13823 as a dataset-restricted surface-water signal, rather than evidence of absolute environmental exclusivity. However, the observed overlap with other lineages highlights public health relevance.

Nevertheless, the predominance of within-source clustering observed in isolates across all three sources, with particular distinctiveness in surface water isolates, suggests that while surface water can capture dominant circulating strains, it does not completely mirror what circulates in the community or clinical setting and preserves its own ecological specificity. Taken together, surface water did not reflect the full ESBL-*E. coli* lineage spectrum present in the community and clinical sources. However, our findings demonstrate that all three sources are connected, with surface water capturing a subset of clinically relevant circulating strains. At the same time, its clearest genomic signal originates from strains circulating among healthy community residents rather than from the clinical population.

Beyond lineage identity, we examined whether the genomic relatedness observed between surface water and human-associated isolates was also reflected in resistance gene content. Overall, the acquired resistome of ESBL-producing *E. coli* showed both shared and source-structured features, with several genes present across all sources but differing in frequency, indicating that surface water isolates partially reflect the human-associated resistome and more closely resemble those from healthy residents than clinical isolates.

A core set of resistance genes was detected across all three sources, including the dominant ESBL determinants $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1B}}$ among β-lactamase genes, and 10 non-β-lactam resistance genes spanning the aminoglycoside, quinolone, macrolide, sulphonamide, and trimethoprim classes. The ubiquity of $bla_{\text{CTX-M-15}}$ across all sources is consistent with its established local and global dominance among ESBL-producing *E. coli* in clinical, community, and environmental niches (Gay et al., 2023; NDoH, 2024). However, beyond this shared core, the resistome overlap between surface water isolates and healthy residents was substantially broader than that between surface water isolates and clinical isolates. Notably, $bla_{\text{CTX-M-27}}$ and $bla_{\text{OXA-1}}$ ARGs were shared exclusively between isolates from surface water and healthy residents, together with twelve non-β-lactam ARGs representing aminoglycoside, tetracycline, amphenicol, and trimethoprim classes. By contrast, no β-lactamase gene nor non-β-lactam ARGs were shared between surface water and clinical isolates. These findings suggest that the surface water resistome is not an equal reflection of both human-associated sources but instead bears a stronger community-associated signature. This pattern is plausibly explained by anthropogenic contamination of local water bodies at the community level. In informal and rural South African settings, like in most parts of sub-Saharan Africa and other LMICs, inadequate sanitation, sewage leakage, greywater disposal, and household runoff can continuously introduce resistant bacteria and ARGs shed by asymptomatic carriers into nearby water bodies (Mukwevho et al., 2025; Tucker et al., 2022). By contrast, clinical isolates are more likely to be shaped by healthcare-associated antibiotic selection and may therefore represent a narrower, more specialised subset of the total circulating resistome. Under this framework, surface water would be expected to overlap more strongly with healthy residents than with clinical isolates, as observed.

ARG diversity patterns further supported this interpretation: healthy resident isolates exhibited the highest β-lactamase diversity, surface water isolates showed an intermediate profile, and clinical isolates displayed a markedly restricted repertoire. This suggests that community-associated populations harbour a broader pool of β-lactamase genes, whereas a narrower set of successful resistance determinants dominates clinical isolates. Despite this low diversity, $bla_{\text{CTX-M-15}}$ was present in all 31 clinical isolates, compared with 50.0% in surface water and 43.7% in healthy resident isolates. This suggests that the clinical resistome in this study was shaped less by broad resistance gene diversity than by the expansion of a limited number of highly successful lineages, most notably the ST38-dominated clinical clade identified in the phylogenetic analysis, uniformly carrying a single high-risk ESBL determinant. By comparison, the broader and more heterogeneous resistance gene pool observed in isolates from surface water and among healthy residents appears to reflect contributions from multiple co-circulating lineages. Statistical analysis reinforced the presence of ecological structuring within the resistome. Significant source-associated variation was detected for tetracycline, macrolide, fluoroquinolone, and amphenicol ARG classes ($p < 0.05$), whereas β-lactamase, aminoglycoside, sulphonamide, and trimethoprim classes showed no significant variation across sources ($p > 0.05$). This indicates that while the core ESBL resistance scaffold is shared, accessory ARG carriage remains source confined (Xu et al., 2025). At the broader functional level, however, resistance mechanism profiles were comparable across all three compartments, with efflux-associated determinants predominating and no significant difference in mechanism distribution detected ($p = 0.595$; Fig. 2). These observations suggest that although the underlying resistance mechanisms are broadly conserved, individual resistance genes exhibit source-structured patterns, being shared across sources but differing in their frequency and distribution.

Overall, the resistome data complement the phylogenetic evidence by showing that surface water is not a complete proxy for either community- or clinical-ESBL-producing *E. coli*. Rather, it captures a partial human-associated signal that aligns more closely with healthy community residents than with clinical isolates. This supports the interpretation that local aquatic systems function as dynamic reservoirs and exchange interfaces for AMR, shaped predominantly by community-derived anthropogenic inputs rather than by direct reflection of the clinical resistome (Bong et al., 2022).

Similarly, virulence determinants were asymmetrically evident

across surface water, healthy-resident, and clinical ESBL-*E. coli* isolates. Although more than three-quarters of virulence genes were shared across isolates from different sources, significant source-associated differences ($p < 0.05$) in gene composition and pathotype structure suggest that surface water shares substantial but selectively filtered virulence traits with both human-associated sources, rather than fully mirroring the virulome of either.

Clinical isolates were distinguished less by unique virulence genes than by greater pathotype complexity and a higher prevalence of exotoxin-associated genes, which were significantly more frequent in clinical isolates than in other sources. Hybrid pathotypes, especially the EPEC + UPEC + NMEC combination, predominated in the clinical collection, whereas no virulence gene was exclusive to clinical isolates. This suggests that the clinical virulome in this study is characterised not by broader gene richness, but by clinically selected combinations of virulence determinants concentrated in successful pathogenic lineages. This interpretation is further supported by the observation that virulence gene clustering was driven primarily by sequence type and phylogroup rather than by source. For example, ST38 retained a conserved virulence repertoire across compartments, indicating that virulence architecture is largely lineage-dependent and can persist across environmental, community, and clinical contexts. Thus, cross-source overlap in virulence factors likely reflects the circulation of shared lineages, whereas source-specific differences in pathotype complexity and exotoxin gene frequency, such as the predominance of hybrid pathotypes and higher exotoxin occurrence in clinical isolates compared to fewer pathotypes in surface water, suggest a selection associated with host infection and environmental contexts.

Healthy-resident isolates contained the greatest virulence gene diversity, accounting for the largest proportion of all detected virulence genes and harbouring 54 source-exclusive virulence genes. This supports asymptomatic human carriage as an important reservoir of virulence determinants, including genes typically associated with extraintestinal pathogenicity. In this context, the healthy community likely represents a broader background pool from which virulence genes can persist and circulate without necessarily causing overt disease. This observation is consistent with previous reports demonstrating that commensal *E. coli* from asymptomatic individuals harbour extraintestinal virulence traits, including adhesins and toxins (Bok et al., 2025). This is further consistent with observations from the Northwest Province, South Africa, where higher pathotypes and complexity were reported in human stool samples compared to water samples, with double EPEC/EHEC pathotypes detected predominantly in human isolates (Chukwu et al., 2020). Commensal gut populations are recognised as major reservoirs where virulence genes can accumulate, persist, and disseminate through horizontal gene transfer, even in the absence of clinical disease (Foster-Nyarko and Pallen, 2022; Ott and Mellata, 2022). The greater richness observed in isolates from healthy residents also helps explain why surface water showed broader functional overlap with this source than with clinical isolates.

By contrast, surface-water isolates were dominated by pure intestinal pathotypes, particularly EPEC associated with phylogroup A, consistent with environmental dissemination and the persistence of faecal-derived colonising lineages. At the same time, the presence of hybrid and ExPEC pathotypes in surface water indicates that these aquatic environments are not merely passive sinks for contamination, but reservoirs of isolates with clinically relevant virulence potential (Jang et al., 2017). Nevertheless, compared with clinical isolates, surface water isolates showed reduced prevalence of toxin genes and lower pathotype complexity, suggesting that the water virulome represents a narrower subset of the total virulence diversity present in human-associated populations. This pattern may reflect ecological filtering after faecal release into the aquatic environment. Potential pressures include dilution, solar irradiation and UV exposure, temperature fluctuation, nutrient limitation, predation by protozoa and bacteriophages, competition with indigenous aquatic microbiota, and the fitness costs associated with maintaining some plasmid-borne or host-adapted virulence traits outside the host (Petersen and Hubbart, 2020). Conversely, intestinal lineages repeatedly introduced through sanitation runoff may be more readily detected because of continual faecal input and/or better persistence under environmental stress, including nutrient limitation or biofilm-associated survival (Alonso-del Valle et al., 2021). Therefore, the lower toxin-gene prevalence and reduced pathotype complexity observed in surface water likely reflect both differential survival after environmental release and continual replenishment from community faecal contamination, rather than the complete absence of clinically relevant strains. However, consistent with our findings, a river impacted by informal settlements in the Western Cape, South Africa, reported the detection of EPEC-associated virulence markers in surface water isolates, reflecting faecal contamination and environmental persistence (Ndlovu et al., 2015).

Taken together, these findings suggest that surface water is not a complete proxy for the virulome of either healthy residents or clinical ESBL-*E. coli* isolates. Rather, it captures a community-linked and lineage-structured subset of virulence traits, shaped by both recurrent faecal inputs and environmental selection pressures that may favour persistence of some intestinal lineages while reducing the detection of more host-adapted or complex pathotype combinations. This supports the interpretation that local aquatic systems function as ecologically filtered interfaces for the circulation of virulence-associated *E. coli*, capturing community-linked lineages while falling short of the full pathotype complexity that characterises the clinical setting. This pattern mirrors and reinforces the phylogenetic and resistome signals observed across sources in this study.

The main limitation of the study is its subset-based genomic design. Surface-water and healthy-resident isolates were selected as balanced, month-stratified subsets to match the smaller number of available clinical isolates. This approach improved comparability across sources and avoided over-representation of the larger non-clinical isolate pools. However, because only a proportion of the surface-water and healthy-resident isolates were included, rare lineages, ARGs, virulence determinants and source-overlap patterns may have been missed, particularly among healthy-resident isolates. Therefore, source-exclusive patterns should be interpreted as subset-based signals rather than evidence of absolute source restriction.

Additional limitations include the focus on culture-confirmed ESBL-producing *E. coli* and the restriction of sampling to one district over eight months, both of which limit generalizability to other resistance mechanisms and settings. To address these gaps, future studies should combine larger and seasonally structured genomic sampling with microbial source tracking, environmental metadata, water-quality measurements, and metagenomic or targeted resistome profiling. Nevertheless, the current design supports comparison of dominant genomic patterns across compartments. It positions surface water as a complementary surveillance matrix rather than a standalone proxy for antimicrobial surveillance in non-sewered communities.

5. Conclusion

This study demonstrates that the genomic profile of ESBL-producing *E. coli* in surface water partially reflects strains circulating in both healthy community residents and clinical populations but aligns more strongly and consistently with isolates from healthy residents across phylogenetic lineage, ARG content, and virulence composition. Evidence from MLST, phylogenomic, resistome, and virulome analyses converged on this pattern: shared sequence types, broader ARG overlap, and more comparable virulence architectures were observed more often between isolates from surface water and healthy residents than between surface water and clinical isolates, while sister-taxon relationships in the phylogeny further supported the closer relationship between surface water and community isolates. However, surface water did not fully reproduce either human-associated source at high genomic resolution,

as differences in clonal composition, ARG diversity, pathotype complexity, and virulence gene richness indicate that aquatic environments impose ecological filters on the strains they retain. These findings support the use of surface water as a useful adjunct surveillance matrix for monitoring lineage distribution and ARG circulation in settings where human and environmental compartments are closely connected, while cautioning against its use as a standalone proxy for fine-scale clonal or pathotype-level epidemiology. Future studies integrating longitudinal sampling and long-read whole-genome sequencing would help clarify the dynamic exchange among aquatic, community, and clinical ESBL-producing *E. coli* populations and strengthen the basis for water-based genomic surveillance in low-resource settings.

Consent to participate

All participants from the community were recruited following explicit, voluntary, written informed consent (Supplementary Material/S1).

Consent to publish

This manuscript contains no personal information or case-specific details requiring consent for publication. All cited sources are publicly available and peer-reviewed publications.

Ethical approval

Ethical approval for this study was obtained from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (Ref: BREC/00003640/2021), the Provincial Health Research Ethics Committee of the KwaZulu-Natal Department of Health (NHRD Ref: KZ_202203_023), and the National Health Laboratory Service (NHLS Ref: PR2225862) for access to clinical isolates. All procedures involving human participants and human-derived specimens were conducted in accordance with the ethical principles of the Declaration of Helsinki and its subsequent amendments, as well as applicable South African ethical and regulatory requirements.

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CRediT authorship contribution statement

Etando Ayukafangha: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Joshua Mbanga:** Conceptualization, Data curation, Formal analysis, Supervision, Validation, Writing – review & editing. **Arshad Ismail:** Data curation, Formal analysis, Methodology, Resources, Validation, Writing – review & editing. **Sabiha Y. Essack:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Akebe Luther King Abia:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sabiha Y. Essack reports financial support was provided by National Research Foundation. Sabiha Y. Essack reports financial support was provided by South African Medical Research Council. Sabiha Y. Essack reports a relationship with Global Respiratory Partnership that includes: board membership. Sabiha Y. Essack reports a relationship with Global Hygiene Council that includes: board membership. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2026.125249.

Data availability

Data will be made available on request.

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List of Supplementary Materials (Manuscript 3)

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Supplementary Material/S2 – Genomic Characteristics of ESBL *E. coli*

BV-BRC Genome ID	Isolate_id	isolate Host	Sources	NCBI_Bio_Accession	MLST	Contigs	Size	GC Content	Contig L50	Contig N50	TRNA	RRNA	CDS	Coarse Consistency	Fine Consistency
562.180622	CAEC1	Blood culture	Clinical	SAMN48072219	ST38	87	5219110	50.56818	10	155814	80	8	5163	99.6	98.7
562.180623	CDEC1	Tissue	Clinical	SAMN48072220	ST38	89	5220916	50.568962	10	155743	80	8	5175	99.6	98.8
562.180624	CDEC2	Catheter tip	Clinical	SAMN48072221	ST13823	87	4912463	50.566895	9	150609	80	7	4901	99.9	98.9
562.180626	CDEC3	Pus	Clinical	SAMN51242266	ST10	123	4808084	50.801857	18	83444	81	7	4772	99.7	98.9
562.180625	CDEC4	Blood culture	Clinical	SAMN51242267	ST10	125	4810852	50.80412	18	87464	79	6	4786	99.7	98.9
562.180628	CJEC1	Sterile sites	Clinical	SAMN48072222	ST38	85	5221543	50.57287	10	155824	80	8	5173	99.6	98.7
562.180627	CJEC2	Pus	Clinical	SAMN51242253	ST38	116	5207843	50.563755	12	148884	80	7	5173	99.6	98.8
562.18063	CJEC3	Sterile sites	Clinical	SAMN51242254	ST38	112	5210079	50.563015	13	141104	80	8	5171	99.6	98.7
562.180629	CJEC4	Blood culture	Clinical	SAMN48072224	ST224	80	4960485	50.859806	8	242008	80	10	4876	99.7	98.4
562.180631	CMEC1	Urine	Clinical	SAMN48072224	ST38	94	5237667	50.556248	9	166039	80	10	5177	99.6	98.7
562.180632	CMEC2	Sterile sites	Clinical	SAMN51242252	ST38	82	5222988	50.575493	9	167373	80	8	5180	99.6	98.7
562.180633	CMEC3	Pus	Clinical	SAMN48072225	ST38	93	5223967	50.565536	10	155743	79	8	5167	99.6	98.8
562.180634	CNEC1	Sterile sites	Clinical	SAMN51242263	ST10	132	4806836	50.79753	19	86897	81	4	4774	99.7	98.7
562.180798	CNEC2	Sterile sites	Clinical	SAMN48072226	ST10	113	4814442	50.80215	17	100191	79	7	4770	99.7	99
562.180635	CNEC22	Sterile sites	Clinical	SAMN51242264	ST38	113	5213185	50.558346	15	130675	79	10	5173	99.6	98.8
562.180799	CNEC3	Blood culture	Clinical	SAMN51242265	ST38	104	5211142	50.557404	12	127872	79	8	5172	99.6	98.8
562.171812	CNEC4	Pus	Clinical	SAMN48072227	ST10	109	4810719	50.804317	16	102534	81	8	4774	99.7	98.9
562.1808	CNEC5	Pus	Clinical	SAMN48072228	ST10	111	4812038	50.803005	15	102534	80	5	4758	99.7	98.9
562.180801	COEC1	Sterile sites	Clinical	SAMN48072229	ST38	98	5229210	50.560696	10	155743	80	8	5173	99.6	98.8
562.180802	COEC2	Blood culture	Clinical	SAMN51242259	ST38	104	5216951	50.56737	12	144611	80	9	5178	99.6	98.5
562.180804	COEC3	Tisue	Clinical	SAMN51242260	ST38	105	5215024	50.56529	11	148097	80	7	5179	99.6	98.8
562.180803	COEC4	Tissue	Clinical	SAMN51242261	ST38	96	5219163	50.561653	11	155728	80	9	5179	99.6	98.8
562.180805	COEC5	Blood culture	Clinical	SAMN48072230	ST38	117	5250323	50.52752	10	155740	80	13	5193	99.6	98.8
562.171811	COEC6	Pus	Clinical	SAMN51242262	ST38	103	5222339	50.566574	13	150479	80	8	5193	99.6	98.7
562.180807	CSEC1	Tissue	Clinical	SAMN48072231	ST38	97	5233023	50.55621	10	155743	78	11	5170	99.6	98.8
562.180806	CSEC2	Pus	Clinical	SAMN48072232	ST38	85	5220224	50.57141	9	166039	79	8	5168	99.6	98.8
562.180808	CSEC3	Pus	Clinical	SAMN51242257	ST38	106	5213977	50.56127	12	148097	80	8	5173	99.6	98.7
562.180809	CSEC4	Blood culture	Clinical	SAMN51242258	ST10	126	4808906	50.801598	19	81541	80	7	4793	99.7	98.8
562.18081	CYEC1	Sputum	Clinical	SAMN48072233	ST38	101	5247375	50.512512	9	166039	80	7	5179	99.6	98.8
562.180812	CYEC2	Sterile sites	Clinical	SAMN51242255	ST38	103	5214830	50.560055	13	135694	81	8	5172	99.6	98.8
562.180811	CYEC3	Sterile sites	Clinical	SAMN51242256	ST38	98	5217161	50.567177	11	150479	81	9	5173	99.6	98.7
562.180701	L01MEC	Stool	Healthy_Residents	SAMN51241999	ST10	70	4869940	50.636456	9	165691	77	6	4766	99.9	99.3
562.180703	L03MEC	Stool	Healthy_Residents	SAMN51242000	ST757	169	4785899	50.844448	25	60926	71	3	4825	99.1	98.5
562.171814	L07MEC	Stool	Healthy_Residents	SAMN51242004	ST44	150	4834912	50.830997	17	87375	79	8	4805	99.7	99
562.171815	L10MEC	Stool	Healthy_Residents	SAMN51242006	ST131	104	5109810	50.76999	11	151859	77	7	5089	99.9	98.7
562.180705	L11JEC	Stool	Healthy_Residents	SAMN51242007	ST224	96	4980811	50.811264	8	217180	79	9	4900	99.7	98.6
562.180691	L13JEC	Stool	Healthy_Residents	SAMN51242009	ST349	615	5469257	50.781742	24	69305	84	6	5698	99.6	90
562.1807	L19JEC	Stool	Healthy_Residents	SAMN51242014	ST131	107	5102848	50.774784	11	183444	79	7	5081	99.9	98.7
562.180689	L20JEC	Stool	Healthy_Residents	SAMN51242015	ST131	118	5122233	50.755775	11	177068	73	8	5108	99.9	98.5
562.180696	L21YEC	Stool	Healthy_Residents	SAMN51242016	ST-	113	5008882	50.470882	10	170018	78	5	4931	99.9	98.7
562.180694	L23YEC	Stool	Healthy_Residents	SAMN51242018	ST69	138	5309134	50.646507	11	136604	81	6	5328	99.6	98.6
562.180693	L24YEC	Stool	Healthy_Residents	SAMN51242019	ST349	265	5131400	50.690357	13	125020	82	4	5134	99.7	96
562.180692	L26YEC	Stool	Healthy_Residents	SAMN51242020	ST10	126	4763087	50.706043	12	129293	78	6	4719	99.6	98.5
562.18068	L31AEC	Stool	Healthy_Residents	SAMN51242023	ST226	128	5063599	50.319843	14	113694	78	8	5118	99.8	98.5
562.180681	L34AEC	Stool	Healthy_Residents	SAMN51242026	ST1141	82	4954014	50.633606	9	187351	79	4	5000	99.3	98.5
562.180682	L37AEC	Stool	Healthy_Residents	SAMN51242029	ST131	76	5135408	50.70902	9	242541	77	6	5139	99.9	98.7
562.180685	L39AEC	Stool	Healthy_Residents	SAMN51242031	ST-	95	5098069	50.79435	8	222419	79	5	5155	99.9	99
562.180687	L41SEC	Stool	Healthy_Residents	SAMN51242033	ST6706	131	5208893	50.517086	14	142846	75	5	5360	99.8	98.9
562.180688	L42SEC	Stool	Healthy_Residents	SAMN51242034	ST1193	78	5005413	50.593086	8	216254	78	5	5003	99.8	98.7
562.180672	L45SEC	Stool	Healthy_Residents	SAMN51242037	ST636	93	4950958	50.636726	9	198199	82	5	4906	99.9	99
562.172013	L47SEC	Stool	Healthy_Residents	SAMN51242039	ST5614	77	5144427	50.888737	9	183897	80	6	5149	99.9	99
562.171821	L51OEC	Stool	Healthy_Residents	SAMN51242041	ST48	185	4786929	50.8117	20	67379	80	5	4784	99.3	98.3
562.180665	L56OEC	Stool	Healthy_Residents	SAMN51242046	ST69	108	5214835	50.691097	11	123278	81	6	5154	99.6	98.9
562.180669	L57OEC	Stool	Healthy_Residents	SAMN51242047	ST155	77	4777616	50.768394	10	156895	80	6	4718	99.9	99.3
562.180668	L60OEC	Stool	Healthy_Residents	SAMN51242050	ST1193	70	5038010	50.621616	10	162758	80	5	5024	99.8	98.7
562.172014	L63NEC	Stool	Healthy_Residents	SAMN51242053	ST13297	104	4980498	50.782894	13	111897	78	7	4975	99.8	99.3

562.180658	L65NEC	Stool	Healthy_Residents	SAMN51242055	ST38	156	5609665	50.37825	13	158903	87	7	5775	99.6	98
562.171825	L68NEC	Stool	Healthy_Residents	SAMN51242058	ST-	117	4905263	50.66911	13	119565	78	7	4905	99.9	99
562.180661	L70NEC	Stool	Healthy_Residents	SAMN51242059	ST38	143	5381546	50.44285	12	141403	80	10	5484	99.6	98.7
562.171826	L71DEC	Stool	Healthy_Residents	SAMN51242060	ST219	80	5366612	50.533875	6	263930	82	7	5262	99.9	99
562.180663	L74DEC	Stool	Healthy_Residents	SAMN51242063	ST88	90	4944657	50.761295	11	156289	76	7	4877	99.9	99.4
562.171828	L75DEC	Stool	Healthy_Residents	SAMN51242064	ST13297	109	4980132	50.779476	13	111901	80	5	4979	99.8	99.3
562.180646	L79DEC	Stool	Healthy_Residents	SAMN51242067	ST155	122	4749331	50.754307	13	110650	75	5	4757	99.9	99.3
562.180707	AEC8EC	River Water	Surface_Water	SAMN51242189	ST13823	82	4901199	50.581417	9	144615	79	7	4912	99.9	98.8
562.180708	AED5EC	River Water	Surface_Water	SAMN51242190	ST38	124	5356834	50.50989	10	151032	85	7	5443	99.6	98.7
562.180709	AOA12EC	River Water	Surface_Water	SAMN51242186	ST13823	77	4900796	50.586926	9	144726	82	7	4912	99.9	98.9
562.180712	AOA13EC	River Water	Surface_Water	SAMN51242187	ST13823	77	4904365	50.582634	8	162055	81	7	4904	99.9	99
562.180717	DOA4EC	River Water	Surface_Water	SAMN51242213	ST761	681	5048354	50.740715	53	28843	78	2	5411	99.5	89.6
562.180721	DOA5EC	River Water	Surface_Water	SAMN51242214	ST7326	515	5478073	50.393124	49	33285	82	7	5869	99.7	94.5
562.180718	DOB10EC	River Water	Surface_Water	SAMN51242219	ST13823	94	4920004	50.581665	9	135115	82	10	4920	99.9	98.9
562.180719	DOB6EC	River Water	Surface_Water	SAMN51242215	ST7326	461	5429096	50.381886	46	36118	82	7	5829	99.7	95.1
562.180729	JED12EC	River Water	Surface_Water	SAMN51242172	ST131	59	4797625	50.660255	7	225760	77	3	4723	99.9	99.1
562.180732	JOA10EC	River Water	Surface_Water	SAMN51242161	ST38	118	5324623	50.52168	10	170768	80	6	5388	99.6	98.7
562.180738	JOB12EC	River Water	Surface_Water	SAMN51242165	ST10	110	4811505	50.801693	16	100191	81	5	4771	99.7	98.9
562.180741	JOB9EC	River Water	Surface_Water	SAMN51242163	ST-	100	5145451	50.512833	9	175986	79	7	5217	99.7	99
562.18074	MEC10_EC	River Water	Surface_Water	SAMN51242150	ST757	169	4785899	50.844448	25	60926	71	3	4825	99.1	98.5
562.180745	MEC4_EC	River Water	Surface_Water	SAMN51242149	ST2705	84	4935032	50.50387	8	167673	78	10	4895	99.3	98.5
562.180748	MED8_EC	River Water	Surface_Water	SAMN51242156	ST-	99	5132663	50.528408	10	175634	78	7	5200	99.7	99.1
562.180752	MOB16_EC	River Water	Surface_Water	SAMN51242148	ST-	127	4668222	50.377144	15	95163	75	2	4637	99.3	98.7
562.180756	NEC18EC	River Water	Surface_Water	SAMN51242208	ST13823	95	4973154	50.4768	9	150609	82	8	5013	99.9	98.5
562.180757	NEC9EC	River Water	Surface_Water	SAMN51242207	ST2705	91	4939836	50.507122	10	175404	77	9	4898	99.3	98.6
562.180758	NED10EC	River Water	Surface_Water	SAMN51242210	ST13823	79	4955507	50.48664	10	144600	81	6	4989	99.9	98.5
562.180759	NED18EC	River Water	Surface_Water	SAMN51242211	ST13823	77	4902908	50.586205	8	162067	81	7	4901	99.9	98.9
562.180766	OEC10EC	River Water	Surface_Water	SAMN51242200	ST13823	75	4956934	50.48907	9	150609	82	7	4990	99.9	98.5
562.180767	OEC9EC	River Water	Surface_Water	SAMN51242199	ST13823	81	4960521	50.490746	10	121143	81	8	4999	99.9	98.5
562.180769	OED4EC	River Water	Surface_Water	SAMN51242201	ST13823	80	4942255	50.519127	10	148382	82	7	4954	99.9	98.5
562.180771	OOB18EC	River Water	Surface_Water	SAMN51242198	ST13823	90	4966778	50.48772	9	135115	82	6	5001	99.9	98.6
562.17181	SEC15EC	River Water	Surface_Water	SAMN51242197	ST2705	79	4879646	50.602707	8	208914	78	9	4804	99.6	98.8
562.180776	SEC3EC	River Water	Surface_Water	SAMN51242195	ST38	123	5400154	50.50921	12	142013	90	5	5499	99.6	98.7
562.180781	SEC8EC	River Water	Surface_Water	SAMN51242196	ST38	117	5318973	50.516895	11	166039	80	6	5379	99.6	98.7
562.180778	SOA12EC	River Water	Surface_Water	SAMN51242193	ST38	84	5217391	50.56742	10	155714	79	9	5165	99.6	98.7
562.180788	YOA11EC	River Water	Surface_Water	SAMN51242173	ST38	135	5410811	50.510914	13	150480	90	5	5524	99.6	98.7
562.180786	YED12EC	River Water	Surface_Water	SAMN51242185	ST13823	80	4897812	50.584015	10	144701	81	7	4899	99.9	99
562.18079	YOA12EC	River Water	Surface_Water	SAMN51242174	ST38	129	5402762	50.521233	13	161136	83	6	5517	99.6	98.7
562.180796	YOB7EC	River Water	Surface_Water	SAMN51242175	ST69	132	5276593	50.656628	15	102236	84	6	5320	99.5	98.7

Supplementary Material/S3 – Resistant Gene Distribution and Diversity Metrices

β-lactamse genes				
sources	genes	isolates_w	total_isolates	prevalence
Clinical	blaCTX-M-15	31	31	1
Clinical	blaTEM-1B	1	31	0.032258065
Healthy_Residents	blaCTX-M-1	2	32	0.0625
Healthy_Residents	blaCTX-M-139	1	32	0.03125
Healthy_Residents	blaCTX-M-14	3	32	0.09375
Healthy_Residents	blaCTX-M-15	14	32	0.4375
Healthy_Residents	blaCTX-M-27	6	32	0.1875
Healthy_Residents	blaCTX-M-55	4	32	0.125
Healthy_Residents	blaDHA-1	3	32	0.09375
Healthy_Residents	blaLAP-1	1	32	0.03125
Healthy_Residents	blaOXA-1	2	32	0.0625
Healthy_Residents	blaOXA-10	1	32	0.03125
Healthy_Residents	blaTEM-141	1	32	0.03125
Healthy_Residents	blaTEM-1B	12	32	0.375
Surface_Water	blaCTX-M-15	16	32	0.5
Surface_Water	blaCTX-M-27	9	32	0.28125
Surface_Water	blaOXA-1	1	32	0.03125
Surface_Water	blaTEM-1B	9	32	0.28125

CHI STATS SUMMARY			
class	chi_square	df	p_value
Tetracyclines	30.99756815	2	0.00000
Macrolides	27.02544172	2	0.00000
Fluoroquinolones	8.607687529	2	0.01352
Amphenicols	7.327364232	2	0.02564
Others_Genes	3.889255276	2	0.14304
Aminoglycoside	3.136029797	2	0.20846
Sulphonamides	2.07265265	2	0.35476
Trimethoprim	1.006620379	2	0.60453
Beta_lactamase	0.021052632	2	0.98953

DIVERSITY SUMMARY				
class	sources	S	H	J
Beta_lactamase	Clinical	2	0.139060798	0.200622324
Beta_lactamase	Healthy_Residents	12	2.063509423	0.830417281
Beta_lactamase	Surface_Water	4	1.15787772	0.835232222
Aminoglycoside	Clinical	3	1.015832175	0.924650293
Aminoglycoside	Healthy_Residents	9	1.718344075	0.782052091
Aminoglycoside	Surface_Water	7	1.476923872	0.75898873
Sulphonamides	Clinical	2	0.68126573	0.98285869
Sulphonamides	Healthy_Residents	3	0.886753368	0.8071577
Sulphonamides	Surface_Water	2	0.658109988	0.949452015
Trimethoprim	Clinical	3	1.015832175	0.924650293
Trimethoprim	Healthy_Residents	6	1.527742598	0.852649379
Trimethoprim	Surface_Water	5	1.111131731	0.690384961
Fluoroquinolones	Clinical	1	0	
Fluoroquinolones	Healthy_Residents	6	1.445541322	0.806771973
Fluoroquinolones	Surface_Water	4	1.108438678	0.799569492
Macrolides	Clinical	1	0	
Macrolides	Healthy_Residents	2	0.552664622	0.79732651
Macrolides	Surface_Water	1	0	
Tetracyclines	Healthy_Residents	2	0.410116318	0.591672779
Tetracyclines	Surface_Water	2	0.514653165	0.74248757
Amphenicols	Healthy_Residents	2	0.673011667	0.970950594
Amphenicols	Surface_Water	1	0	
Others_Genes	Healthy_Residents	2	0.673011667	0.970950594
Others_Genes	Surface_Water	1	0	

Non β-lactamse genes			
sources	class	genes	isolates_wi
Clinical	Aminoglycoside	aac(3)-IIa	22
Clinical	Aminoglycoside	aadA2	22
Clinical	Aminoglycoside	aadA5	8
Clinical	Fluoroquinolones	qnrS1	8
Clinical	Macrolides	mph(A)	29
Clinical	Sulphonamides	sul1	30
Clinical	Sulphonamides	sul2	22
Clinical	Trimethoprim	dfrA12	22
Clinical	Trimethoprim	dfrA17	8
Clinical	Trimethoprim	dfrA5	22
Healthy_Residents	Aminoglycoside	aac(3)-IIa	2
Healthy_Residents	Aminoglycoside	aac(3)-IId	2
Healthy_Residents	Aminoglycoside	aac(6')-Ib-cr	3
Healthy_Residents	Aminoglycoside	aadA2	3
Healthy_Residents	Aminoglycoside	aadA5	13
Healthy_Residents	Aminoglycoside	aph(3")-Ib	17
Healthy_Residents	Aminoglycoside	aph(3')-IIa	2
Healthy_Residents	Aminoglycoside	aph(3')-Ia	1
Healthy_Residents	Aminoglycoside	aph(6)-Id	21
Healthy_Residents	Amphenicols	FloR	2
Healthy_Residents	Amphenicols	catA1	3
Healthy_Residents	Fluoroquinolones	ARR-3	2
Healthy_Residents	Fluoroquinolones	OqxA	1
Healthy_Residents	Fluoroquinolones	OqxB	1
Healthy_Residents	Fluoroquinolones	ant(3")-Ia	3
Healthy_Residents	Fluoroquinolones	qnrB4	3
Healthy_Residents	Fluoroquinolones	qnrS1	10
Healthy_Residents	Macrolides	erm(B)	7
Healthy_Residents	Macrolides	mph(A)	22
Healthy_Residents	Others_Genes	bleO	2
Healthy_Residents	Others_Genes	fosA3	3
Healthy_Residents	Sulphonamides	sul1	20
Healthy_Residents	Sulphonamides	sul2	23
Healthy_Residents	Sulphonamides	sul3	3
Healthy_Residents	Tetracyclines	tet(A)	18
Healthy_Residents	Tetracyclines	tet(B)	3
Healthy_Residents	Trimethoprim	dfrA1	5
Healthy_Residents	Trimethoprim	dfrA12	3
Healthy_Residents	Trimethoprim	dfrA14	7
Healthy_Residents	Trimethoprim	dfrA17	16
Healthy_Residents	Trimethoprim	dfrA5	3
Healthy_Residents	Trimethoprim	dfrA7	2
Surface_Water	Aminoglycoside	aac(3)-IIa	1
Surface_Water	Aminoglycoside	aac(3)-IId	1
Surface_Water	Aminoglycoside	aadA2	1
Surface_Water	Aminoglycoside	aadA5	20
Surface_Water	Aminoglycoside	aph(3")-Ib	13
Surface_Water	Aminoglycoside	aph(3')-Ia	4
Surface_Water	Aminoglycoside	aph(6)-Id	13
Surface_Water	Amphenicols	catA1	1
Surface_Water	Fluoroquinolones	OqxA	3
Surface_Water	Fluoroquinolones	OqxB	3
Surface_Water	Fluoroquinolones	ant(3")-Ia	6
Surface_Water	Fluoroquinolones	qnrS1	17
Surface_Water	Macrolides	mph(A)	10
Surface_Water	Others_Genes	bleO	3
Surface_Water	Sulphonamides	sul1	24
Surface_Water	Sulphonamides	sul2	14
Surface_Water	Tetracyclines	tet(A)	15
Surface_Water	Tetracyclines	tet(B)	4
Surface_Water	Trimethoprim	dfrA1	3
Surface_Water	Trimethoprim	dfrA12	1
Surface_Water	Trimethoprim	dfrA15	2
Surface_Water	Trimethoprim	dfrA17	20
Surface_Water	Trimethoprim	dfrA5	6

Supplementary Material/S4 – Distribution of Resistant Mechanisms and AMR Gene Families

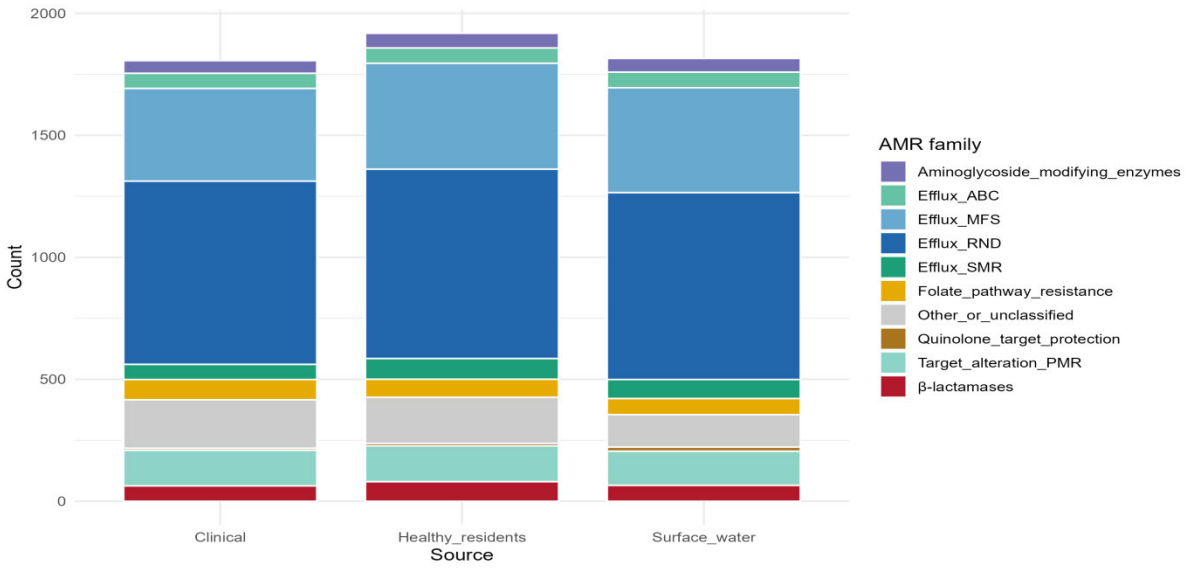

Figure 2 B: Showing AMR Gene family distribution across ESBL Ec isolate sources. Surface water is similar comparative proportions to healthy residents and clinical isolates across multiple AMR gene families.

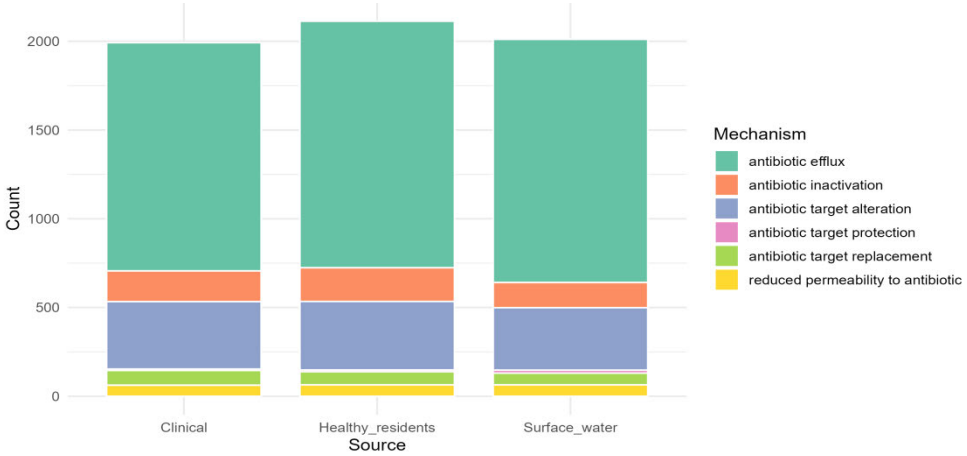

Figure 2 C: Showing Distribution of resistant mechanism exhibited by ESBL Ec isolate across sources. Surface water is similar comparative proportions to healthy residents and clinical isolates across multiple AMR gene families. Efflux-associated determinants dominated in all three compartments,

Supplementary Material/S5 – Distribution of Phylogroups, MLST and Serotypes of ESBL *E. coli* across Sources

(A) Phylogroup By Source

sources	phylogroup	n	percent
Clinical	A	8	25.81
Clinical	B1	1	3.23
Clinical	D	22	70.97
Healthy_Residents	A	9	28.12
Healthy_Residents	B1	6	18.75
Healthy_Residents	B2	9	28.12
Healthy_Residents	C	1	3.12
Healthy_Residents	D	6	18.75
Healthy_Residents	E	1	3.12
Surface_Water	A	18	56.25
Surface_Water	B1	4	12.5
Surface_Water	B2	1	3.12
Surface_Water	D	8	25
Surface_Water	U	1	3.12

(B) MLST By Sources

sources	mlst	n	percent
Clinical	ST10	7	22.58
Clinical	ST13823	1	3.23
Clinical	ST224	1	3.23
Clinical	ST38	22	70.97
Healthy_Residents	ST10	2	6.25
Healthy_Residents	ST1141	1	3.12
Healthy_Residents	ST1193	2	6.25
Healthy_Residents	ST131	4	12.5
Healthy_Residents	ST131-1LV	1	3.12
Healthy_Residents	ST13297	2	6.25
Healthy_Residents	ST155	2	6.25
Healthy_Residents	ST15519	1	3.12
Healthy_Residents	ST219	1	3.12
Healthy_Residents	ST224	1	3.12
Healthy_Residents	ST226	1	3.12
Healthy_Residents	ST349	2	6.25
Healthy_Residents	ST38	2	6.25
Healthy_Residents	ST44	1	3.12
Healthy_Residents	ST48	1	3.12
Healthy_Residents	ST5614	1	3.12
Healthy_Residents	ST636	1	3.12
Healthy_Residents	ST6706	1	3.12
Healthy_Residents	ST69	2	6.25
Healthy_Residents	ST73-1LV	1	3.12
Healthy_Residents	ST757	1	3.12
Healthy_Residents	ST88	1	3.12
Surface_Water	ST10	1	3.12
Surface_Water	ST10-1LV	1	3.12
Surface_Water	ST131	1	3.12
Surface_Water	ST13823	11	34.38
Surface_Water	ST155-1LV	2	6.25
Surface_Water	ST226-1LV	1	3.12
Surface_Water	ST2705	3	9.38
Surface_Water	ST38	7	21.88
Surface_Water	ST69	1	3.12
Surface_Water	ST7326	2	6.25
Surface_Water	ST757	1	3.12
Surface_Water	ST761	1	3.12

(C) SEROTYPE BY SOURCES

sources	o_h_serotype	n	percent
Clinical	H2	1	3.23
Clinical	O86:H18	22	70.97
Clinical	O9:H10	7	22.58
Clinical	O9:H30	1	3.23
Healthy_Residents	H20	1	3.12
Healthy_Residents	H23	1	3.12
Healthy_Residents	H30	1	3.12
Healthy_Residents	H4	1	3.12
Healthy_Residents	H48	1	3.12
Healthy_Residents	H9	1	3.12
Healthy_Residents	O106:H18	1	3.12
Healthy_Residents	O113:H4	1	3.12
Healthy_Residents	O141:H5	1	3.12
Healthy_Residents	O149:H12	1	3.12
Healthy_Residents	O15:H11	1	3.12
Healthy_Residents	O15:H18	1	3.12
Healthy_Residents	O16:H5	1	3.12
Healthy_Residents	O21:H7	1	3.12
Healthy_Residents	O25:H4	4	12.5
Healthy_Residents	O27:H14	1	3.12
Healthy_Residents	O27:H18	1	3.12
Healthy_Residents	O6:H1	1	3.12
Healthy_Residents	O75:H5	2	6.25
Healthy_Residents	O86:H18	2	6.25
Healthy_Residents	O86:H2	2	6.25
Healthy_Residents	O8:H19	1	3.12
Healthy_Residents	O8:H25	2	6.25
Healthy_Residents	O9:H10	1	3.12
Healthy_Residents	O9:H37	1	3.12
Surface_Water	H10	3	9.38
Surface_Water	H2	11	34.38
Surface_Water	H20	1	3.12
Surface_Water	H4	1	3.12
Surface_Water	O111:H4	1	3.12
Surface_Water	O15:H1	1	3.12
Surface_Water	O169:H45	1	3.12
Surface_Water	O16:H5	1	3.12
Surface_Water	O181:H16	2	6.25
Surface_Water	O86:H18	7	21.88
Surface_Water	O88:H25	2	6.25
Surface_Water	O9:H10	1	3.12

Supplementary Material/S6 – Public Database search of ST13823

Database	Uberstrain	Name	Source Niche	Source Type	Source Details	Collecti	Continent	Country	City	Species	Bio Project ID	Project ID	Sample ID	Secondary Sam	Date Entere	ST	ST Complex	Lineage
Enterobase	ESC_CB0688AA	1729794	Human	Human	Homo sapiens; human	2022	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN31407517	SRS15504227	23/10/2022	13823	ST165 Cplx	A
Enterobase	ESC_CB7280AA	1768320	Human	Human	Homo sapiens; human	2022	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN31885721	SRS15876440	28/11/2022	13823	ST165 Cplx	A
Enterobase	ESC_IB2478AA	202313493	Human			2023	Europe	France		Escherichia coli					26/12/2023	13823	ST165 Cplx	A
Enterobase	ESC_JB0416AA	1878162	Human	Human	Homo sapiens; human	2023	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN40209956	SRS20625254	08/03/2024	13823	ST165 Cplx	A
Enterobase	ESC_KB2422AA	01301733	Human	Human	Homo sapiens; human	2023	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN41465210	SRS21352626	22/05/2024	13823	ST165 Cplx	A
Enterobase	ESC_KB2565AA	01304228	Human	Human	Homo sapiens; human	2023	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN41465033	SRS21352434	22/05/2024	13823	ST165 Cplx	A
Enterobase	ESC_KB6241AA	01162304	Human	Human	Homo sapiens; human	2023	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN41629664	SRS21489270	02/06/2024	13823	ST165 Cplx	A
Enterobase	ESC_OB3131AA	01633749	Human	Human	Homo sapiens; human	2024	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN44596390	SRS23115653	07/11/2024	13823	ST165 Cplx	A
Enterobase	ESC_PB3850AA	DAMRST0175_ES_PINKP_Ecoli				2021	Africa	Tanzania		Escherichia coli	PRJEB65607	ERP150742	SAMEA114317369	ERS16306482	21/12/2024	13823	ST165 Cplx	A
Enterobase	ESC_PB3940AA	DAMRST0174_ES_PINKD_Ecoli				2021	Africa	Tanzania		Escherichia coli	PRJEB65607	ERP150742	SAMEA114317368	ERS16306481	21/12/2024	13823	ST165 Cplx	A
Enterobase	ESC_PB3942AA	DAMRST0161_ES_PINK_Ecoli				2021	Africa	Tanzania		Escherichia coli	PRJEB65607	ERP150742	SAMEA114317349	ERS16306462	21/12/2024	13823	ST165 Cplx	A
Enterobase	ESC_QB3864AA	TJ24WQ0192				2024									26/02/2025	13823	ST165 Cplx	A
Enterobase	ESC_QB9777AA	01714770	Human	Human	Homo sapiens; human	2025	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN47759170	SRS24583335	04/04/2025	13823	ST165 Cplx	A
Enterobase	ESC_RB0019AA	ESW-27				2024									06/04/2025	13823	ST165 Cplx	A
Enterobase	ESC_RB7765AA	F128				2023									11/05/2025	13823	ST165 Cplx	A
Enterobase	ESC_VB0639AA	01844486	Human	Human	Homo sapiens; human	2025	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN52904160	SRS26912037	26/10/2025	13823	ST165 Cplx	A
Enterobase	ESC_YB3203AA	fS25a	Human	Human	ENVO:01001829; Homo sapiens	2024	Africa	Malawi		Escherichia coli	PRJNA1399404	SRP660383	SAMN54493225	SRS27684390	16/02/2026	13823	ST165 Cplx	A
Enterobase	ESC_YB3238AA	fS100a	Human	Human	ENVO:01001829; Homo sapiens	2024	Africa	Malawi		Escherichia coli	PRJNA1399404	SRP660383	SAMN54493193	SRS27684482	16/02/2026	13823	ST165 Cplx	A
Enterobase	ESC_YB3289AA	fS95a	Human	Human	ENVO:01001829; Homo sapiens	2024	Africa	Malawi		Escherichia coli	PRJNA1399404	SRP660383	SAMN54493255	SRS27684424	16/02/2026	13823	ST165 Cplx	A
Enterobase	ESC_YB3298AA	fS80a	Human	Human	ENVO:01001829; Homo sapiens	2024	Africa	Malawi		Escherichia coli	PRJNA1399404	SRP660383	SAMN54493247	SRS27684416	16/02/2026	13823	ST165 Cplx	A
Enterobase	ESC_YB3323AA	fS177a	Human	Human	ENVO:01001829; Homo sapiens	2024	Africa	Malawi		Escherichia coli	PRJNA1399404	SRP660383	SAMN54493221	SRS27684388	16/02/2026	13823	ST165 Cplx	A
Enterobase	ESC_YB3324AA	fS174a	Human	Human	ENVO:01001829; Homo sapiens	2024	Africa	Malawi		Escherichia coli	PRJNA1399404	SRP660383	SAMN54493220	SRS27684387	16/02/2026	13823	ST165 Cplx	A
Enterobase	ESC_YB3332AA	fS155a	Human	Human	ENVO:01001829; Homo sapiens	2024	Africa	Malawi		Escherichia coli	PRJNA1399404	SRP660383	SAMN54493213	SRS27684379	16/02/2026	13823	ST165 Cplx	A
Enterobase	ESC_YB3333AA	fS14a	Human	Human	ENVO:01001829; Homo sapiens	2024	Africa	Malawi		Escherichia coli	PRJNA1399404	SRP660383	SAMN54493212	SRS27684378	16/02/2026	13823	ST165 Cplx	A
Enterobase	ESC_ZB1319AA	01249166	Human	Human	Homo sapiens; human	2023	Europe	United Kingdom		Escherichia coli	PRJNA315192	SRP071789	SAMN57103470	SRS28650408	07/04/2026	13823	ST165 Cplx	A
Enterobase	ESC_ZB5680AA	ISG-EC-1730	Human	Human		2023	Europe	Spain		Escherichia coli	PRJEB102514	ERP183906	SAMEA120862311	ERS28397118	15/04/2026	13823	ST165 Cplx	A
BV-BRC	562.148078	Escherichia coli MRSN110015	Human	Human	Homo sapiens; human	2022	Africa	Kenya		Escherichia coli	PRJNA1078534	JBBGHJ00000	SAMN40004727		2024-04-22T17:43:02.973Z			

CHAPTER FIVE - MANUSCRIPT 4

EVALUATING SURFACE WATER FOR GENOMIC SURVEILLANCE OF COMMUNITY-LINKED ANTIMICROBIAL RESISTANCE IN ESBL-PRODUCING KLEBSIELLA PNEUMONIAE

Author Contributions

Etando Ayukafangha - as principal investigator, co-conceptualised the study, performed the laboratory work, analysed the data, and drafted the manuscript.

Joshua Mbanga - as co-supervisor, co-conceptualised the study, assisted with sample collection and processing, vetted the results, and critically reviewed the manuscript.

Arshad Ismail - conducted data analysis, vetted the results and critically reviewed manuscript.

Sabiha Y. Essack - as co-supervisor, co-conceptualised the study, secured funding, guided the literature review and ethical approval application, facilitated data collection, vetted the results, and critically reviewed the manuscript.

Akebe Luther King Abia - as main supervisor, co-conceptualised the study, guided the literature review, data collection, processing and analysis, vetted the results, and critically reviewed the manuscript.

Objectives met – This chapter answers the ESBL-producing K. pneumoniae component of objective 2

Evaluating Surface Water for Genomic Surveillance of Community-Linked Antimicrobial Resistance in ESBL-Producing *Klebsiella pneumoniae*

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Evaluating surface water for genomic surveillance of community-linked antimicrobial resistance in ESBL-producing *Klebsiella pneumoniae*

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ABSTRACT

A major limitation in wastewater-based epidemiology is its application in communities without centralised sewage systems necessitating alternative approaches.

We evaluated surface water as an alternate antimicrobial resistance (AMR) monitoring interface by comparing the resistome, virulome, and phylogeny of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-Kp) from surface water (SW), healthy residents (HR), and clinical (CL) settings.

Ninety-six (SW = 32, HR = 32, CL = 32) ESBL-Kp isolates collected over eight-months underwent comparative genomic analysis of antibiotic resistance genes (ARGs), virulence determinants, multilocus sequence types, and phylogenetic relatedness.

Sixteen sequence types (STs) were identified across all sources. ST983 was shared by all sources, ST25 and ST219 were exclusive to SW-HR, and ST152 to SW-CL. More SW (93.8%) isolates shared STs with HR than with CL (9.4%). Phylogenomic analysis was lineage-driven rather than source-structured, with SW isolates forming stronger subclades with HR through ST25 and ST219. Resistome composition showed substantial overlap with differences ($p = 0.003$). The dominant β-lactamase ARG in all sources was $bla_{CTX\text{-}M\text{-}15}$, alongside non-β-lactam *sul2* and *dfrA14*. Pairwise exclusive ARGs totalled 20 (CL-HR), seven (SW-HR), and two (SW-CL); source-restricted ARGs totalled fourteen, nine, and none for CL, HR, and SW, respectively. SW-HR. Virulome overlap was driven by ST25/KL2 and ST219/KL114, a limited SW-CL link was observed through ST152/KL149, and source-restricted lineages were concentrated mainly in clinical and healthy resident isolates.

SW shared major ESBL-Kp AMR features with both HR and CL, but distribution differed across sources. SW more closely resembled HR, supporting its use as a complementary tool rather than a standalone approach for AMR surveillance.

1. Introduction

Antimicrobial resistance (AMR) is a major public-health threat that compromises the effective treatment of infections worldwide. Its impact is particularly severe in low- and middle-income countries (LMICs), where a high infectious disease burden often intersects with limited diagnostic capacity, weak laboratory infrastructure, fragmented health information systems and uneven access to routine microbiological testing (Iskandar et al., 2021; Ondoa et al., 2025). Conventional AMR surveillance is largely based on clinical isolates obtained through healthcare systems and therefore predominantly represents individuals who access formal care, have appropriate specimens collected and are linked to functioning laboratory-reporting systems (Holt et al., 2025). Consequently, facility-based surveillance may underrepresent resistance circulating among asymptomatic community populations and does not adequately capture environmental reservoirs that may contribute to AMR persistence and dissemination.

These limitations have increased interest in environmental surveillance as a complementary component of AMR monitoring. Wastewater-based epidemiology has gained particular attention because wastewater

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can integrate microbial and resistance signals from large sewered catchment populations. Large-scale wastewater metagenomic studies have demonstrated the value of sewage for mapping antimicrobial-resistance gene distributions across countries, while subsequent studies have identified associations between sewage resistomes and clinical resistance patterns (Hendriksen et al., 2019; Karkman et al., 2020). However, wastewater surveillance is most readily implemented where waste from a defined population is collected through centralised sewerage and can be sampled at common points, such as wastewater-treatment-plant influent. Its applicability may therefore be limited in LMIC and resource-constrained settings where sewer connectivity is incomplete or absent and human waste enters pit latrines, septic systems, open drains, stormwater channels, canals, runoff and surrounding surface waters (Shrestha et al., 2021; Truyens et al., 2025; WHO/UNICEF, 2023).

Surface water may represent a contextually relevant environmental surveillance matrix in communities without comprehensive sewage infrastructure. In such settings, rivers and streams may receive diffuse inputs from households, informal sanitation systems, stormwater runoff, healthcare-associated activities and direct waste discharge, potentially integrating AMR signals from multiple human-associated sources (Meradji et al., 2025). Previous studies have demonstrated the occurrence of multidrug-resistant and ESBL-producing Enterobactera,les in surface waters affected by wastewater discharge, poor sanitation and other anthropogenic activities, including clinically relevant resistance genes and bacterial lineages (Bartley et al., 2019; Blaak et al., 2015; Guyomard-Rabenirina et al., 2017). However, these studies primarily established surface water as a receiving environment or reservoir for AMR by comparing surface water and wastewater compartments or assessing anthropogenic contamination. Evidence remains limited on whether surface-water isolates reflect the genomic resistance characteristics, circulating lineages and phylogenetic relationships observed among spatially connected community-carriage and clinical populations.

The need to evaluate such environmental matrices is particularly relevant in sub-Saharan Africa, where AMR surveillance remains uneven and many populations are not comprehensively represented by routine facility-based systems. South Africa has made important progress through national AMR surveillance initiatives aligned with global frameworks such as GLASS, particularly within public-sector hospital and laboratory networks (Engler et al., 2021; NDoH, 2024). Nevertheless, surveillance coverage may remain constrained in some rural and informal settlement settings by unequal access to healthcare, microbiological testing and centralised sanitation infrastructure (Mataboge et al., 2016; NDoH, 2024; Stats SA, 2025). In 2024, Stats SA reported that 76.3% of South Africans had safely managed sanitation, indicating that nearly one in four still lacked such access, with shared sanitation facilities particularly common in urban areas. In such communities where domestic and human waste may enter nearby rivers and streams, surface water may constitute a more relevant environmental receiving matrix than wastewater collected through formal sewer networks. Its capacity to reflect AMR circulating in connected human populations, however, requires direct evaluation.

Within this context, ESBL-producing *K. pneumoniae* is a clinically relevant target organism for evaluating whether surface water reflects human-associated AMR. *K. pneumoniae* is an opportunistic gut-associated pathogen and an important cause of healthcare- and increasingly community-associated infections. It also has a flexible accessory genome and frequently carries acquired resistance determinants on plasmids and other mobile genetic elements, making ESBL-producing strains useful for tracking multidrug-resistant Enterobacterales across human and environmental compartments (Caneiras et al., 2019). Its public health importance is reinforced by the 2024 WHO Bacterial Priority Pathogens List, which ranks carbapenem-resistant *K. pneumoniae* among the highest-priority bacterial threats for research, development, and public health action (WHO, 2024). Beyond clinical settings, resistant *K. pneumoniae* and members of the *K. pneumoniae* species complex have been recovered from wastewater and surface waters, with genomic studies showing that wastewater pathways can contribute to the persistence and environmental release of clinically relevant resistant lineages (Davidova-Gerzova et al., 2024; King et al., 2020; Verburg et al., 2024).

This study evaluated whether surface water can serve as an alternative matrix for human AMR surveillance in low-resource settings by comparing the resistome, virulome, and phylogenetic relatedness of ESBL-producing *K. pneumoniae* isolates from healthy residents of an informal settlement, surface water flowing through the community, and a proximate healthcare facility.

2. Materials and methods

2.1. Ethical statement

The protocol was approved by the Biomedical Research Ethics Committee, University of KwaZulu-Natal (BREC/00003640/2021), the KwaZulu-Natal Department of Health Provincial Health Research Ethics Committee (NHRD Ref. KZ_202203_023), and the National Health Laboratory Service (NHLS Ref. PR2225862). Written informed consent was secured from all community volunteers prior to study enrolment.

2.2. Study site and sample collection

This study was conducted in the uMgungundlovu District Municipality (UMDM), KwaZulu-Natal, South Africa. It was part of a broader longitudinal investigation evaluating the phenotypic and genomic characteristics of ESBL-producing *K. pneumoniae* across three interconnected sources: surface water, healthy community residents, and clinical settings. This sub-study examined concordance in genomic antimicrobial resistance profiles across these sources at higher resolution, with a particular focus on comparative analysis of the resistome, virulome and phylogenetic relationships among selected isolates.

Community samples consisted of stool specimens collected from healthy residents in an informal settlement. Clinical isolates were obtained from bacteriological cultures of various specimen types such as stool, urine, blood, cerebrospinal fluid, pus, and other body fluids from hospitalised adult patients (>18 years old), processed by the Medical Microbiology service of the NHLS serving the UMDM. Surface water samples were collected from the Kwapata River, which drains into the Msunduzi River, a major tributary of the uMngeni River flowing across the district as previously described (Ayukafangha et al., 2026).

2.3. *K. pneumoniae* identification, ESBL confirmation and antimicrobial susceptibility testing (AST)

Eighty surface-water samples, 1978 stool specimens, and 2154 clinical cultures yielded 462, 1667, and 244 presumptive *K. pneumoniae* isolates, respectively, following phenotypic isolation and identification on Simmons Citrate Agar supplemented with 1% (w/v) myo-inositol (SCAI) (Oxoid™, Basingstoke, United Kingdom; Sigma-Aldrich, St. Louis, Missouri, USA). Presumptive isolates were subjected to DNA extraction using the boiling method as previously described (Mbanga et al., 2020). Molecular confirmation was performed using qualitative real-time PCR targeting the *phoE* housekeeping gene. Briefly, each 10-µL reaction contained 5 µL Luna® Universal qPCR Master Mix (New England Biolabs, Ipswich, MA, USA), 1 µL nuclease-free water, 0.5 µL each of the forward and reverse primers, and 3 µL of template DNA. The *phoE*-specific primers were phoE-F (5′-TGCCCAGACCGATAACTTTA-3′) and phoE-R (5′-CTGTTTCTTCGCTTCACGG-3′) (Sun et al., 2010). Amplification comprised an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, followed by melting-curve analysis. Isolates showing amplification and a single melt peak

corresponding to that of the *K. pneumoniae* ATCC 35657 positive control were considered *phoE*-positive. A no-template control was included in each run to detect possible contamination.

The PCR-confirmed *K. pneumoniae* were then subjected to ESBL screening and AST studies, yielding 60, 74, and 37 ESBL producers in surface water, healthy residents, and clinical sources, respectively, as per the ESBL screening and confirmation protocol previously described (Ayukafangha et al., 2026). Briefly, isolates were screened for ESBL production using CHROMID® ESBL agar (bioMérieux, Marcy l'Étoile, France). Phenotypic confirmation was performed using the double-disk synergy test, with cefotaxime (30 µg) and ceftazidime (30 µg) disks positioned 20 mm from a centrally placed amoxicillin/clavulanic acid disk (20/10 µg). An increase of ≥5 mm in the inhibition-zone diameter around either cephalosporin disk was interpreted as a positive ESBL result. Antimicrobial susceptibility testing was performed using the VITEK® 2 system with the AST-N256 card (bioMérieux, Marcy l'Étoile, France) for Gram-negative bacteria, following the manufacturer's instructions. Results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2021).

Confirmed ESBL-producing *K. pneumoniae* isolates from each source underwent genomic DNA extraction using the GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St Louis, Missouri, USA). DNA concentration and purity were assessed using a NanoDrop microvolume spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at a 260/280 nm ratio in accordance with the manufacturer's protocol.

2.4. Whole-genome sequencing (WGS) and selection of study isolates

The extracted DNA of confirmed ESBL-producing *K. pneumoniae* isolates underwent whole-genome sequencing (WGS) at the National Institute for Communicable Diseases (NICD) Sequencing Core Facility. Sequenced isolates were screened for non-*K. pneumoniae* contamination, after which 32 clinical isolates remained. As this represented the smallest source group, all 32 clinical isolates were retained for comparative analysis. To obtain balanced source groups, 32 isolates were randomly selected from each of the larger healthy-resident and surface-water isolate pools using month-stratified sampling, with four isolates selected per source per month. This balanced, month-stratified selection reduced disproportionate representation of the larger isolate pools while preserving temporal coverage. The final comparative genomic dataset, therefore, comprised 96 ESBL-producing *K. pneumoniae* isolates: 32 clinical, 32 healthy-resident, and 32 surface-water isolates.

2.5. Bioinformatic analyses

Multiplexed paired-end libraries were prepared using the Illumina DNA Prep Kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions. Library quality and fragment size distribution were assessed using an Agilent TapeStation (Agilent Technologies, Santa Clara, CA, USA), and library quantification was performed using a Qubit 4.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Standardised libraries were pooled at 4 nM to achieve approximately 100 × coverage per sample and sequenced at NICD (Johannesburg, South Africa) on the Element Biosciences AVITI and Illumina NextSeq 1000/2000 platforms to generate 2 × 150 bp paired-end reads. Raw sequence reads were quality-checked using FASTQC v0.12.0 (Andrews, 2010) and trimmed with Sickle v1.33 (https://github.com/najoshi/sickle) before de novo assembly with SKESA v2.4.0 (https://github.com/ncbi/SKESA). Draft assemblies were included in downstream analyses if they had a minimum read depth of 30 ×, at least 95% estimated genome coverage, and an assembled genome size within the expected range of 4.4-6.5 Mb. Contigs shorter than 200 bp were removed. The resulting quality-filtered draft genomes were submitted to NCBI GenBank under BioProject PRJNA1253337, and isolate-specific Genome and BioSample accession numbers, together with assembly-quality metrics, are provided in Supplementary Material S1.

To characterise the resistome, acquired antimicrobial resistance genes, point mutations, and disinfectant resistance genes were identified using ResFinder v4.7.2 (http://genepi.food.dtu.dk/resfinder) (Bortolaia et al., 2020) with default parameters, while resistance mechanisms and associated genes were further examined using the Comprehensive Antimicrobial Resistance Database (CARD) (https://card.mcmaster.ca) (Alcock et al., 2023). Sequence types were assigned using MLST v2.0.

(https://bitbucket.org/genomicepidemiology/mlst). To characterise the virulome and surface antigens, virulence determinants together with capsular (K) and lipopolysaccharide (O) loci were identified using Kleborate v3.2.4 with the integrated Kaptive v3 module (Lopes et al., 2025). In addition to broader virulome characterisation, isolates were specifically screened for the major hypervirulent *K. pneumoniae*-associated markers aerobactin (*iuc*), salmochelin (*iro*), and the hypermucoidy regulators *rmpA* and *rmpA2*. Phylogenomic analysis was performed on isolates across sources together with a reference genome (*K. pneumoniae* HS11286, ST11) from BV-BRC. All genomes were retrieved, annotated, and processed using the Bacterial and Viral Bioinformatics Resource Center (BV-BRCB; https://www.bv-brc.org/). Maximum-likelihood phylogenetic reconstruction was carried out within BV-BRC, and resulting trees were visualised in Interactive Tree of Life (iTOL; https://itol.embl.de). To quantify relatedness within sequence types shared across sources, assembled genomes underwent lineage-specific core-genome multilocus sequence typing (cgMLST) analysis using BV-BRC. Pairwise core-genome allelic distances were summarised by source pair, with ≤10 allelic differences used as an indicative threshold for close relatedness based on previous *K. pneumoniae* studies (Zhou et al., 2017). Annotation, customisation, and incorporation of associated metadata were performed in R (Zhou et al., 2023)

2.6. Statistical analyses

Statistical analyses were performed in R version 4.5.0 using RStudio version 2025.09.0.387. Genomic determinants were summarised at the isolate level as frequencies, proportions, andpresence-absence variables, as appropriate. Associations between isolate source and categorical genomic variables were assessed using Pearson's chi-square test implemented with the "chisq.test" function in the R stats package. Where the assumptions of the chi-square test were not met because of sparse expected cell counts, Fisher's exact test was applied using the "fisher.test" function, with Monte Carlo simulation for larger contingency tables where required. Standardised residuals were examined to identify categories contributing to statistically significant associations. *P*-values of multiple comparisons were adjusted using the Benjamini-Hochberg false discovery rate procedure (Benjaminit and Hochberg, 1995).

Diversity in the distributions of resistance genes, resistance mechanisms, and virulence-related determinants was summarised using the Shannon diversity index and Pielou's evenness (Pielou, 1966; Shannon, 1948), implemented using established functions in the vegan package. Principal component analysis (PCoA) was performed on centred and scaled isolate-by-gene presence-absence matrices using the "prcomp" function in the R stats package to visualise similarities in resistome composition among sources (Jolliffe and Cadima, 2016).

Differences in multivariate resistome composition among sources were tested using permutational multivariate analysis of variance based on Bray-Curtis dissimilarities, implemented with the "adonis2" function in the vegan package (Anderson, 2001; Oksanen et al., 2025). Homogeneity of multivariate dispersion was evaluated using "betadisper" followed by permutation testing to support interpretation of the PERMANOVA results. Statistical significance was defined as $p < 0.05$. Data visualisations, including bar plots, ordinations, and heatmaps, were generated using ggplot2 and ComplexHeatmap in R (Gu et al., 2016; Wickham, 2016).

3. Results

3.1. Genomic features of ESBL-Kp isolates

Assembly metrics for the 96 quality-filtered draft genomes are summarised in Supplementary Material S1. Genome sizes ranged from 5.31 to 5.66 Mb, with GC contents ranging from 56.38% to 57.81%. Draft assemblies comprised 38–199 contigs, with N50 values ranging from 22.27 to 477.31 kb and L50 values from 3 to 19. Genome annotation identified 5,124-6,427 coding sequences, 73-90 tRNA genes, and 2-14 rRNA genes per genome. BV-BRC quality assessment yielded coarse-consistency scores of 97.5-99.6% and fine-consistency scores of 91.0-98.7%.

3.2. MLST and phylogenetic relationships

MLST analysis identified 16 distinct sequence types (STs) across all sources. Only one sequence type, ST983, was shared across surface water (SW), healthy residents (HR), and clinical (CL) isolates. Two STs (ST25 and ST219) were exclusive to SW and HR, and one (ST152) to SW and CL. Five ST were unique to CL, four to HR, and one to SW, as shown in Fig. 1a.

Consistent with this distribution, codon-based maximum-likelihood phylogenetic analysis of the 96 isolates and one reference genome (*K. pneumoniae* HS11286, ST11) revealed a population structure primarily shaped by clonal lineage rather than by source of isolation (Fig. 1b). The majority of isolates ($n = 94$) clustered within a single dominant clade (BS = 93%), while two independently divergent lineages, both exclusively clinical, branched outside this clade (the dotted black lines).

Within the dominant clade, several well-supported substructures were evident. The largest was an ST25 subclade (yellow clade) (BS = 100%, $n = 31$), composed predominantly of SW isolates ($n = 24$) together with a smaller number of HR isolates ($n = 7$) and notably lacking any clinical isolate. A second major lineage centred on ST983 (Pink clade) grouped isolates from all three sources (15 CL, 6 HR, and 2 SW) without clear internal separation by source, representing the most prominent source connectedness. A third lineage, defined by ST152 (sky-blue subclade, BS = 100%, $n = 8$), consisted mainly of clinical isolates ($n = 7$) connected SW with CL.

Additional structure further highlighted source relationships. A secondary cluster (green subclade, BS = 50%, $n = 11$) grouped all ST219 isolates (4 SW, 2 HR) with five HR-associated ST607 isolates, again without clinical representation, reinforcing the close relationship between SW and HR populations. In contrast, ST29 (4 HR, 2 CL) and ST1026-1LV (1 HR, 1 CL) linked HR and CL isolates within shared lineages.

At the finest phylogenetic resolution, direct clustering analysis defined as sister-taxon relationships or terminal groupings connected by minimal branch lengths (red-highlighted clades) showed two cross-source clusters linking SW and HR within the ST25 lineage, though both were supported by short branch lengths rather than strong bootstrap values and no direct SW-CL sister pair was observed. In contrast, HR isolates formed multiple direct cross-source relationships with CL isolates, positioning HR as the most phylogenetically connected source.

Overall, there was generally more self-source clustering (SW = 14/32, HR = 12/32, CL = 16/32) than cross-source clustering. Among the few cross-source clustering, both subclade-level structure and fine-scale clustering indicate that surface water isolates are most closely aligned with those from healthy residents, while also maintaining more limited but detectable connections to clinical lineages. This is reflected in the high proportion of SW isolates sharing sequence types with HR isolates (30/32; 93.8%) compared to CL isolates (3/32; 9.4%), and in the absence of direct SW-CL sister relationships.

3.2.1. Lineage-specific core-genome multilocus sequence typing (cgMLST)

cgMLST was used to quantify allelic relatedness within the four sequence types shared across isolate sources: ST25, ST152, ST219, and ST983 (Fig. 2). Within ST25, comprising 24 surface-water and seven healthy-resident isolates, cross-source distances ranged from 0 to 8 alleles, with a median of 4 alleles. Ten surface-water-healthy-resident pairs had 0 allelic differences across the loci analysed, including AOA9-L55OKP, OEC18KP-L51OKP, and YED1KP-L04JKP.

Within ST983, surface-water-healthy-resident isolate distances ranged from 0 to 8 alleles (median, 4), compared with 3 to 11 alleles between surface-water and clinical isolates (median, 7) and 3 to 10 alleles between clinical and healthy-resident isolates (median, 5). The closest ST983 surface-water-healthy-resident isolate pair, MED10KP and L09YKP, had 0 allelic differences, while MED10KP and the closest clinical isolate, CAKP2, differed by 3 alleles.

Within ST152, the single surface-water isolate, OEC1-KP, differed from the seven clinical isolates by 0 to 5 alleles (median, 1); OEC1-KP and CYKP2 had 0 allelic differences. In contrast, ST219 surface-water and healthy-resident isolates differed by 18 to 21 alleles (median, 19), with the smallest distances observed between L14AKP and the surface-water isolates JED7KP and NOA12KP, each differing by 18 alleles.

3.3. Detected resistance genes of ESBL K. pneumoniae isolates

Across the 96 isolates analysed, a total of 79 antimicrobial resistance genes (ARGs) were identified. β-lactamase genes were the most abundant class, followed by aminoglycoside, then fluoroquinolone, and trimethoprim resistance genes, as seen in Fig. 2a. Macrolide resistance was the least represented.

A core set of 27 resistance genes resistance were shared across all three sources. Among β-lactamases, $bla_{CTX\text{-}M\text{-}15}$ was detected in 27 (84.4%) clinical isolates, 31 (96.9%) healthy resident isolates, and 31 (96.9%) surface water isolates. Similarly, $bla_{TEM\text{-}1B}$ was highly prevalent across all sources (clinical: 78.1%; healthy residents: 75.0%; surface water: 84.4%). $bla_{OXA\text{-}1}$ was more frequent in clinical (71.9%) and surface water isolates (84.4%) than in healthy residents (40.6%). Outside the β-lactamase class, among fluoroquinolone-associated determinants, *OqxA* and *OqxB* were highly prevalent in clinical isolates (93.8% and 96.9%, respectively), moderately represented in healthy residents (75% each), and less common in surface water isolates (25% each). In contrast, $bla_{SHV\text{-}110}$ and $bla_{SHV\text{-}81}$ were highly prevalent in surface water isolates (75% each), less frequent in healthy residents (21.9% each), and rare in clinical isolates (3.1% each). Similarly, sulphonamide (*sul2*) and trimethoprim (*dfrA14*) resistance genes were widely distributed across all sources, each occurring in >60% of isolates in all groups.

Pairwise analysis revealed the largest number of exclusively shared genes between clinical and healthy resident isolates ($n = 20$), including *aac(3)-IId*, *aadA2*, $bla_{CTX\text{-}M\text{-}3}$, $bla_{SHV\text{-}172}$, $bla_{SHV\text{-}187}$, $bla_{SHV\text{-}33}$, $bla_{TEM\text{-}141}$, $bla_{TEM\text{-}1A}$, and multiple additional bla_{TEM} variants, as well as *dfrA12*, *dfrA15*, *qnrB6*, and *qnrS1*. Surface water and healthy resident isolates shared seven resistance genes not observed in clinical isolates, comprising several bla_{SHV} variants ($bla_{SHV\text{-}145}$, $bla_{SHV\text{-}179}$, $bla_{SHV\text{-}194}$, $bla_{SHV\text{-}199}$, $bla_{SHV\text{-}26}$, $bla_{SHV\text{-}78}$, and $bla_{SHV\text{-}98}$). In contrast, only two resistance determinants were exclusively shared between surface water and clinical isolates: *catA1* and *tet(D)*.

Source-restricted resistance genes were also observed. Clinical isolates contained 14 unique genes, including multiple bla_{SHV} variants (e.g., $bla_{SHV\text{-}103}$, $bla_{SHV\text{-}11}$, $bla_{SHV\text{-}13}$, $bla_{SHV\text{-}40}$, $bla_{SHV\text{-}56}$, $bla_{SHV\text{-}70}$), as well as *aph(3')-Ia*, *catA2*, and *fosA7*. Healthy resident isolates harboured nine unique genes, including $bla_{OKP\text{-}B\text{-}15}$, $bla_{OXA\text{-}18}$, and several *bla*SHV variants (e.g., $bla_{SHV\text{-}65}$, $bla_{SHV\text{-}75}$, $bla_{SHV\text{-}94}$, $bla_{SHV\text{-}96}$), in addition to *dfrA17*. No resistance gene was found to be exclusive to surface water isolates. Additional details, including the proportional distribution of resistance genes and chromosomal point mutations across sources, are provided in Supplementary Material S2.

Principal component analysis (PCA) demonstrated substantial

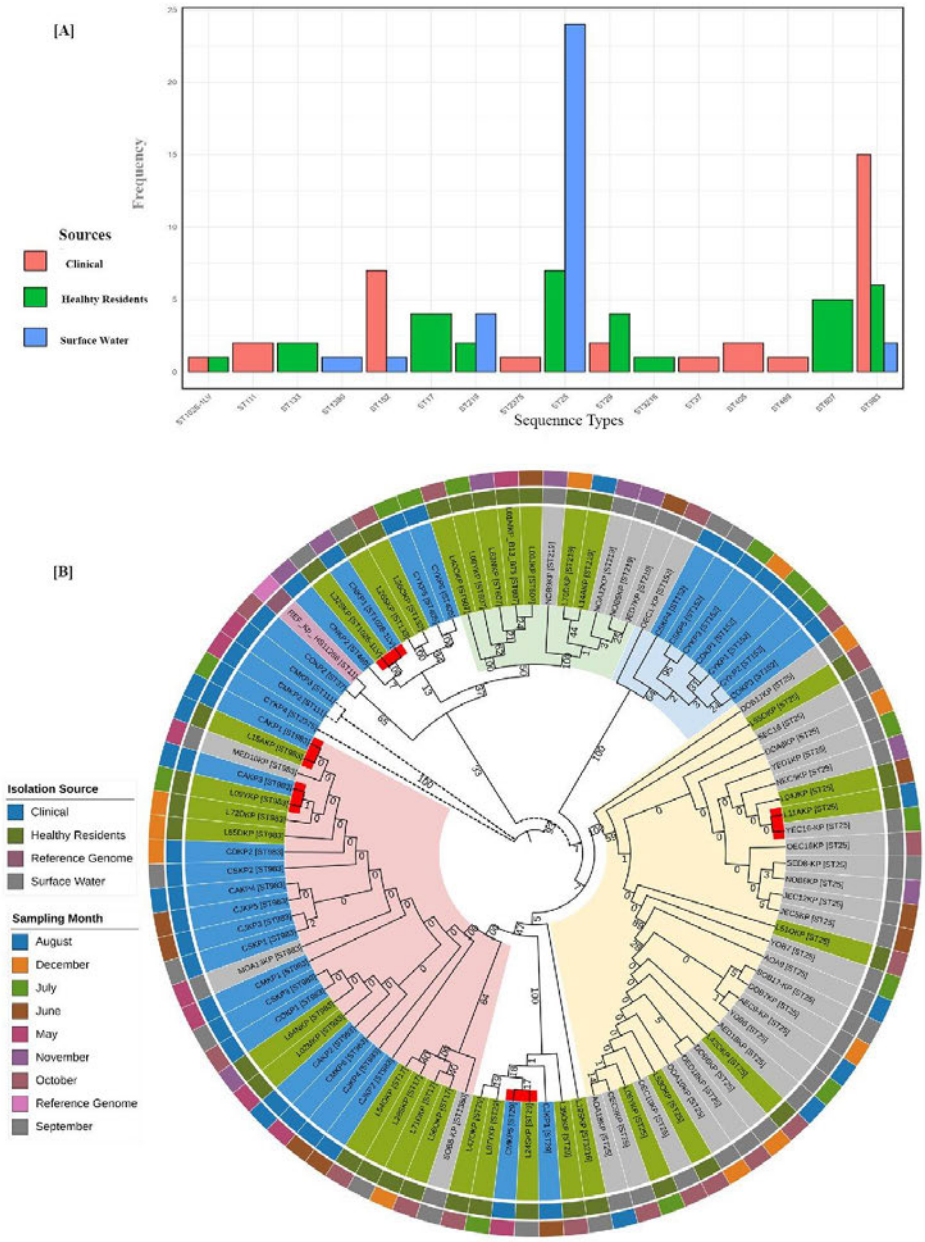

Fig. 1. [A] Distribution of sequenced types across isolates sources. [B] Phylogenetic tree shows the relationships among study isolates based on a maximum-likelihood analysis. Isolate IDs and sequence types (STs) are displayed at the tips. Tip background colours indicate source of isolation: clinical (blue), healthy residents (dark green), and surface water (grey). Concentric metadata rings, shown from inner to outer, represent isolation source and sampling month. The four major mixed-source clades are shaded (yellow, pink, green, and sky-blue), within source clades are highlighted in sky-bue, and tight or sister cross-source clustering is highlighted in red. Internal node values indicate bootstrap support (0–100).

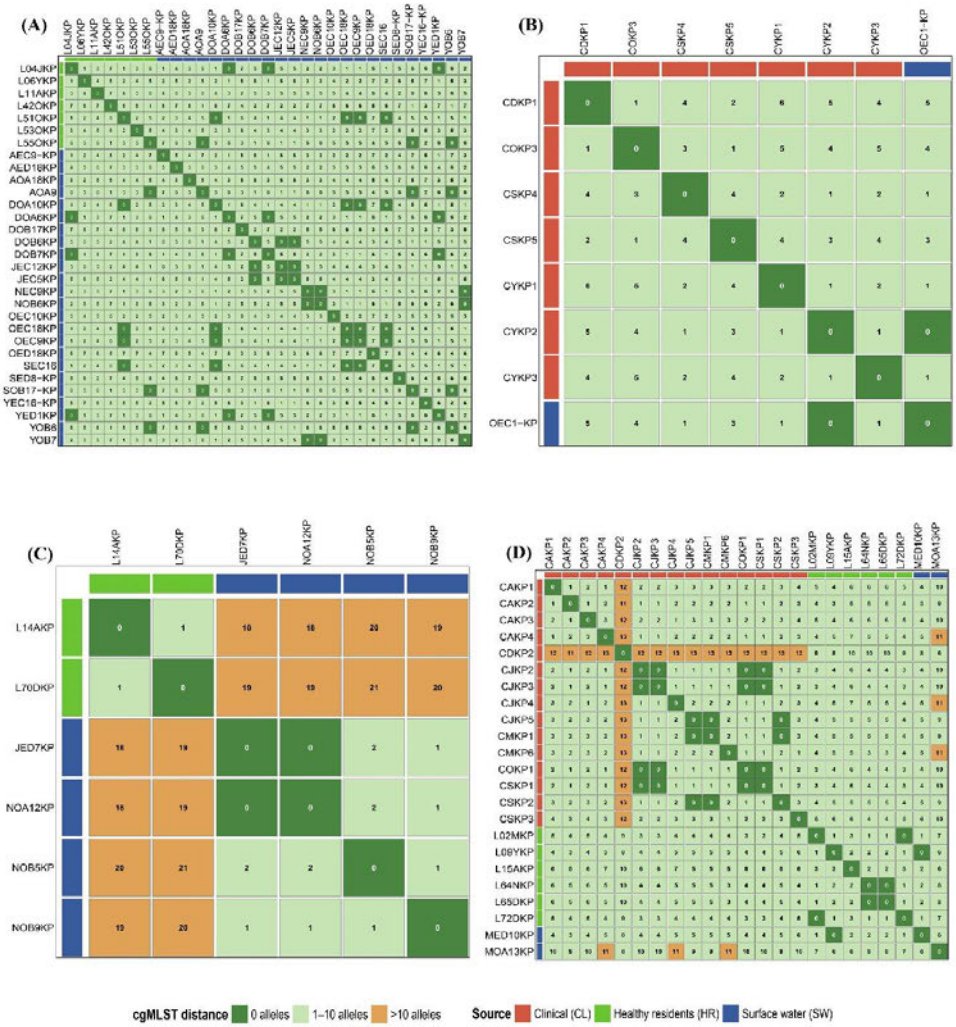

Fig. 2. Pairwise cgMLST allelic distances within sequence types shared across isolate sources. Matrices show pairwise allelic differences among (A) ST25 – SW-HR, (B) ST152 – SW-CL, (C) ST219 – SW-HR, and (D) ST983 - HR-SW-CL isolates. Source annotation strips indicate clinical (red), healthy-resident (green), and surface-water (blue) isolates labels. Cell colours represent 0 allelic differences (dark green), 1–10 differences (light green), and >10 differences (orange).

overlap in resistome composition across all three sources (Fig. 2C). PERMANOVA analysis identified a statistically significant effect of source on resistome composition ($R^2 = 0.075$, $p = 0.003$). Chi-square analysis of ARG classes showed significant differences in the distribution of fluoroquinolone, tetracycline, amphenicol, and macrolide resistance genes across sources ($p < 0.001$), whereas β-lactamase, aminoglycoside, sulphonamide, and trimethoprim resistance classes were similarly distributed across sources ($p > 0.05$) (Fig. 3).

3.3.1. *Resistance mechanism of ESBL K. pneumoniae*

CARD analysis revealed 75 distinct resistance genes distributed across seven mechanism categories. Genes including *rsmA*, *marA*, *emrA*, *beaR*, *adeF*, *ompA*, *LptD*, *K. pneumoniae OmpK37*, and *E. coli AcrAB-TolC* with *MarR* mutation conferring resistance to CIP and TET were consistently detected in all 32 isolates from each source. The top 60 genes and corresponding isolate counts across sources are shown in Fig. 4A. With the exception of antibiotic target protection, multidrug resistance mechanisms, predominantly antibiotic efflux and antibiotic inactivation, were present in every isolate across all sources (100%). Surface water isolates ($n = 2$, 6.25%) exhibited the lowest representation of antibiotic target protection (Supplementary Material S3/Table 1). Genes belonging to MDR efflux families (ABC, MFS, RND, SMR), reduced permeability, target alteration PMR, and β-lactamases were detected in all isolates (100%) from all sources (Supplementary Material/S3/

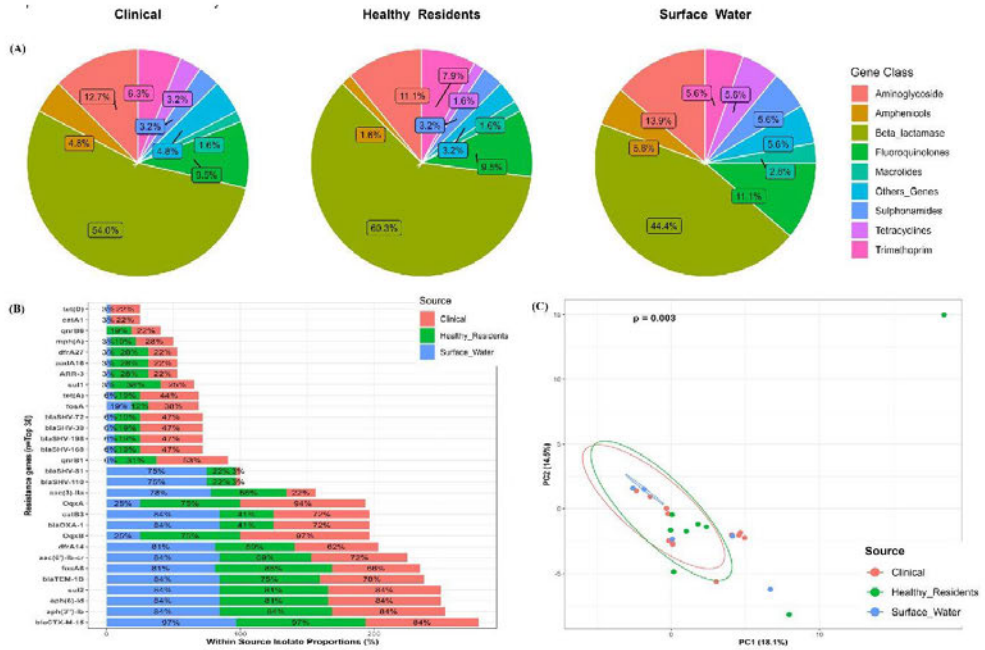

Fig. 3. [A] Showing distribution of antibiotic resistance genes across sources by gene classes (B), within sources isolate proportion of top 30 genes, and (C) Principal component analysis (PCA) on the presence–absence matrix of resistance genes detected among the isolates. PCA shows that Clinical (red), healthy residents (green), and surface water (blue) isolates largely occupy the same central region of the ordination space, but resistome composition differs significantly between sources ($p < 0.05$).

Fig. 4b/c)

Resistance mechanism profiles were highly similar across the three sources. All sources showed the same richness value (6), with comparable Shannon diversity values for clinical (H = 1.532), healthy resident (H = 1.540), and surface water isolates (H = 1.472), as well as similar Pielou's evenness values (0.855, 0.859, and 0.822, respectively). Chi-square analysis showed no significant difference in the distribution of resistance mechanisms across sources ($p = 0.969$).

Heatmap showing the distribution of the top 60 antibiotic resistance genes across Clinical, Healthy Resident, and Surface Water isolates. Genes and corresponding isolate counts are colour-coded from green (low) to red (high), with each row representing a gene. Genes are annotated by resistance mechanism, indicated by colour-coded bars on the right. Efflux, inactivation, and target alteration mechanisms dominate across all sources, while target replacement and reduced permeability are less frequent.

3.4. *Virulence determinants of ESBL K. pneumoniaeisolates*

Virulence-associated determinants were screened across 96 isolates. The siderophore systems *aerobactin* (*iuc*) and *salmochelin* (*iro*) and the hypermucoidy regulators *rmpA/rmpA2* were not detected in any isolates. Fig. 5 (a–f) shows the distribution of respective virulence determinants across sources, while Supplementary Material/S4 details isolate lineages associated with the virulence determinant across sources.

Virulence-associated loci, including capsular (K) loci, O-antigen loci, and yersiniabactin-associated *ICEKp* elements, were distributed across a limited number of shared and source-restricted determinants (Fig. 5). Overall, 79/96 isolates (82.3%) carried a virulence score of 1, indicating the presence of yersiniabactin-associated virulence determinants. The only virulence trait shared across all three sources was *KL127/OL13/O13*, corresponding to ST983, detected in 15 CL, 6 HR, and 2 SW isolates, all carrying *ybt5: ICEKp6* (virulence score = 1)

Pairwise sharing was most frequent between surface water and healthy resident isolates. The dominant shared background was ST25/KL2/OL2α.2/O1αβ,2β, present in 24 SW and 7 HR isolates, all carrying ybt14; ICEKp5. A second SW-HR shared background was ST219/KL114/OL2α.1/O1αβ,2α, detected in four SW and two HR isolates, also carrying *ybt14; ICEKp5*. In contrast, the main SW-CL shared background was ST152/KL149/OL4/O4, represented by seven CL and one SW isolate, all carrying *ybt10: ICEKp4*. Healthy resident and clinical isolates also shared ST29/KL30 and ST1026-1LV/KL8, both lacking yersiniabactin (virulence score = 0).

Source-restricted virulence backgrounds were observed mainly in clinical and healthy resident isolates. Clinical-only lineages included ST405/KL151, carrying ybt27; *ICEKp22*, as well as ST37/KL7, ST111/KL63, ST469/KL139, and ST2375/KL14, which lacked yersiniabactin. Healthy resident-exclusive traits included ST133/KL116 with ybt16; *ICEKp12*, and ST17/ST607 with KL25, which variably carried *ybt14* or lacked yersiniabactin. Surface water showed minimal exclusivity, with only ST1380/KL155 lacking yersiniabactin.

Overall, the virulence determinants showed a shared ST983/KL127/OL13 core, broader SW-HR overlap through ST25/KL2 and ST219/KL114, a limited SW-CL link through ST152/KL149, and a small set of source-restricted lineages concentrated mainly in clinical and healthy-resident isolates. At the source level, virulence-positive isolates were detected in 31/32 (96.9%) SW, compared with 24/32 (75.0%) HR and 24/32 (75.0%) CL isolates.

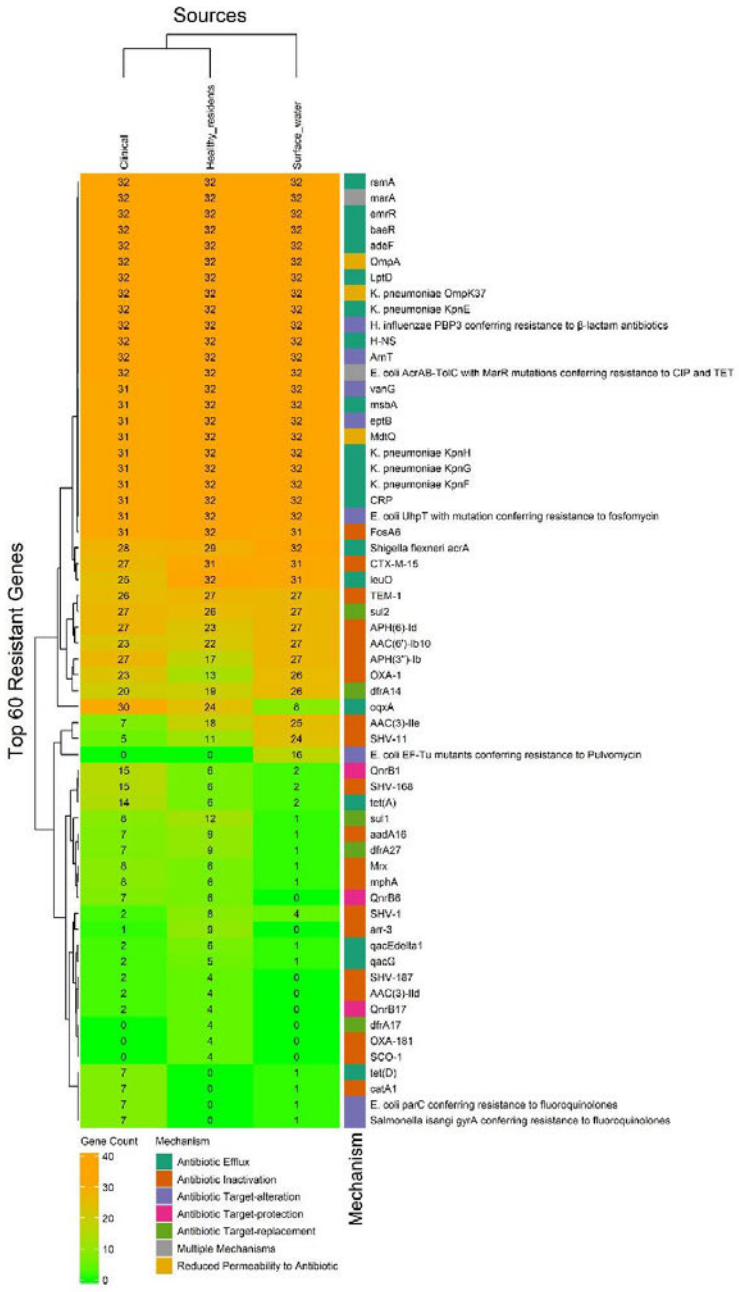

Fig. 4. A: Heatmap of resistance genes associated with MDR mechanism across sources.

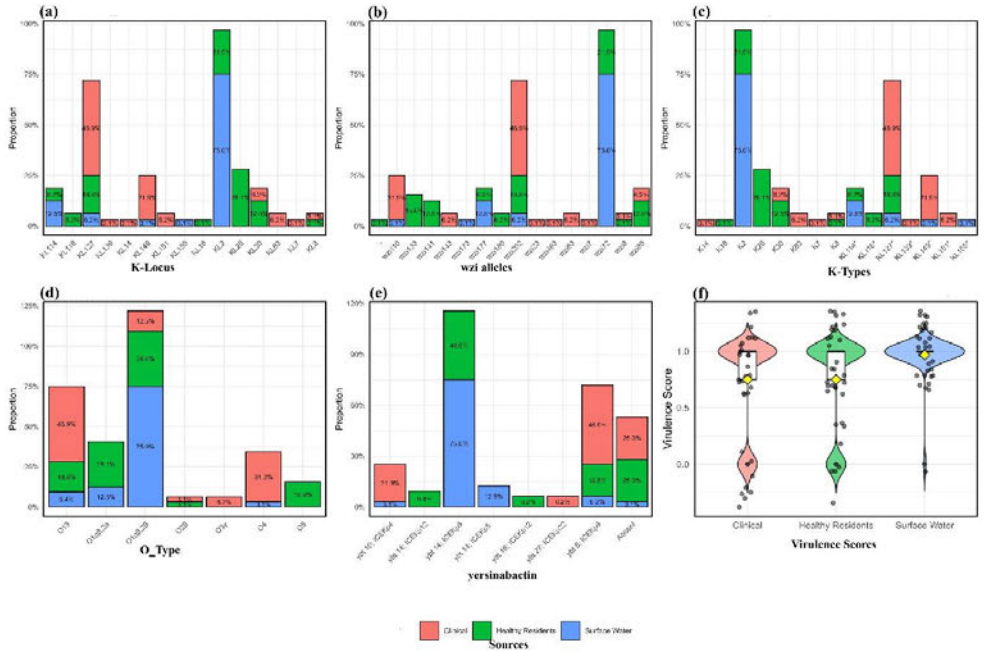

Fig. 5. Stacked bar chats of (a) KL-Locus, (b)Wzi alleles, (c) Capsular types, (d) O- Antigens (e)yersiniabactin locus, and (f) virulence score plot of ESBL-Kp Virulence Factors.

Healthy resident isolates showed the highest virulence-associated diversity and evenness (KL, O loci, O-types, and yersiniabactin; H up to 1.83, J up to 0.83), followed by clinical isolates with intermediate diversity, while surface water isolates had the lowest diversity and evenness across all markers ($H \leq 0.87$, $J \leq 0.50$). Differences in KL, O loci/types, and yersiniabactin distributions were highly significant across sources (all $p < 0.001$)

4. Discussion

Antimicrobial resistance surveillance in sub-Saharan Africa and other low- and middle-income countries remains critically constrained by weak health systems, limited laboratory capacity, and an over-reliance on clinical case detection conditions that render conventional surveillance both incomplete and geographically uneven. Wastewater-based approaches have been proposed as a scalable complement to clinical surveillance, yet their applicability depends on functioning sewage infrastructure that is frequently absent in rural settings (Balcázar, 2025; Punch et al., 2025). In these settings, domestic and community waste drains directly into surface water bodies, making surface water not only a potential reservoir for circulating resistance determinants but also a more contextually appropriate and accessible surveillance matrix (Meradji et al., 2025). To evaluate the potential of surface water as an AMR surveillance proxy, we compared the resistome, virulome, and phylogenetic relationships of ESBL-Kp isolates recovered from surface water, healthy community residents, and nearby healthcare facilities within a spatially connected informal settlement in South Africa to provide local context if AMR profiles in surface water reflect what circulates in the community or clinical setting.

4.1. Multilocus sequence typing (MLST) and phylogenomic analysis of isolates

From our findings, ESBL *K. pneumoniae* isolates showed limited overlap in circulating sequence types across three sources, with few pairwise between surface water and either healthy residents or clinical-associated sources lineage-based rather than source-based phylogenetic clustering with isolates from healthy residents and clinical setting. The sequence type, ST983, a lineage associated with hospital infection, was the only notable lineage overlap across all three sources in this study. This is consistent with its previously reported local presence among hospitalised patients and their surrounding hospital environment within the same district (Founou et al., 2019). Beyond local studies, ST983 has been reported among healthy farmers and livestock in a Peruvian One-Health farm study (Benavides et al., 2026), during a neonatal intensive care unit outbreak in Zambia (Nadimpalli et al., 2026), and hospital wastewater in South West England (Gibbon et al., 2021), supporting the broader dissemination across environmental, animal and human niches. Although ST983 isolates in the current study formed a distinct monophyletic clade in the phylogenetic analysis, previous studies have reported a close evolutionary relationship with other clones such as ST17 and ST15 clones associated with bloodstream infections and gastrointestinal colonisation in South Africa (Kopotsa et al., 2020; Salvador-Oke et al., 2024), highlighting the observed overlap as potential of surface water in detecting clinically relevant circulating lineages.

Cross-source pairwise ST analysis showed that surface water isolates were more closely aligned with healthy residents than with clinical isolates. Specifically, 93.8% (30/32) of surface water isolates shared sequence types with healthy residents, primarily through the dominant lineages ST25 and ST219, whereas only 9.4% (3/32) shared sequence

types with clinical isolates through ST152. This was further supported phylogenetically, where ST25 and ST219 formed strongly bootstrapped subclades with healthy resident isolates (BS = 100%), compared to the ST152 surface water - clinical association, collectively positioning surface water as more closely aligned with the healthy resident than the clinical isolates. Lineage-specific cgMLST (Fig. 2) provided quantitative support for this pattern: surface-water-healthy-resident pairs differed by 0–8 alleles within ST25 (median, 4; ten pairs with 0 allelic differences) and by 0–8 alleles within ST983 (median, 4). These distances fall within the ≤10-allele range previously used to identify closely related *K. pneumoniae* cgMLST clusters, although such thresholds remain dependent on the cgMLST scheme and epidemiological context (Zhou et al., 2017). Although ST25, ST219, and ST152 have been sporadically reported in South African hospital settings, including paediatric contexts in Durban, Pretoria, Cape Town and Thembisa (Hetsa et al., 2025; Marais et al., 2024; Mbelle et al., 2020; Osei Sekyere et al., 2024), community prevalence remains poorly characterised, likely reflecting surveillance bias toward clinical populations rather than true community absence. While the phylogenetic proximity of surface water to healthy resident isolates is consistent with the informal settlement context of this study where community faecal contamination and waste disposal into surface water are expected, the bootstrapped subclade structure provides genomic-level evidence beyond ST co-occurrence, confirming true clonal relatedness between these compartments. The stronger overlap between surface-water and healthy-resident isolates is consistent with community linkage, but *K. pneumoniae* occurs across human, animal, and environmental reservoirs, and surface water isolates may represent either inputs from human or animal sources or strains established in natural habitats (Rocha et al., 2022). Notably, ST152 has previously been documented in both carriage and clinical isolates locally (Founou et al., 2019; Masalane et al., 2025), and recovered from a wastewater treatment plant and its receiving water body in the same geographical area (Ramsamy et al., 2022), corroborating its role as an ecologically versatile lineage bridging carriage, environmental, and clinical compartments and lending direct precedent to our findings.

Notwithstanding the cross-source overlaps, self-source clustering was the predominant pattern overall, with 14/32 (43.8%) of surface water, 12/32 (37.5%) of healthy residents, and 16/32 (50.0%) of clinical isolates forming within-source groupings, suggesting that each source retains a degree of ecological distinctiveness despite shared lineages. This was most evident among healthy resident and clinical isolates, where several maximally supported (BS = 100%) exclusively within-source subclades were identified. Healthy residents formed self-exclusive clades encompassing ST17, ST607, and ST133 lineages, none of which were recovered from surface water or clinical samples, while clinical isolates similarly formed exclusive, well-supported groupings within ST405 and ST111. Surface water likewise harboured its own exclusive sequence type, with ST1380 absent from both healthy resident and clinical ST repertoires. Notably however, despite its ST-level exclusivity, ST1380 clustered phylogenetically within the healthy resident neighbourhood, grouping with ST17 isolates at a bootstrap of 84%, indicating that even the surface water-restricted lineage maintains greater genomic affinity to the community compartment than to clinical isolates. The absence of surface water representatives from the healthy residents and clinical exclusive clades, combined with the community-directed phylogenetic positioning of its own exclusive lineage, argues against surface water being a passive mirror of either source, and instead supports its role as a selective environmental reservoir that captures specific shared lineages, particularly ST25, ST219, and ST983, while remaining ecologically distinct in its overall population composition. Overall, the combined MLST and phylogenomic evidence suggests that surface water in this setting does not fully recapitulate the community or clinical *K. pneumoniae* population, but instead captures a meaningful subset of circulating lineages, with its closest phylogenetic relationship being to healthy residents rather than clinical isolates.

4.2. Antibiotic resistance genes (ARGs) resistance mechanism of ESBL-Kp isolates

Comparative resistome analysis of the study isolates (Fig. 3) revealed a substantial shared core set of determinants across clinical, healthy resident, and surface water sources, indicating the interconnected circulation of resistance determinants across sources. This was reflected by the detection of 27 ARGs common to all sources, including the globally disseminated ESBL gene $bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1B}$, and non-β-lactam determinants such as *sul2* and *dfrA14*, with no significant difference in the distribution of resistance gene categories such as β-lactamase, aminoglycoside, sulphonamide, and trimethoprim resistance across sources. The high prevalence of $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1B}$ across all three sources complements earlier reports on increased prescription and usage of β-lactam antibiotics such as penicillins and cephalosporins in South Africa (Alabi and Essack, 2022) while the ubiquity of *sul2* and *dfrA14* similarly mirrors reported widespread co-trimoxazole (trimethoprim-sulfamethoxazole) use for urinary tract infection management and HIV prophylaxis in the South Africa (Fourie et al., 2021; Nel et al., 2023). Consequently, recent local studies on Klebsiella species from clinical (Hetsa et al., 2025; Masalane et al., 2025; NDoH, 2024), community (Ogunbosi et al., 2020), and environmental (Motlhaping et al., 2026; Richter et al., 2021) sources have noted high prevalence of these genes. This shared genetic determinant was matched by a highly conserved resistance mechanism profile, with multidrug efflux systems detected universally across all sources (Fig. 4A). The substantial overlap observed in the PCA (Fig. 3C) and the small but statistically significant PERMANOVA effect size (R^2 = 0.075) indicate a modest source-associated shift rather than strong biological separation, with most resistome variation not explained by source. However, this overall similarity does not preclude source-specific variation in individual resistance traits.

Despite this common backbone, several source-dependent patterns indicate ecological differentiation of the resistome. Clinical isolates were enriched for fluoroquinolone-associated determinants (*oqxA, oqxB*), likely reflecting stronger antibiotic selection pressure in the clinical setting from high-watch antibiotic category prescribing (Mthombeni et al., 2023; Spinickum et al., 2024), and the markedly lower prevalence of these determinants in surface water isolates suggests weaker maintenance in the aquatic environment, possibly due to reduced antimicrobial selection and differing ecological trade-offs rather than a fundamental ecological discontinuity between sources (Larsson and Flach, 2022). In contrast, surface water isolates showed marked enrichment of multiple *bla*SHV variants, consistent with the accumulation of diverse resistance lineages introduced by mixed anthropogenic inputs, including domestic wastewater, community faecal discharge, and hospital effluent, each potentially contributing distinct *bla*SHV alleles. Healthy resident isolates displayed an intermediate profile, sharing the greatest number of exclusive ARGs with clinical isolates, suggesting that asymptomatic carriage populations may function as an epidemiological bridge between hospitals and the wider environment. The significant between-source differences in fluoroquinolone, tetracycline, amphenicol, and macrolide resistance classes, contrasted with the similar distribution of β-lactamase, aminoglycoside, sulphonamide, and trimethoprim genes, further indicate that while the core resistome is conserved, accessory resistance traits remain responsive to local antimicrobial selection pressures.

From a surveillance perspective, surface water partially mirrored the human-associated resistome but did not fully reproduce the community and clinical relationship. Notably, no ARG was unique to surface water isolates, whereas both clinical and healthy resident isolates harboured source-restricted genes, suggesting that ESBL *K. pneumoniae* from surface water profiles largely reflect redistributed community and clinical resistance rather than an independent reservoir of novel determinants. However, the limited overlap of exclusive genes between surface water and clinical isolates indicates that aquatic surveillance alone would

underestimate certain clinically relevant resistance signatures. Notably, surface-water-only surveillance would not have captured 14 resistance genes detected only in clinical isolates and nine detected only in healthy-resident isolates or several sequence types absent from surface water, including ST17, ST29, ST111, ST133, ST405, ST469, ST607, ST2375, and ST3216. Nevertheless, the consistent detection of widely circulating ARGs in surface water supports its role as a complementary surveillance matrix, particularly for population-level monitoring of dominant resistance trends. Surface water should therefore be considered an adjunct, rather than a substitute, within integrated One Health AMR surveillance frameworks.

4.3. Virulome of ESBL – K. pneumoniae across sources

None of the isolates carried *aerobactin* (*iuc*), *salmochelin* (*iro*), or the hypermucoidy regulators *rmpA/rmpA2*, confirming the absence of the hypervirulent *Klebsiella pneumoniae* (hvKp) pathotype across all sources. Virulence was instead dominated by yersiniabactin, with 82.3% of isolates carrying ICEKp-encoded ybt loci (virulence score = 1). This is consistent with a South African finding showing that multidrug-resistant clinical *K. pneumoniae* exhibit substantial capsular diversity but lack hypervirulence genes, reflecting predominantly classical lineage (Mbelle et al., 2020). These findings indicate a population in which virulence is primarily mediated by iron acquisition rather than hypervirulence-associated plasmids. However, variation in yersiniabactin lineages and capsular backgrounds across sources indicates that the virulome is not uniform and remains source-structured. Similar patterns have been reported elsewhere, where overlap between clinical and environmental *K. pneumoniae* populations coexists with uneven distribution of virulence determinants, with yersiniabactin widespread while *iuc*, *iro*, and *clb* are largely restricted to clinical lineages (Rocha et al., 2022).

The strongest inter-source relationship was observed between surface water and healthy resident isolates, driven primarily by ST25/KL2/*ybt14*; *ICEKp5* and, to a lesser extent, ST219/KL114/*ybt14*; *ICEKp5*, whereas overlap between surface water and clinical isolates was limited. Given that ST25 is a clinically documented (Hetsa et al., 2025; Olwagen et al., 2025; Zhao et al., 2022) but epidemiologically heterogeneous lineage, its dominance in surface water supports the interpretation that aquatic isolates capture broader community-associated backgrounds rather than hospital-enriched populations. This interpretation is reinforced by the near-universal detection of virulence-positive isolates in surface water (96.9%), coupled with markedly reduced diversity and evenness relative to healthy residents. Together, these patterns are consistent with clonal enrichment of a limited number of successful lineages in the aquatic environment, rather than broad representation of the underlying human virulome.

Source-restricted lineages were concentrated in clinical and healthy resident isolates, while surface water showed minimal exclusivity. The significantly lower diversity observed in surface water compared to healthy residents indicates that, although dominant community-associated lineages are captured, the broader virulome diversity present in the human population is not. Consequently, surface water does not provide a comprehensive proxy for either the clinical or the community virulome composition. Rather, it reflects a restricted subset of circulating lineages, with stronger alignment to community-associated backgrounds. Surface water surveillance may therefore be informative for detecting dominant or emerging lineages but should be interpreted as complementary to, rather than a substitute for, direct human sampling within integrated One Health surveillance frameworks.

This study has some limitations to consider when interpreting findings. First, sampling one informal settlement and one river catchment limits generalisability. Findings are thus most applicable to settings with comparable hydrology, sanitation, land use, climate, and healthcare connectivity. Future studies should include multiple communities and river catchments spanning contrasting conditions to test whether these patterns hold more broadly. Second, all 32 available clinical genomes were analysed, whereas only balanced, month-stratified subsets of 32 isolates were selected from the larger surface-water and healthy-resident isolate pools. Although this improved comparability, the subsets may not have captured the full genomic diversity present in the larger pools, and alternative random subsets could have produced different estimates of source overlap and diversity, particularly for rare sequence types, low-frequency resistance or virulence genes, and source-restricted features. Future studies should include larger isolate numbers, repeated resampling, and extended seasonal coverage to assess the stability of the findings, formally evaluate temporal changes in resistance genes and sequence types, and determine the plasmid or chromosomal location and genetic context of key resistance determinants. Thirdly, isolate-level genotype-phenotype concordance was not assessed, limiting evaluation of how reliably detected resistance genes predicted phenotypic resistance. Future studies should integrate paired antimicrobial susceptibility and genomic analyses to quantify this concordance. Finally, livestock, wildlife, agricultural, stormwater, and other environmental inputs were not sampled, precluding formal source attribution. Parallel sampling of these inputs, combined with hydrological and microbial source tracking, would strengthen attribution, and operational research should compare cost, turnaround time, sensitivity, and surveillance value.

5. Conclusion

This study evaluated whether surface water can serve as an alternative matrix for human AMR surveillance by comparing the resistome, virulome, and phylogenetic relatedness of ESBL-producing *K. pneumoniae* from surface water, healthy residents, and clinical sources. The findings show that surface water can provide useful surveillance information on ESBL-producing *K. pneumoniae* circulating within the study setting, particularly by detecting dominant community-associated lineages and commonly shared resistance determinants. However, surface water did not fully represent the genomic, resistance, or virulence diversity observed among healthy-resident and clinical isolates.

Surface-water isolates were more closely aligned with healthy-resident isolates than with clinical isolates, indicating that surface water in this setting more closely reflected community-associated carriage than clinical infection. Although shared resistance and virulence features were detected across sources, source-specific lineages, resistance determinants, and virulence profiles were underrepresented or absent in surface water.

Surface water may therefore provide a useful complementary surveillance matrix in communities where sewer-based wastewater surveillance is unavailable and community-level AMR data are limited. Its findings should be interpreted alongside clinical and community surveillance data within an integrated One Health AMR surveillance framework.

6. Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

Generative AI and AI-assisted writing tools, including ChatGPT and Grammarly Premium, were used to support language editing, improve clarity and coherence of writing, assist with refining, summarise and clarify complex scientific concepts. Generative AI was not used for data generation, statistical analysis or reference management. All AI-assisted content was critically reviewed, edited, and verified for scientific accuracy, relevance, and consistency with the study findings.

CRediT authorship contribution statement

Etando Ayukafangha: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Joshua Mbanga:** Writing – review & editing,

Writing – original draft, Visualization, Validation, Supervision, Methodology, Data curation. **Arshad Ismail:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis, Data curation. **Sabiha Y. Essack:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Akebe Luther King Abia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

S.Y.E. is a chairperson of the Global Respiratory Partnership and a member of the Global Hygiene Council, both supported by unrestricted educational grants from Reckitt (Pty.) Ltd., UK. All other authors have no competing interests.

All authors have read, understood, and complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2026.100744.

Data availability

Data will be made available on request.

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List of Supplementary Materials (Manuscript 4)

- **Supplementary Material/S1** – Ethical Consideration and Informed Consent; Refer to Appendices 1.1 to 1.4 of this Thesis. **Page 284 - 287.**
- Supplementary Material/S2 - Assembly metrics for the sequenced *K. pneumoniae* isolates. **Pages 263 - 264.**
- Supplementary Material/S3 - Distribution of ARGs and chromosomal point mutations of ESBL *K. pneumoniae* isolates across sources. **Page 265 - 267.**
- Supplementary Material/S4: Distribution of Resistant Mechanisms and AMR Gene Families of ESBL *K. pneumoniae* isolates across sources. **Page 268.**
- Supplementary Material/S5: ESBL *K. pneumoniae* lineages associated with the virulence determinants across sources. **Page 269**
- **Others:** Extended phylogeny of ESBL *K. pneumoniae* and 100 genomes from ten LMIC African countries **Page 270**

Supplementary Material/S2 - Assembly metrics for the sequenced *K. pneumoniae* isolates.

Isolate Source	Study_Isolate I	BV-BRC Genom	NCBI Genome /	NCBI Biosampl	MLST	Contigs	Size	GC Content	Contig L50	Contig N50	TRNA	RRNA	CDS	Coarse Consist	Fine Consisten
Clinical	CAKP1	573.90325	JBNFSO000000	SAMN4807225	ST983	58	5312597	57.388504	5	358594	76	9	5165	99.5	98.1
Clinical	CAKP4	573.90326	JBQZGU000000	SAMN5124227	ST983	44	5300013	57.43305	4	473765	77	5	5157	99.5	98.1
Clinical	CDKP2	573.90327	JBQZGN000000	SAMN5124228	ST983	99	5596750	57.510735	4	456063	77	2	5507	99.3	94
Clinical	CMKP2	573.90328	JBNFSU000000	SAMN4807226	ST111	79	5520810	57.18983	9	252493	76	8	5507	99.4	97.9
Clinical	COKP1	573.90329	JBQZGQ000000	SAMN5124227	ST983	39	5300890	57.435467	3	473622	77	4	5146	99.5	98.2
Clinical	CSKP4	573.90330	JBQZGT000000	SAMN5124227	ST152	95	5383441	57.453644	11	187983	78	5	5274	99.1	97.9
Clinical	CSKP5	573.90331	JBQZGS000000	SAMN5124227	ST152	101	5383779	57.45667	11	180172	77	6	5281	99.1	97.9
Clinical	CYKP1	573.90332	JBNFSZ000000	SAMN4807226	ST152	103	5392362	57.430325	10	180171	79	6	5285	99.2	97.9
Clinical	CYKP3	573.90333	JBNFTB000000	SAMN4807226	ST152	106	5389891	57.437767	11	180191	79	7	5299	99.2	97.9
Clinical	CYKP2	573.90334	JBNFTA000000	SAMN4807226	ST152	102	5380792	57.456577	11	180182	78	6	5280	99.2	98
Clinical	CYKP4	573.90335	JBNFTC000000	SAMN4807226	ST2375	65	5398673	57.236805	8	284651	78	4	5374	99.2	97.6
Clinical	CNKP1	573.90399	Pending (@NCI	SAMN5124228	ST-	96	5507921	57.36709	10	226482	80	6	5462	99.2	98.2
Clinical	CSKP1	573.90400	JBNFSW000000	SAMN4807226	ST983	41	5298966	57.43145	4	477310	76	8	5153	99.4	98.1
Clinical	CAKP2	573.90663	JBNFSP000000	SAMN4807225	ST983	51	5313189	57.396717	4	473562	77	7	5169	99.5	98.2
Clinical	CAKP3	573.90664	JBNFSQ000000	SAMN4807225	ST983	52	5308474	57.390316	4	456023	76	8	5160	99.5	98.2
Clinical	CJKP1	573.90665	JBQZGZ000000	SAMN5124227	ST29	66	5387418	57.369392	6	298400	78	7	5299	99.5	98.6
Clinical	CJKP2	573.90666	JBNFSR000000	SAMN4807225	ST983	54	5315242	57.39814	4	456479	77	11	5157	99.5	98.2
Clinical	CJKP4	573.90667	JBQZGY000000	SAMN5124227	ST983	39	5299311	57.43252	4	455366	75	5	5153	99.5	98.1
Clinical	CJKP5	573.90668	JBQZGX000000	SAMN5124227	ST983	42	5299943	57.43292	4	473579	77	4	5159	99.5	98.1
Clinical	CDKP1	573.90669	JBQZGO000000	SAMN5124228	ST152	99	5429460	57.443245	11	180191	78	3	5350	99.3	97.1
Clinical	CJKP3	573.90670	JBNFSS000000	SAMN4807225	ST983	46	5300232	57.43141	5	358698	77	5	5150	99.5	98.2
Clinical	CMKP1	573.90671	JBNFST000000	SAMN4807225	ST983	45	5306919	57.41659	4	456023	77	7	5164	99.5	98.2
Clinical	CMKP3	573.90672	JBNFSV000000	SAMN4807226	ST-	199	7749410	57.481884	16	133221	79	11	9124	98.7	57.8
Clinical	CNKP2	573.90673	Pending (@NCI	SAMN5124228	ST469	198	6429097	57.809097	9	267851	90	14	6427	97.5	81
Clinical	COKP2	573.90674	JBQZGQ000000	SAMN5124227	ST-	115	6143624	56.38063	589	2270	73	2	9500	98.3	64.8
Clinical	CMKP5	573.90676	Pending (@NCI	SAMN5124226	ST29	73	5386922	57.371277	6	298509	78	7	5305	99.5	98.6
Clinical	COKP3	573.90677	JBNFSY000000	SAMN4807226	ST152	100	5384320	57.455948	11	164064	78	7	5289	99.2	97.9
Clinical	CSKP2	573.90678	JBNFSX000000	SAMN4807226	ST983	51	5313853	57.392876	5	455716	77	6	5166	99.5	98.1
Clinical	CYKP5	573.90679	JBQZGW000000	SAMN5124227	ST405	64	5644921	57.03913	6	288621	77	9	5546	99.6	98
Clinical	CSKP3	573.90680	JBNFSY000000	SAMN4807226	ST983	42	5301632	57.435257	4	477310	77	6	5157	99.5	98.4
Clinical	CYKP6	573.90681	JBQZGV000000	SAMN5124227	ST405	69	5654594	57.026657	7	294902	77	7	5551	99.6	97.9
Clinical	CMKP6	573.91124	Pending (@NCI	SAMN5124226	ST983	38	5299748	57.43339	4	473562	78	4	5159	99.4	98
Healthy Reside	L06YKP	573.90312	JBQZOJ000000	SAMN5124207	ST25	121	5500861	57.176414	16	117269	79	10	5472	99.5	97.7
Healthy Reside	L14AKP	573.90313	JBQZOC000000	SAMN5124208	ST219	65	5525910	57.10922	7	325342	77	7	5463	99.6	98.2
Healthy Reside	L42OKP	573.90316	JBQZNB000000	SAMN5124211	ST25	109	5504584	57.18016	15	136040	79	10	5465	99.4	97.9
Healthy Reside	L32SKP	573.90317	JBQZNL000000	SAMN5124210	ST-	97	5508270	57.366795	10	226482	80	6	5463	99.2	98.2
Healthy Reside	L72DKP	573.90596	JBQZLX000000	SAMN5124214	ST983	41	5299637	57.43269	3	473597	77	4	5151	99.5	98.2
Healthy Reside	L70DKP	573.90600	JBQZLZ000000	SAMN5124213	ST219	68	5524469	57.110214	9	178315	74	4	5469	99.5	98.2
Healthy Reside	L71DKP	573.90601	JBQZLY000000	SAMN5124213	ST17	65	5632696	57.093212	4	427403	76	9	5592	99.6	97.8
Healthy Reside	L63NKP	573.90607	JBQZMG000000	SAMN5124213	ST607	76	5521096	57.264137	8	271865	76	8	5446	99.3	98.2
Healthy Reside	L65DKP	573.90609	JBQZME000000	SAMN5124213	ST983	46	5299282	57.433044	5	396893	77	6	5157	99.5	98.2
Healthy Reside	L64NKP	573.90610	JBQZMF000000	SAMN5124213	ST983	43	5301582	57.434063	3	473985	77	5	5157	99.5	98.2
Healthy Reside	L51OKP	573.90612	JBQZMS000000	SAMN5124211	ST25	106	5503475	57.180798	14	136636	78	11	5463	99.4	98
Healthy Reside	L54OKP	573.90613	JBQZMP000000	SAMN5124212	ST17	75	5629918	57.091595	8	288549	75	10	5596	99.6	97.7
Healthy Reside	L53OKP	573.90615	JBQZMQ000000	SAMN5124212	ST25	107	5507009	57.178116	15	133705	80	13	5460	99.4	97.9
Healthy Reside	L56OKP	573.90616	JBQZMN000000	SAMN5124212	ST17	73	5630767	57.09446	6	305423	76	9	5603	99.6	97.8
Healthy Reside	L55OKP	573.90618	JBQZMO000000	SAMN5124212	ST25	114	5504090	57.178555	15	133705	79	10	5471	99.4	97.9
Healthy Reside	L47OKP	573.90622	JBQZMW000000	SAMN5124211	ST29	75	5386120	57.36831	7	270290	78	8	5310	99.6	98.7
Healthy Reside	L40OKP	573.90626	JBQZND000000	SAMN5124210	ST607	80	5531715	57.26799	8	263061	76	8	5439	99.6	98.3
Healthy Reside	L35OKP	573.90630	JBQZNI000000	SAMN5124210	ST29	77	5385966	57.366962	8	232597	78	7	5310	99.5	98.6
Healthy Reside	L36OKP	573.90631	JBQZNH000000	SAMN5124210	ST133	71	5584660	57.2497	10	237572	77	9	5492	99.5	98.1
Healthy Reside	L28SKP	573.90635	JBQZNP000000	SAMN5124209	ST17	82	5629303	57.094616	6	327861	76	9	5598	99.6	97.6
Healthy Reside	L19SKP	573.90641	Pending (@NCI	SAMN5124208	ST3216	233	5497255	57.39943	10	179176	79	5	5574	99.4	95.9
Healthy Reside	L24SKP	573.90645	JBQZNT000000	SAMN5124209	ST29	73	5413107	57.23382	5	376126	78	9	5362	99.5	98.6

Healthy Reside	L26SKP	573.90646	JBQZNR000000	SAMN5124209	ST133	77	5584171	57.24837	8	305259	77	8	5489	99.5	98.2
Healthy Reside	L09YKP	573.90647	JBQZOG000000	SAMN5124207	ST983	45	5299206	57.432587	5	455543	77	4	5160	99.5	98.1
Healthy Reside	L11AKP	573.90649	JBQZOE000000	SAMN5124207	ST25	104	5504500	57.179436	14	136040	79	10	5468	99.4	98
Healthy Reside	L15AKP	573.90652	JBQZOB000000	SAMN5124208	ST983	43	5300866	57.433956	5	408443	77	6	5151	99.5	98.1
Healthy Reside	L02MKP	573.90655	JBQZON000000	SAMN5124207	ST983	43	5300573	57.432377	4	473644	77	6	5157	99.5	98.1
Healthy Reside	L01MKP_B13_E	573.90657	JBQZOO000000	SAMN5124206	ST607	70	5378671	57.40764	11	175783	77	9	5255	99.6	98.5
Healthy Reside	L03JKP	573.90658	JBQZOM000000	SAMN5124207	ST607	77	5533098	57.266743	8	258544	77	8	5446	99.6	98.2
Healthy Reside	L07YKP	573.90659	JBQZOI000000	SAMN5124207	ST29	85	5464327	57.088512	6	298400	78	7	5443	99.5	98.6
Healthy Reside	L04JKP	573.90660	JBQZOL000000	SAMN5124207	ST25	125	5499157	57.17498	19	101480	76	11	5481	99.4	97.9
Healthy Reside	L08YKP	573.90661	JBQZOH000000	SAMN5124207	ST607	73	5377887	57.406727	12	150804	77	7	5260	99.5	98.5
Surface Water	AOA9KP	573.90321	JBNFSL000000	SAMN4807225	ST25	110	5503665	57.178715	14	136042	79	9	5467	99.5	98.1
Surface Water	AOA18KP	573.90322	JBNFSM000000	SAMN4807225	ST25	108	5504674	57.179646	14	134480	79	10	5471	99.4	98
Surface Water	OEC18KP	573.90324	JBQZII000000	SAMN5124223	ST25	100	5504458	57.179146	14	136635	77	9	5469	99.5	98
Surface Water	YOB7KP	573.90556	JBQZIP000000	SAMN5124222	ST25	94	5503523	57.18041	13	141143	79	9	5465	99.4	98
Surface Water	YOB6KP	573.90557	JBNFTK000000	SAMN4807227	ST25	108	5502980	57.17998	14	135524	77	9	5464	99.4	98
Surface Water	SEC16KP	573.90558	JBNFTE000000	SAMN4807227	ST25	102	5504196	57.180122	13	141830	77	10	5463	99.4	97.9
Surface Water	SED8-KP	573.90560	JBNFTG000000	SAMN4807227	ST25	110	5507618	57.16987	13	141143	79	10	5479	99.4	98
Surface Water	SOB8-KP	573.90561	JBNFTH000000	SAMN4807227	ST1380	171	5662576	57.11007	13	127307	81	6	5732	99.3	98.2
Surface Water	SOB17-KP	573.90562	JBNFTI000000	SAMN4807227	ST25	106	5504684	57.17834	13	141830	79	10	5468	99.5	98
Surface Water	YEC16-KP	573.90563	JBNFTJ000000	SAMN4807227	ST25	107	5503993	57.179344	14	135530	78	10	5472	99.4	98
Surface Water	NOB9KP	573.90564	JBQZIC000000	SAMN5124223	ST219	59	5430347	57.21207	9	220400	76	7	5334	99.6	98.4
Surface Water	YED1KP	573.90565	JBQZIO000000	SAMN5124222	ST25	100	5503300	57.180965	14	136635	79	10	5459	99.5	98.1
Surface Water	OEC1-KP	573.90566	JBNFTD000000	SAMN4807226	ST152	95	5378958	57.455997	10	191572	79	6	5268	99.3	98.1
Surface Water	OEC10KP	573.90567	JBQZIK000000	SAMN5124223	ST25	100	5505477	57.17997	14	136042	79	10	5467	99.4	98
Surface Water	OEC9KP	573.90568	JBQZIL000000	SAMN5124223	ST25	99	5505286	57.1801	13	141830	77	9	5465	99.5	98.1
Surface Water	OED18KP	573.90570	JBQZIG000000	SAMN5124223	ST25	103	5504803	57.17905	14	136635	78	11	5466	99.4	98
Surface Water	MOA13KP	573.90571	JBQZIV000000	SAMN5124222	ST983	197	5481873	57.454285	4	473805	80	7	5473	99.1	95.6
Surface Water	NEC9KP	573.90572	JBQZIB000000	SAMN5124224	ST25	97	5503369	57.182446	13	141143	79	9	5457	99.4	98
Surface Water	NOA12KP	573.90574	JBQZIF000000	SAMN5124223	ST219	58	5428841	57.212986	9	223368	75	6	5337	99.6	98.2
Surface Water	NOB5KP	573.90576	JBQZIE000000	SAMN5124223	ST219	57	5429465	57.213444	8	264472	76	5	5330	99.6	98.3
Surface Water	NOB6KP	573.90577	JBQZID000000	SAMN5124223	ST25	100	5504038	57.18029	14	136636	79	9	5464	99.5	98.1
Surface Water	JED7KP	573.90579	JBQZIQ000000	SAMN5124222	ST219	59	5429206	57.214077	8	262029	76	6	5323	99.6	98.3
Surface Water	MED10KP	573.90580	JBQZIU000000	SAMN5124222	ST983	43	5300573	57.432377	4	473644	77	6	5157	99.5	98.1
Surface Water	DOB7KP	573.90581	JBQZHU000000	SAMN5124224	ST25	98	5506538	57.1795	13	141143	79	10	5461	99.4	98
Surface Water	DOB17KP	573.90585	JBQZHQ000000	SAMN5124225	ST25	91	5504055	57.180405	12	146139	79	9	5463	99.5	98.1
Surface Water	JEC5KP	573.90586	JBQZIT000000	SAMN5124222	ST25	98	5504180	57.18147	13	141143	78	9	5465	99.4	98
Surface Water	JEC12KP	573.90587	JBQZIS000000	SAMN5124222	ST25	97	5503523	57.181282	13	141143	79	8	5469	99.4	98
Surface Water	DOB6KP	573.90588	JBQZHV000000	SAMN5124224	ST25	98	5505408	57.180176	12	141143	79	9	5470	99.4	97.9
Surface Water	DOA10KP	573.90591	JBQZHW000000	SAMN5124224	ST25	99	5502575	57.181065	14	136635	78	9	5462	99.4	98
Surface Water	AEC9-KP	573.90592	JBNFSH000000	SAMN4807224	ST25	107	5505506	57.177578	13	136042	78	10	5464	99.5	98.1
Surface Water	AED18KP	573.90597	JBQZIN000000	SAMN5124222	ST25	99	5505171	57.180096	13	141143	79	9	5472	99.6	98.2
Surface Water	DOA6KP	573.90599	JBQZHZ000000	SAMN5124224	ST25	98	5505688	57.181282	12	141830	79	9	5462	99.5	98.1
Reference Gen	Klebsiella pneu	1125630.4	BV-BRC Refere	BV-BRC Refere	ST11	7	5682322	57.12	1	5333942	88	25	5523	99.5	96.6

Supplementary Material/S3 - Distribution of ARGs and chromosomal point mutations of ESBL *K. pneumoniae* isolates across sources.

Isolate_ID	Sources	Disinfectant	Point Mutaton Gene	Mutation_type
AEC9-KP	Surface_Water			
AED18KP	Surface_Water		acrR; ompK36; ompK37	Known
AOA18-KP	Surface_Water		acrR; ompK36; ompK37	Known
AOA9-KP	Surface_Water		acrR; ompK36; ompK37	Known
CAKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CAKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CAKP3	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CAKP4	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CDKP1	Clinical	OqxB; OqxA	ompK36; ompK37; gyrA; parC	Known
CDKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP3	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP4	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP5	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP3	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP5	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP6	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CNKP1	Clinical	qacE	acrR; ompK36; ompK37	Known
CNKP2	Clinical	OqxB; OqxA; qacE	acrR; ompK36; ompK37	Known
COKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
COKP2	Clinical	OqxB	acrR; ompK36; ompK37	Known
COKP3	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CSKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CSKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CSKP3	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CSKP4	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CSKP5	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CYKP1	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CYKP2	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CYKP3	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CYKP4	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CYKP5	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CYKP6	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
DOA10KP	Surface_Water			
DOA6KP	Surface_Water		acrR; ompK36; ompK37	Known
DOB17-KP	Surface_Water		acrR; ompK36; ompK37	Known
DOB6KP	Surface_Water		acrR; ompK36; ompK37	Known
DOB7KP	Surface_Water		acrR; ompK36; ompK37	Known
Isolate_ID		Resistance gene		
JEC12KP	Surface_Water		acrR; ompK36; ompK37	Known
JEC5KP	Surface_Water		acrR; ompK36; ompK37	Known
JED7KP	Surface_Water	OqxB; OqxA	acrR; ompK36; ompK37	Known
L01MKP_B13_B75	Healthy_Residents	OqxB; OqxA	acrR; ompK36; ompK37	Known
L02MKP	Healthy_Residents			

Isolate_ID	Sources	Disinfectant	Point Mutaton Gene	Mutation_type
AEC9-KP	Surface_Water			
AED18KP	Surface_Water		acrR; ompK36; ompK37	Known
AOA18-KP	Surface_Water		acrR; ompK36; ompK37	Known
AOA9-KP	Surface_Water		acrR; ompK36; ompK37	Known
CAKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CAKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CAKP3	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CAKP4	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CDKP1	Clinical	OqxB; OqxA	ompK36; ompK37; gyrA; parC	Known
CDKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP3	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP4	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CJKP5	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP3	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP5	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CMKP6	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CNKP1	Clinical	qacE	acrR; ompK36; ompK37	Known
CNKP2	Clinical	OqxB; OqxA; qacE	acrR; ompK36; ompK37	Known
COKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
COKP2	Clinical	OqxB	acrR; ompK36; ompK37	Known
COKP3	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CSKP1	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CSKP2	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CSKP3	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CSKP4	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CSKP5	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CYKP1	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CYKP2	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CYKP3	Clinical	OqxB; OqxA; qacE	ompK36; ompK37; gyrA; parC	Known
CYKP4	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CYKP5	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
CYKP6	Clinical	OqxB; OqxA	acrR; ompK36; ompK37	Known
DOA10KP	Surface_Water			
DOA6KP	Surface_Water		acrR; ompK36; ompK37	Known
DOB17-KP	Surface_Water		acrR; ompK36; ompK37	Known
DOB6KP	Surface_Water		acrR; ompK36; ompK37	Known
DOB7KP	Surface_Water		acrR; ompK36; ompK37	Known
Isolate_ID		Resistance gene		
JEC12KP	Surface_Water		acrR; ompK36; ompK37	Known
JEC5KP	Surface_Water		acrR; ompK36; ompK37	Known
JED7KP	Surface_Water	OqxB; OqxA	acrR; ompK36; ompK37	Known
L01MKP_B13_B75	Healthy_Residents	OqxB; OqxA	acrR; ompK36; ompK37	Known
L02MKP	Healthy_Residents			

YOB7KP	Surface_Water		acrR; ompK36; ompK37	Known
YOB8-KP	Surface_Water		acrR; ompK36; ompK37	Known

Supplementary Material/S4: Distribution of Resistant Mechanisms and AMR Gene Families of ESBL *K. pneumoniae* isolates across sources.

Table 1. Distribution of mechanism across sources.

S/N	Sources	Mechanism	Isolat	Sourc	Proportion
1	Clinical	antibiotic efflux	32	32	100
2	Clinical	antibiotic inactivation	32	32	100
3	Clinical	antibiotic target alteration	32	32	100
4	Clinical	reduced permeability to antibic	32	32	100
5	Clinical	antibiotic target replacement	28	32	87.5
6	Clinical	antibiotic target protection	25	32	78.12
7	Healthy_residen	antibiotic efflux	32	32	100
8	Healthy_residen	antibiotic inactivation	32	32	100
9	Healthy_residen	antibiotic target alteration	32	32	100
10	Healthy_residen	reduced permeability to antibic	32	32	100
11	Healthy_residen	antibiotic target replacement	30	32	93.75
12	Healthy_residen	antibiotic target protection	17	32	53.12
13	Surface_water	antibiotic efflux	32	32	100
14	Surface_water	antibiotic inactivation	32	32	100
15	Surface_water	antibiotic target alteration	32	32	100
16	Surface_water	reduced permeability to antibic	32	32	100
17	Surface_water	antibiotic target replacement	27	32	84.38
18	Surface_water	antibiotic target protection	2	32	6.25

Shannon Diversity Indices

	source	S	H	E
1	Clinical	6	1.53	0.86
2	Healthy_residents	6	1.54	0.86
3	Surface_water	6	1.47	0.82

Chi Square Stats

Statistic	DF	²_value
3.434966475	10	0.97

Figure 3 (B) and 3 (C) - Distribution of AMR Gene Families

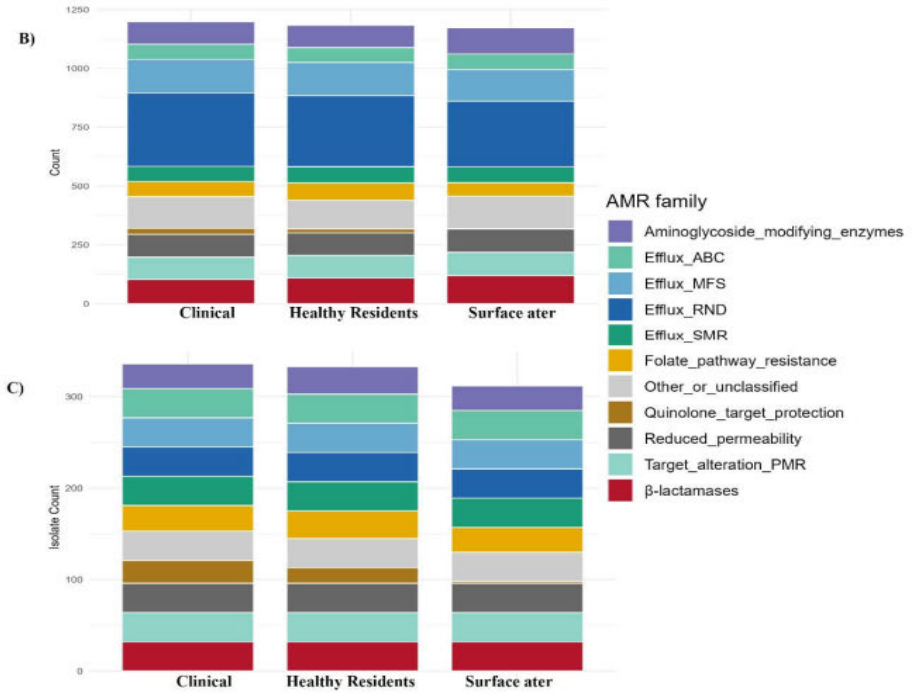
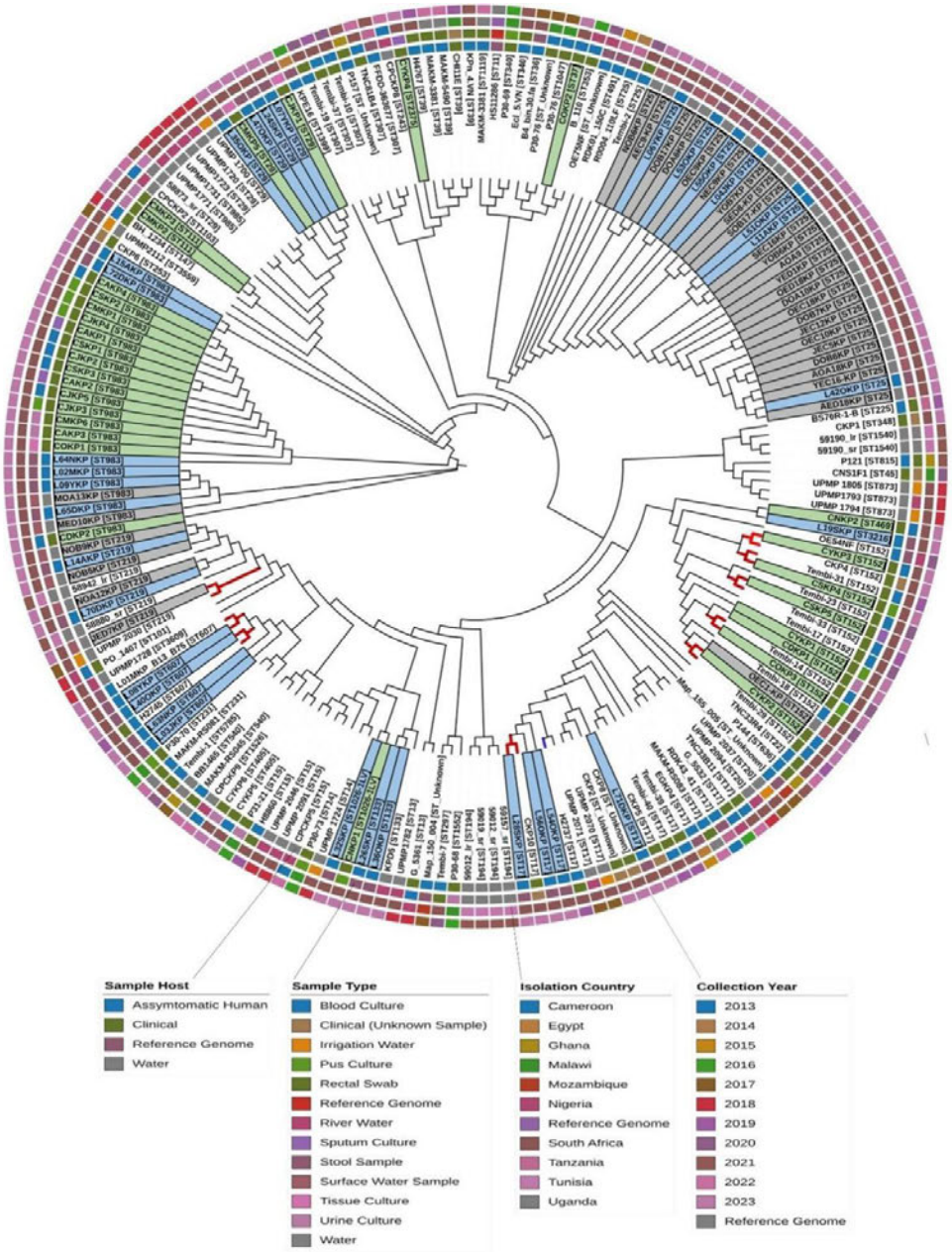


Figure 3B. Showing distribution of AMR gene family distribution (gene based) across sources.
Figure 3C. Showing distribution of AMR gene family distribution (isolate count based) across sources. Each bar stack represents the total count of AMR genes (A) or isolate (B) per source, segmented by gene family. Efflux-related families (RND, MFS, ABC, SMR) were predominant across all sources, with Clinical isolates showing the highest overall gene count. Quinolone_target_protection family genes were least represent with least seen among surface water isolates

Supplementary Material/S5: ESBL *K. pneumoniae* lineages associated with the virulence determinants across sources.

sources	ST	KL_WZI	No of Isolates	Proportion
Surface Water	ST25	KL2/wzi72	24	0.75
Clinical	ST983	KL127/wzi202	15	0.46875
Clinical	ST152	KL149/wzi110	7	0.21875
Healthy Residents	ST25	KL2/wzi72	7	0.21875
Healthy Residents	ST983	KL127/wzi202	6	0.1875
Healthy Residents	ST607	KL25/wzi133	5	0.15625
Healthy Residents	ST17	KL25/wzi141	4	0.125
Surface Water	ST219	KL114/wzi177	4	0.125
Healthy Residents	ST29	KL30/wzi85	4	0.125
Clinical	ST111	KL63/wzi63	2	0.0625
Healthy Residents	ST133	KL116/wzi180	2	0.0625
Healthy Residents	ST219	KL114/wzi177	2	0.0625
Clinical	ST29	KL30/wzi85	2	0.0625
Clinical	ST405	KL151/wzi143	2	0.0625
Surface Water	ST983	KL127/wzi202	2	0.0625
Clinical	ST1026-1L	KL8/wzi8	1	0.03125
Healthy Residents	ST1026-1L	KL8/wzi8	1	0.03125
Surface Water	ST1380	KL155/wzi173	1	0.03125
Surface Water	ST152	KL149/wzi110	1	0.03125
Clinical	ST2375	KL14/wzi23	1	0.03125
Healthy Residents	ST3216	KL16/-	1	0.03125
Clinical	ST37	KL7/wzi7	1	0.03125
Clinical	ST469	KL139/wzi493	1	0.03125

Others: Extended phylogeny of ESBL *K. pneumoniae* and 100 genomes from ten LMIC African countries **Page 270**



Extended phylogeny of our isolates and 100 genomes from ten LMIC African countries. Isolate from current study are highlighted in light green (clinical), light blue (healthy residents) and grey (surface water). Close clustering between our isolates and others is indicated with red nodes while circular colour strips (left (inside) to right (outside) represent metadata linked to the isolates as per the legend. The extended phylogeny shows broad intermixing of current-study isolates with publicly available African genomes, with several close clustering events indicating relatedness between local isolates and regionally circulating lineages across clinical, community, and environmental sources.

CHAPTER SIX: CONCLUSION, LIMITATIONS, RECOMMENDATIONS AND SIGNIFICANCE

6.1 Conclusion

The following conclusions were reached based on the study objectives.

6.1.1 Objective 1 - To determine the phenotypic AMR profiles of ESBL-producing *E. coli* and *K. pneumoniae* isolates recovered from surface water, healthy community residents, and clinical isolates.

a) Phenotypic AMR profiles of ESBL-producing *E. coli*

- ESBL-producing *E. coli* was detected across all three sources, confirming phenotypic co-occurrence. However, the prevalence differed significantly, It was significantly ($p < 0.05$) highest among healthy residents (27.1%), followed by surface water (16.8%), and lowest among clinical isolates (5.3%).
- Surface-water ESBL *E. coli* isolates showed closer phenotypic resemblance to healthy-resident isolates, sharing resistance profiles across ampicillin, cephalosporins, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, and piperacillin-tazobactam compared to clinical isolates, where similarity was limited to ampicillin, cephalosporins, and trimethoprim-sulfamethoxazole alone.
- Antibiogram overlap was limited across sources. Out of 44 distinct patterns, only two were shared across all three compartments, and no MDR pattern was shared exclusively between isolates from surface water and either of the human compartments.
- Multidrug resistance was detected across all three sources but at markedly different ($p < 0.05$) proportions, highest among clinical isolates (64.5%), followed by healthy residents (41.6%), and lowest in surface-water isolates (5.7%).

b) Phenotypic AMR Profiles of ESBL-producing *K. pneumoniae*

- ESBL-producing *K. pneumoniae* co-occurred across all three compartments at significantly different proportions ($p < 0.05$): clinical isolates (15.2%), surface water (13.0%) and healthy residents (6.3%).
- Surface-water ESBL-producing *K. pneumoniae* isolates showed greater antibiotic-resistance similarity to healthy-resident compared to clinical isolates.
- Surface water and healthy residents shared profiles for amoxicillin-clavulanic acid, piperacillin-tazobactam, cephalosporins, carbapenems, gentamicin, and ciprofloxacin ($p> 0.05$), differing only for tobramycin, trimethoprim-sulfamethoxazole, and tigecycline ($p < 0.05$), whereas surface water–clinical concordance was narrower and limited to cephalosporins, carbapenems, amikacin, and tigecycline, with significant divergence across amoxicillin-clavulanic acid, piperacillin-tazobactam, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole ($p< 0.05$)
- Antibiogram analysis identified 31 distinct patterns, of which 18 were MDR, with surface-water isolates sharing more patterns with healthy-resident than with clinical isolates.
- Four MDR patterns were shared between isolates from surface water and healthy residents, no MDR pattern was shared between surface water and clinical isolates, and only one non-MDR pattern was common to all three sources with most antibiogram patterns remaining source-specific.
- Multidrug-resistant ESBL-producing *K. pneumoniae* was detected across all three sources at markedly different proportions, highest among clinical isolates (94.6%), followed by healthy residents (41.9%), and lowest among surface-water isolates (25.0%) ($p < 0.001$)

6.1.2 Objective 2: To investigate the genomic concordance of AMR profiles of ESBL-producing *E. coli* and *K. pneumoniae* isolates from surface water to isolates from healthy residents and clinical facility through analysis of the resistome, virulome, and phylogeny.

a) ESBL-producing *E. coli* Genomic AMR Profiles

- Surface-water ESBL *E. coli* captured part of the community and clinical resistome but was more closely associated with isolates from healthy residents than with clinical isolates. Forty-five ARGs were detected, including nine core-shared ARGs: *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1B}}$, *aadA5*, *qnrS*1, *mph(A), sul1*, *sul*2, *dfrA*17, and *dfrA5*. ARGs observed in isolates from surface water and healthy residents included *bla*$_{\text{CTX-M-27}}$, *bla*$_{\text{OXA-1}}$, *oqxA/B*, *tetA/B*, and *dfrA*1, while no ARGs were observed in both surface water and clinical isolates.
- Although 285 virulence genes were widely distributed across sources, diversity and pathotype structure differed significantly ($p < 0.05$). Fifty-four virulence genes were only observed in isolates from healthy residents, five were only observed in surface water isolates, with none exclusive to clinical isolates despite their pathotype complexity, including hybrid pathotypes (EPEC + UPEC + NMEC), while surface-water isolates were dominated by a single pathotype (EPEC), albeit still carrying selected clinically relevant virulence signatures.
- MLST and phylogenomic analyses identified three core-shared phylogroups (A, B1, and D), with phylogroup B2 observed in isolates from both surface water and healthy residents and none observed in isolates from both surface water and clinical isolates.
- Among 29 STs, ST10 and ST38 were shared across all sources, ST131, ST69, and ST757 were observed in isolates from surface water and healthy residents, and ST13823 in surface water and clinical isolates with phylogeny largely ST-driven

rather than source-structured, and a small subset showing closer surface water–healthy-resident than surface water-clinical clustering.

b) ESBL-producing *K. pneumoniae* Genomic AMR Profiles

- Surface-water ESBL-producing *K. pneumoniae* captured a substantial but incomplete part of the shared resistome, with overlap stronger with healthy residents than with clinical isolates.
- Of the 79 ARGs detected, 27 were shared across all three sources, dominated by $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, *sul*2, and *dfrA*14, resistome composition differed significantly by source ($R^2 = 0.075$, $p = 0.003$), no ARG was exclusive to surface water isolates, and pairwise ARG sharing was greatest between clinical and healthy-resident isolates (n = 20), followed by surface water-healthy residents (n = 7), and lowest between surface water and clinical isolates (n = 2).
- Virulome analysis showed that surface water captured dominant shared virulence backgrounds but with lower diversity and evenness than healthy-resident and clinical isolates. Yersiniabactin-associated determinants were predominant across the dataset (79/96 isolates; virulence score = 1). The only background shared across all three sources was ST983/KL127/OL13/O13, the strongest surface water-healthy-resident overlap was driven by ST25/KL2 and ST219/KL114, and surface water-clinical overlap was limited to ST152/KL149
- MLST and phylogenomic analyses confirmed that surface-water isolates were more closely related to healthy resident than to clinical isolates. Of the 16 STs identified, only ST983 was shared across all three sources; ST25 and ST219 were observed in isolates from both surface water and healthy residents, and, ST152 in isolates from surface water and clinical isolates. Interestingly, 93.8% of surface-water isolates shared STs with healthy-resident isolates compared with only 9.4% with clinical isolates, with

phylogenomic clustering being lineage-driven rather than source-structured and surface-water isolates forming strongly supported subclades with isolates from healthy residents through ST25 and ST219. No direct surface water-clinical sister pair was detected. Several clinically important lineages, including ST405, ST37, ST111, ST469, and ST2375, were source-restricted, with two divergent lineages that were exclusively observed in clinical isolates.

6.1.3 Objective 3: To determine whether surface water is a feasible AMR monitoring proxy at phenotypic and genomic levels.

a) Phenotypic Conclusions Addressing the Feasibility of Surface Water as an AMR Monitoring Proxy

i) Surface water is phenotypically feasible as a co-occurrence surveillance matrix but does not proportionally reflect ESBL burden across human compartments.

- ESBL-producing *E. coli* and *K. pneumoniae* co-occurred in surface water, healthy-resident, and clinical isolates across both studies, confirming that surface water participates in the local AMR ecology and provides proof that environmentally based surveillance can detect the local circulation of clinically relevant resistant Enterobacterales. However, ESBL prevalence differed significantly across compartments for both species, with surface water neither consistently mirroring community nor clinical burden, suggesting that it cannot be relied upon as a quantitative proxy for ESBL carriage in human populations.

ii) Surface water provides only selective phenotypic concordance with human compartments, with stronger alignment to healthy residents than to clinical isolates.

- Across both organisms, surface-water isolates shared more antibiotic-specific resistance profiles and antibiogram patterns with healthy-resident than with clinical

isolates, suggesting that environmental AMR signatures are more reflective of community-level than healthcare-associated resistance. However, this concordance was partial, with resistance to aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole differing significantly between surface water and at least one human compartment, and the majority of antibiogram patterns remaining source-specific, further constraining the interpretive value of surface-water phenotypic data alone.

iii) Surface water consistently underestimates multidrug resistance burden, precluding its use as a standalone phenotypic proxy for AMR monitoring.

- MDR was the most pronounced area of divergence across both species, with surface-water isolates exhibiting the lowest proportions in both datasets, markedly below clinical isolates, where MDR reached 64.5% (*E. coli*) and 94.6% (*K. pneumoniae*) and no MDR antibiogram patterns were shared between surface water and clinical isolates for either species. This underestimation of the most clinically consequential resistance phenotype represents a fundamental limitation of surface water as a standalone surveillance proxy.
- Overall, the phenotypic evidence supports surface water as a complementary environmental surveillance matrix within a One Health framework capable of signalling AMR presence and capturing selected resistance profiles but insufficient as a standalone proxy for the prevalence, MDR burden, or full antibiogram diversity observed in community or clinical human compartments.

b) Genomic Conclusions Addressing the Feasibility of Surface Water as an AMR Monitoring Proxy

i) Surface water genomically captures dominant circulating resistance determinants shared across compartments, but with an incomplete and community-biased resistome that underrepresents the clinical ARG repertoire.

- Across both species, surface water harboured core shared ARGs including *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1B}}$, *sul2*, and *dfrA* variants which were distributed across all three compartments, confirming its capacity to detect dominant circulating resistance determinants.
- However, resistome overlap was consistently stronger between isolates from surface water and healthy residents than with clinical isolates. For *E. coli*, ARGs observed in both surface water and healthy residents (e.g., *bla*$_{\text{CTX-M-27}}$, *oqxA/B*, tetA/B) had no surface water-clinical equivalent, while for *K. pneumoniae,* pairwise ARG sharing was greatest between clinical and healthy-resident isolates (n = 20), followed by surface water–healthy residents (n = 7), and was minimal between surface water and clinical isolates (n = 2).
- This indicates that surface water is feasible for capturing community-circulating resistance gene pools but does not adequately represent the breadth of resistance determinants associated with clinical infection, limiting its standalone utility as a genomic proxy for clinical AMR surveillance.

ii) Phylogenomic and MLST analyses demonstrated that surface water is more genomically concordant with community-resident isolates than with clinical isolates, with lineage-driven clustering supporting a shared community-environment AMR ecology.

- For both species, phylogenomic analyses revealed lineage-driven rather than source-structured clustering, with surface-water isolates consistently forming closer phylogenetic relationships with healthy-resident than with clinical isolates.
- In *E. coli*, phylogroup B2, encompassing globally disseminated lineages such as ST131, was observed in isolates from both surface water and healthy residents, with no phylogroup identified in both surface water and clinical isolates. In *K. pneumoniae*, 93.8% of surface-water isolates shared sequence types with healthy-resident isolates compared with only 9.4% with clinical isolates, with surface-water isolates forming strongly supported subclades with healthy residents through ST25 and ST219 and no direct surface water-clinical sister pair detected.
- Virulome analyses reinforced this pattern, with surface water capturing predominantly community-associated virulence backgrounds while exhibiting lower diversity than both human compartments, providing genomic evidence that surface water is more reflective of community-associated AMR ecology than of clinical AMR population structure.

iii) Genomic Surveillance using Surface Water is Feasible but Insufficient as a Standalone Proxy: It Functions as a Partial Genomic Sentinel for Community-Associated AMR, not a Comprehensive Monitor of Clinical AMR.

- Consistent with the phenotypic findings, surface water captured a meaningful but restricted subset of the broader resistome, virulome, and lineage diversity observed in human sources, with several clinically important lineages including ST405, ST37,

ST111, ST469, and ST2375 for *K. pneumoniae* and hybrid pathotypes (EPEC + UPEC + NMEC) for *E. coli* either source-restricted or entirely absent, indicating that genomic surveillance of surface water would systematically miss a proportion of the most clinically consequential AMR signals.

- Critically, the genomic data provide molecular validation of the phenotypic observations. The partial surface water-healthy-resident overlap was not incidental but underpinned by shared ARGs, conserved resistance mechanisms, and shared lineages, confirming biological connectivity across community and environmental compartments.
- Therefore, surface water is feasible as a complementary sentinel matrix for capturing dominant community-circulating ARGs and lineages within a One Health framework, but insufficient as a standalone proxy for the full AMR burden observed in community or clinical human populations.

6.2 Limitations

This study has limitations that should be considered when interpreting the findings.

- First, the genomic analysis was based on a relatively small, sequenced subset compared with the larger parent phenotypic dataset. Although the sampling was balanced across sources and allowed direct comparison between surface-water, healthy-resident, and clinical isolates, rare resistance genes and virulence profiles may have been missed. In addition, random sub-sampling of surface-water and healthy-resident isolates to match the smaller clinical group may have reduced the representation of the full diversity present in the larger community and environmental isolate pools.

- Second, the study was conducted in a single geographic setting linked to a specific informal settlement and nearby healthcare facilities. Therefore, the findings may not be directly generalisable to other informal settlements, rivers, healthcare facilities, or environmental contexts with different sanitation infrastructure, hydrology, antimicrobial use patterns, and population dynamics.
- Third, the study was based on culture-confirmed ESBL-producing isolates. While this approach enabled detailed phenotypic and genomic characterisation of priority organisms, it may have missed non-culturable bacteria, low-abundance lineages, mixed-isolate populations, and resistance genes carried by other bacterial hosts in the broader environmental resistome.
- Fourth, the surface-water component had limited environmental resolution. Grab sampling and isolate-based prevalence estimates do not fully capture microbial load, temporal variation, rainfall effects, dilution, persistence, or environmental selection pressures. As a result, the study provides a comparative snapshot of ESBL-producing isolates across sources rather than a complete assessment of the dynamic environmental resistome.
- Finally, although the study provides evidence of phenotypic and genomic concordance between isolates from surface water, healthy residents, and clinical settings, it does not capture transmission dynamics and directionality. Shared sequence types, resistance genes, and virulence determinants may reflect recent exchange, but they may also result from common ancestry, shared selective pressures, or common contamination sources. Therefore, the findings should be interpreted as evidence of relatedness and overlap across compartments, rather than definitive proof of transmission pathways.

6.3 Recommendations

- Future studies should include a larger genomic sample size, particularly from surface-water and healthy-resident isolates to better capture low-frequency lineages, rare antimicrobial resistance genes, virulence profiles, and temporal changes in AMR circulation. Where possible, genomic sampling should align with seasonal variation to improve understanding of how ESBL-producing organisms and resistance determinants persist or fluctuate over time.
- Future work should also incorporate microbial source tracking to improve attribution of surface-water contamination. Sampling should include potential contributing sources such as hospital effluent, wastewater treatment plants, sewage discharge points, household drainage, upstream and downstream river sites, animal sources, and agricultural runoff. This would help determine whether ESBL-producing lineages and resistance genes detected in surface water are primarily human-derived or influenced by healthcare, animal, agricultural, sewage, or mixed environmental inputs.
- Environmental surveillance should be strengthened by collecting relevant metadata and quantitative water-quality measurements. These should include rainfall, river flow, temperature, physicochemical water-quality parameters, faecal indicator bacteria, antibiotic residues, heavy metals, sanitation discharge points, water-use practices, and proximity to household or healthcare waste streams. Such information would help explain why surface water captures some AMR signals but not others, and would support better distinction between dilution effects, environmental persistence, and true changes in AMR burden.
- Future studies should combine isolate-based whole-genome sequencing with metagenomic or targeted resistome profiling of surface water. Isolate-based WGS provides organism-specific and strain-level resolution, while metagenomics can

capture the broader environmental resistome, including from non-culturable organisms, low-abundance bacterial populations, mixed communities, and resistance genes carried by bacterial hosts not recovered through culture.

6.4 Significance of the Study

- This study provides important One Health evidence that ESBL-producing Enterobacterales occur across surface water, healthy community residents, and clinical settings within a spatially connected informal-settlement context. By integrating phenotypic antimicrobial susceptibility testing with whole-genome sequencing, the study demonstrates that AMR in this setting is not confined to healthcare facilities but is distributed across community, clinical, and environmental compartments.
- A significant finding of the study is that it highlights healthy community residents as an important reservoir of ESBL-producing organisms with broad phenotypic and genomic diversity. Surface water appeared to capture an environmentally filtered subset of this community-associated AMR reservoir, including selected resistance determinants and epidemiologically relevant lineages. In contrast, clinical isolates represented a more infection-associated subset, characterised by higher multidrug resistance and greater selection of clinically important resistance profiles. This distinction is important because clinical surveillance alone may underestimate the wider diversity of AMR circulating silently in the community, while surface-water surveillance alone may underestimate the burden and intensity of clinical multidrug resistance.
- The findings also support the potential role of surface water as a complementary AMR surveillance tool in informal settlements and other low-resource settings where conventional wastewater-based surveillance is limited by poor sewer infrastructure.

The detection of shared resistance determinants, including *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1B}}$, *sul*2, and *dfrA*14, together with shared lineages such as ST983 across surface water, healthy residents, and clinical isolates, shows that surface water can detect selected AMR signals relevant to both community and clinical compartments. The presence of lineages shared between surface water and healthy residents further supports the value of surface water for detecting community-linked AMR circulation beyond formal healthcare systems.

- However, the significance of surface-water surveillance lies in its use as a complementary early-warning system rather than as a direct substitute for clinical or community carriage surveillance. The study shows that surface water can reflect selected dominant resistance patterns and lineages but may not fully capture the diversity of human carriage or the high MDR burden observed in clinical isolates. Therefore, the findings support a combined surveillance approach in which clinical, community, and environmental data are interpreted together.
- The study has practical public health relevance for sanitation improvement, wastewater management, environmental monitoring, and AMR risk communication in communities with high exposure to contaminated water. It provides a genomic foundation for integrating surface-water sampling into AMR surveillance strategies in informal settlements, particularly as an early-warning or supplementary system for detecting community-linked resistant Enterobacterales.
- Overall, this study contributes to the development of an alternate AMR monitoring system (AIARMS) for low-resource and poorly sewered settings. The findings support a tiered surveillance model in which surface-water testing is used as an environmental screening tool, followed by targeted clinical, community, and genomic investigations when important resistance determinants or high-risk lineages are detected.

APPENDICES

Appendix 1: Ethical Approval Letters, Authorisations and Informed Consent

Appendix 1.1 Biomedical Research Ethics Committee of the University of KwaZulu-Natal

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

27 March 2022

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 Non-Degree Purposes

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 27 March 2022. Please ensure that any outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Amended_Lockdown_Level_1_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 27 March 2022. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 12 April 2022.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

INSPIRING GREATNESS

Appendix 1.2: KwaZulu-Natal Department of Health Provincial Health Research Ethics Committee



KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:
Health Research & Knowledge Management Unit

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

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 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 & Imbalenhle clinics and Edendale, Northdale and Greys hospitals.

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 manager BEFORE your research begins in order to ensure that conditions in the facility 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 and hard copies) when your research is complete to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy 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: 22/03/2022

GROWING KWAZULU-NATAL TOGETHER

Appendix 1.3: Ethics Approval letter from National Health Laboratory Service (NHLS)

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 555 0367/0406
Email: babatyi.kgokong@nhls.ac.za
academic.research@nhls.ac.za
Web: www.nhls.ac.za

28 November 2022

Applicant: Sabiha Essack
Institution: University of KwaZulu-Natal
E-mail Address: essacks@ukzn.ac.za
Tel: 031 260 7785 **Cell:** 082 343 9892

CC: Sumayya Haffejee

Project Title: One Health AIARMS in the COVID-19 World
Reference Number: PR2225862

Research Application Type(s):
1. Request for Data

RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES

This letter serves to advise that the application requesting permission to conduct the above-mentioned research using the listed NHLS resources has been reviewed and **"Approved"**. Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available ***without patient names and other patient identifiers (including, but not limited to, national identity numbers, hospital/clinic file numbers, addresses and telephone numbers)***.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. Data and/or material shall not be shared with other parties unless approved by the NHLS
5. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
6. Processes shall be discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) and agreed upon.
7. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – https://aarms.nhls.ac.za.
8. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
9. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

[redacted]

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix 1.4: AIARMS- Participant Information and Consent

Antimicrobial Research Unit
College of Health Sciences
University of KwaZulu-Natal
Private Bag X54001, Durban,
4000, South Africa
Telephone: +27 (0) 31 260 7785
Telefax: +27 (0) 31 260 7792
Email: essacks@ukzn.ac.za

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

Isimemezelo Sokuvuma i-One Health AIARMS Esikhathini se-COVID-19

Mina [redacted] Inombolo kamazisi [redacted] ohlala e [redacted] ngazisiwe ngocwaningo olubizwa nge"One Health AIARMS emhlabeni weCOVID-19" unompilo.

Ngiyaqonda ngenhloso kanye nezinqubo zocwaningo futhi ngiyavuma uku (1) nika amasampuli ami asemakhaleni, emphinjeni kanye nawendle njalo ezinyangeni ezimbili, (2) ukunika imininingwane yami yokuxhumana nami mayelana namasampuli ngokuzayo, (3) ngiqcwalise uhlu lwemibuzo futhi (3) ngivumele nomlekeleli wocwaningo abhekisise ekhaya lami. Ngiyavuma ukuthi lonke lolulwazi luzosetshenziswa ngobumfihlo obuqinile.

Nginikeziwe ithuba lokuphendula imibuzo mayelana nocwaningo futhi ngiphendulile ngokunganelisayo. Ngazisiwe ngezindlela zokunxephezela ezikhona.

Ngiyavuma ukuthi ukubamba kwami iqhaza kulolucwaningo ukuzikhethela ngokuphelele futhi ngingahoxa noma ingasiphi isikhathi ngaphandle komphumela.

Uma ngineminye imibuzo/ukukhathazeka noma imibuzo mayelana nocwaningo ngiyaqonda ukuthi ngingathinta umcwaningi kule kheli elingezansi:

Phrofesa Sabiha Essack
Usihlalo we-South African Research kwi Antibiotic Resistance & One Health
Antimicrobial Research Unit
Ikolishi leSayenzi Yezempilo
Inyuvesi yaKwaZulu-Natali, Private Bag X54001
Durban, 4000
Ucingo: [redacted]
Isikhahlamezi: [redacted]
I-imeyili: [redacted]

Ngiyaqonda futhi ukuthi ukukhathazeka mayelana namalungelo ami njengombambiqhaza noma ngomcwaningi, ngingathinta iKomidi Lezimiso Zocwaningo Lokwelashwa kule kheli elingezansi:

UKUPHATHWA KWEZIMISO ZOCWANINGO LOKWELASHWA
Ihovisi locwaningo, Westville Campus
Govan Mbeki Building
ENyuvesi yaKwaZulu-Natal
Private Bag X 54001, Durban, 4000
KwaZulu-Natali, NINGIZIMU AFRIKA
Ucingo: [redacted]
Isikhahlamezi: [redacted]

[redacted]
Isiginisha

Kusayinwe 15 usuku luka January 2023 e [redacted]

Ufakazi 1 [redacted] **Ufakazi 2:** [redacted]

Appendix 2: Ethics and Research Training Certificates

Appendix 2.1: TRREE Training Certificate – Introduction to Research Ethics



TRREE

Zertifikat
Certificat
Certificado
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

Etando Ayukafangha

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

[redacted]

Professeur Dominique Sprumont
Coordinateur TRREE Coordinator

Release Date: 2024/07/22

CID : XQKyKOjQC

APPROVED BY
SIWF FMH
ISFM

Programmes de formation continue (2 crédits)
Continuing Education Programs (2 credits)

Foederatio Pharmaceutica Helvetiae

FPH
Programmes de formation postgraduée et continue

Programmes de formation continue
Continuing Education Programs

Ce programme est soutenu par - This program is supported by :

European and Developing Countries Clinical Trials Partnership (EDCTP) (www.edctp.org) - Swiss National Science Foundation (www.snf.ch) - Canadian Institutes of Health Research (http://www.cihr-irsc.gc.ca/e/2561.html) - Swiss Academy of Medical Science (SAMS/ASSM/SAMS) (www.samw.ch) - Commission for Research Partnerships with Developing Countries (www.kfpe.ch)

IRIV : 20120171

Appendix 2.2: TRREE Training Certificate – Research Ethics Evaluation



TRREE

Zertifikat
Certificat
Certificado
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

Etando Ayukafangha

a complété avec succès - has successfully completed

Module 2 (2023) - Research Ethics Evaluation

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

[redacted]

Release Date: 2024/07/23

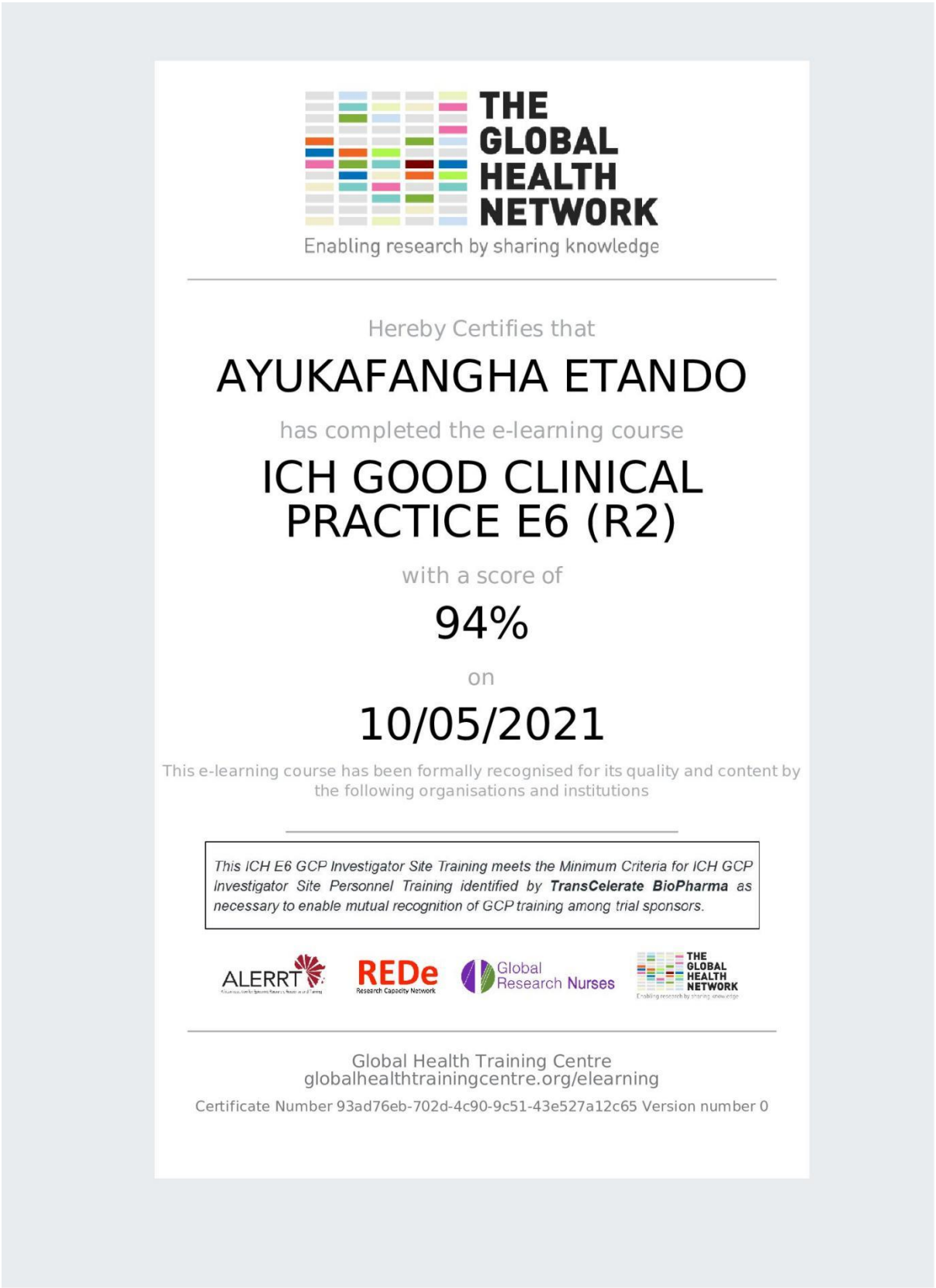
Professeur Dominique Sprumont
Coordinateur TRREE Coordinator

APPROVED BY SIWF ISFM

Foederatio Pharmaceutica Helvetiae FPH
Programmes de formation postgraduée et continue

Ce programme est soutenu par - This program is supported by :

Appendix 2.3: Introduction to Good Clinical Laboratory Practice certificate



THE GLOBAL HEALTH NETWORK

Enabling research by sharing knowledge

Hereby Certifies that

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has completed the e-learning course

ICH GOOD CLINICAL PRACTICE E6 (R2)

with a score of

94%

on

10/05/2021

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ALERRT REDe Research Capacity Network Global Research Nurses THE GLOBAL HEALTH NETWORK

Global Health Training Centre
globalhealthtrainingcentre.org/elearning

Certificate Number 93ad76eb-702d-4c90-9c51-43e527a12c65 Version number 0

Appendix 2.4: Step-by-Step Guide on How to Prepare a Successful Paper



CERTIFICATE

PROUDLY PRESENTED TO

Ayukafangha Etando

Step-by-step Guide on How to Prepare a Successful Paper

Aug 11, 2020

Date of Completion

Leen Samarah

Organizer

SPRINGER NATURE

Appendix 3: Study Data Collection Tools and Guidelines

Appendix 3.1: Guidelines to Participants on How to Use Potable Camping Toilets

Things to know <u>prior to using a portable camping Toilet.</u>

- *The camping toilet will be a bucket with a toilet seat attached to it. There will be a biodegradable bag that can be placed inside the camping bucket to collect your waste. You will be shown how to tie up and throw away the waste after the sample is taken.*

<u>Using a camping Toilet</u>

1. A camping toilet will be given to you at the beginning of the project. It is yours to keep and must be looked after.
2. Plastic bags and gloves will be provided to you monthly – one plastic bag and one pair of gloves for each day of the month.
   Store the bags safely and out of reach of children.
3. Secure a comfortable spot (where you feel it is private) and place the camping toilet on a flat surface. Check that the camping toilet is stable.
4. Place the bag inside the bucket.
   Make sure the bag reaches the bottom of the bucket and that the top of the bag completely covers your toilet seat.
5. Use the toilet as you normally do.
6. Wearing the gloves provided, take the bag out of the toilet at the end of the day or when it is full, and tie a knot at the top to prevent leaks as you take it to the skip provided by the Msunduzi Municipality.
7. Dispose of your waste in the skip provided by Msunduzi Municipality.
8. Wash out the bucket with soap and water, and put it in a new, clean plastic bag for the next day.
9. Wash your hands with soap and clean water before continuing your normal daily activities.

References

Fitterman, S. (2022, November 16). *3 Ways to Make a Camping Toilet - wikiHow*. WikiHow. https://www.wikihow.com/Make-a-Camping-Toilet

Muanda, C., Goldin, J., & Haldenwang, R. (2020). Factors and impacts of informal settlements residents' sanitation practices on access and sustainability of sanitation services in the policy context of Free Basic Sanitation. *Journal of Water, Sanitation and Hygiene for Development*, *10*(2), 238–248. https://doi.org/10.2166/washdev.2020.123

Schwarz, A. (2022, August 22). *A Beginner's Guide to Camping Toilets - Magazine - camping.info*. Camping.Info. https://www.camping.info/en/magazine/a/a-beginners-guide-to-camping-toilets

Appendix 3.2: Guidelines on How to Collect and Provide Stool Samples

Prior to the collection of specimens

- *Notify the Research Assistant if you are or have taken antibiotic treatment.*
- *Do not use a laxative before collecting the stool sample.*
- *Obtain sterile collection containers from the researcher.*
- *Remember to wash your hands with soap and water before and after collecting the sample.*

Collecting Stool Samples for Bacterial Enteric Pathogen Testing

1. Stool collection containers and a plastic sheet will be provided. The container is made of plastic, and the lid has a spoon attached to it.
2. You will be contacted a day or two before the sample collection day by the Research Assistant assigned to you.
3. On the day of sample collection, collect your stool sample as follows:
    - ✓ Place a clean bag in the toilet.
    - ✓ Wash your hands with soap and water.
    - ✓ Place the stool collection container near the toilet.
    - ✓ Wear a pair of gloves.
    - ✓ If you also need to urinate, please do this before you pass stool.
    - ✓ Once you have urinated, place a plastic sheet over the waste that is already in the bucket.
    - ✓ Pass stool as you would normally do on the plastic sheet.
    - ✓ When you are done, use the spoon attached to the lid of the plastic container to transfer a small amount of your stool (two scoops), without any urine or water, into the container.

      Do not attempt to fit the whole stool into the container, as this can cause it to leak.
    - ✓ Close the sample container.
    - ✓ Place the container in the small plastic bag provided for the Research Assistant to collect.
    - ✓ Wash your hands with soap and water.

References:

Abrahamson, M., Hooker, E., Ajami, N. J., Petrosino, J. F., & Orwoll, E. S. (2017). Successful collection of stool samples for microbiome analyses from a large community-based population of elderly men. Contemporary Clinical Trials Communications, 7, 158–162. https://doi.org/10.1016/j.conctc.2017.07.002

National Health Laboratory Service (NHLS). (2018). Primary Healthcare Laboratory Handbook: A step-by-step Guide. www.itldesign.co.za

Appendix 3.3: Stool Container Label

STOOL SAMPLE CONTAINER

S. Code: _____, Age: ____Yrs., Sex: ☐M / ☐F

Sample Collection: Date: __/__/_____ Time: ___H: ___M
Place of Collection: _____
Doctor/Researcher: _____
ATB: ☐Yes/ ☐NO, **Duration:** ☐1Day, ☐2-3 Days, ☐1Wk, ☐> 1Wk

NB: Please tighten Lid securely and place container in the plastic bag provided

Appendix 3.4: Sample Collection Itenery

AIARMS PROJECT ITINERARY

Sample collection dates

All dates may be shifted to Sunday*

Sample collection	Ward 22	Date
1	Group 1	Saturday 11 March 2023
1	Group 2	Saturday 25 March 2023
2	Group 1	Saturday 15 April 2023
2	Group 2	Saturday 29 April 2023
3	Group 1	Saturday 13 May 2023
3	Group 2	Saturday 27 May 2023
4	Group 1	Saturday 10 June 2023
4	Group 2	Saturday 24 June 2023
5	Group 1	Saturday 15 July 2023
5	Group 2	Saturday 29 July 2023
6	Group 1	Saturday 12 August 2023
6	Group 2	Saturday 26 August 2023
7	Group 1	Saturday 16 September 2023
7	Group 2	Saturday 30 September 2023
8	Group 1	Saturday 14 October 2023
8	Group 2	Saturday 28 October 2023
9	Group 1	Saturday 11 November 2023
9	Group 2	Saturday 25 November 2023
10	Group 1	Saturday 9 December 2023
10	Group 2	Sunday 17 December 2023
11	Group 1	Saturday 13 January 2024
11	Group 2	Saturday 27 January 2024
12	Group 1	Saturday 9 February 2024
12	Group 2	Saturday 23 February 2024

Appendix 4: Conference Attendance and Presentation

Appendix 4.1: Conference Attendance



Appendix 4.2 : Conference Attendance and Presentation

Gmail Etando Ayuk. <etta5013@gmail.com>

Re: SeMar Conference Registration, 8-10 April 2026, Durban South Africa

1 message

Lyn Elizabeth Middleton <MiddletonL@ukzn.ac.za> 2 March 2026 at 11:38
To: "etta5013@gmail.com" <etta5013@gmail.com>
Cc: Carl-Fredrik Flach <carl-fredrik.flach@microbio.gu.se>, Melissa Ramtahal <Ramtahal@ukzn.ac.za>, Sabiha Yusuf Essack <ESSACKS@ukzn.ac.za>

Dear Mr Ayukafangha

Thank you for registering for the International Conference on Sewage Surveillance of Antibiotic Resistance in Sub-Saharan Africa, taking place 8–10 April 2026 in Durban, South Africa. We're pleased to confirm that we have successfully received your registration, and we thank you for your interest in the conference.

Please note that participants are responsible for arranging and covering their own travel, accommodation, and local transport. A block of rooms has been reserved at the conference hotel. If you wish to stay at the venue, use the booking reference 0804UN_006 when making your reservation by emailing Pinkie.Pato@southernsun.com.

We look forward to connecting with you soon and to welcoming you in Durban.

Sincerely,
The Conference Organizing Committee

Dr Lyn Middleton (Solomon)
Associate Professor (hon) College of Health Sciences, Antimicrobial Research Unit, Discipline of Pharmaceutical Sciences, University of KwaZulu-Natal, Durban, South Africa
Consultant, Gender Equality, Social Inclusion, and Intersectionality in IDRC Innovet-2 AMR research projects
Technical Support, Gender and Equity, ICARS Climate change and AMR innovation research projects

UKZN email disclaimer:
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Appendix 5: List of Publications within Study Window Not Included in this Thesis

1. Abia, A. L. K., & **Ayukafangha, E.** (2025). Wastewater-based monitoring as an early warning for viral infections in Africa: Challenges and prospects. In J. A. Shenge & A. L. K. Abia (Eds.), *Viral diseases and One Health in Africa* (pp. 151-168). Springer. https://doi.org/10.1007/978-3-032-09613-5_12
2. Abia, A. L. K., Mulyangote, T., & **Ayukafangha, E**. (2025). Environmental reservoirs of viral infections in Africa. In J. A. Shenge & A. L. K. Abia (Eds.), *Viral diseases and One Health in Africa* (pp. 103-118). Springer. https://doi.org/10.1007/978-3-032-09613-5_8
3. Adnan, N., Haque, M., Mou, T. J., Islam, S., Nahar, S., Chowdhury, K., Zaman, T., Akter, F., Sharmin, S., Nusrat, N., Kabir, R., Biswas, N. K., Kumar, S., Sharma, P., Lutfor, A. B., Siddiqui, T. H., **Etando, A.,** Ahmad, R., Abdullah, A., & Godman, B. (2022). The impact of COVID-19 on the teaching of non-medical healthcare professionals in Bangladesh: Observations and implications from a pilot study. *Bangladesh Journal of Medical Science, 21*(4), 731-743. https://doi.org/10.3329/bjms.v21i4.61361
4. Ahmad, R., Oli, A. N., **Etando, A.,** Sharma, P., Sinha, S., Chowdhury, K., Rowaiye, A. B., Akter, S., Mou, T. J., Dhingra, S., Ahmed, M. F., Nusrat, N., Azmuda, N., Lugova, H., Adnan, N., & Haque, M. (2023). Lactic acid bacteria fermented foods: Impact on immune system and consequences over type 2 diabetes mellitus. *Journal of Applied Pharmaceutical Science, 13*(6), 018-056. https://doi.org/10.7324/JAPS.2023.142387
5. Chowdhury, K., **Etando, A.,** Shahwan, M., Škrbić, R., Jairoun, A. A., Haque, M., & Godman, B. (2022). COVID-19 and the impact on the education of healthcare professionals across countries with a particular focus on developing countries.

Bangladesh Journal of Medical Science, 21(2), 221-232. https://doi.org/10.3329/bjms.v21i2.58053

6. Chowdhury, K., Haque, M., **Etando, A**., Kumar, S., Lugova, H., Shahwan, M., Škrbić, R., Jairoun, A. A., & Godman, B. (2022). The global impact of the COVID-19 pandemic on the education of healthcare professionals, especially in low- and middle-income countries. *Advances in Human Biology, 12*(2), 87-92. https://doi.org/10.4103/aihb.aihb_60_22
7. Chowdhury, K., Haque, M., Lutfor, A. B., Siddiqui, T. H., Ahmad, R., Sultana, I., Sharma, P., Lugova, H., **Etando, A.,** & Godman, B. (2022). Impact of the COVID-19 pandemic on dental and medical education in Bangladesh: A pilot study and the implications. *Bangladesh Journal of Medical Science, 21*(2), 444-454. https://doi.org/10.3329/bjms.v21i2.58080
8. **Etando, A.,** & Abia, A. L. K. (2023). Climate change: Emerging driver of infectious diseases. In S. Nazneen, A. L. K. Abia, & S. Madhav (Eds.), *Emerging pandemics: Connections with environment and climate change* (pp. 17-32). CRC Press. https://doi.org/10.1201/9781003288732-2
9. **Etando, A.,** Amu, A. A., Haque, M., Schellack, N., Kurdi, A., Alrasheedy, A. A., Timoney, A., Mwita, J. C., Rwegerera, G. M., Patrick, O., Niba, L. L., Boahen-Boaten, B. B., Tabi, F. B., Amu, O. Y., Acolatse, J., Incoom, R., Sefah, I. A., Guantai, A. N., Opanga, S., … Godman, B. (2021). Challenges and innovations brought about by the COVID-19 pandemic regarding medical and pharmacy education especially in Africa and implications for the future. *Healthcare, 9*(12), Article 1722. https://doi.org/10.3390/healthcare9121722
10. Kamal, Z. M., Dutta, S., Rahman, S., **Etando, A.,** Hasan, E., Nahar, S. N., Wan Ahmad Fakuradzi, W. F. S., Sinha, S., Haque, M., & Ahmad, R. (2022). Therapeutic application

of lithium in bipolar disorders: A brief review. *Cureus, 14*(9), Article e29332. https://doi.org/10.7759/cureus.29332

11. Krishnapillai, A., Lugova, H., Haque, M., Ariaratnam, S., Abdul Aziz, A. F. B., Hasan Maziz, M. N., Patil, S. S., Daher, A. M., Ali, S. M., Rahman, N. A. A., Rahman, N. I. A., Chowdhury, K., Sharma, P., **Etando, A.,** Kumar, S., Abdullah, A., Shahwan, M., & Godman, B. (2022). The shock of the SARS-CoV-2 pandemic on health professionals' education: A pilot qualitative study in Malaysia. *Journal of Applied Pharmaceutical Science, 12*(10), 161-171. https://doi.org/10.7324/JAPS.2022.121018

12. Lutfor, A. B., Jahan, F., Siddiqui, T. H., Chowdhury, K., Ahmad, R., Nusrat, N., Akter, F., Adnan, N., Islam, S., Kumar, S., Sharma, P., Lugova, H., **Etando, A.,** Sultana, I., Sarkar, S., Begum, F., Chowdhury, C. S., Mou, T. J., Nahar, S., … Haque, M. (2023). The impact of the COVID-19 pandemic on the education of medical, dental and non-medical healthcare professionals in Bangladesh: Findings and connotation. *Advances in Human Biology, 13*(Suppl. 1), S85-S95. https://doi.org/10.4103/aihb.aihb_53_23

13. Nipa, N. J., Aktar, N., Hira, H. M., Akter, F., Jahan, D., Islam, S., **Etando, A.,** Abdullah, A., Chowdhury, K., Ahmad, R., Haq, A., & Haque, M. (2022). Intestinal parasitic infections among pediatric patients in a metropolitan city of Bangladesh with emphasis on cryptosporidiosis. *Cureus, 14*(7), Article e26927. https://doi.org/10.7759/cureus.26927

14. Ogunleye, O. O., Godman, B., Fadare, J. O., Mudenda, S., Adeoti, A. O., Yinka-Ogunleye, A. F., Ogundele, S. O., Oyawole, M. R., Schönfeldt, M., Rashed, W. M., Galal, A. M., Masuka, N., Zaranyika, T., Kalungia, A. C., Malande, O. O., Kibuule, D., Massele, A., Chikowe, I., Khuluza, F., **…** Meyer, J. C. (2022). Coronavirus disease 2019 (COVID-19) pandemic across Africa: Current status of vaccinations and implications for the future. *Vaccines, 10*(9), Article 1553. https://doi.org/10.3390/vaccines10091553

15. Rabiu Abubakar, A., Ahmad, R., Rowaiye, A. B., Rahman, S., Iskandar, K., Dutta, S., Oli, A. N., Dhingra, S., Tor, M. A., **Etando, A.,** Kumar, S., Irfan, M., Gowere, M., Chowdhury, K., Akter, F., Jahan, D., Schellack, N., & Haque, M. (2022). Targeting specific checkpoints in the management of SARS-CoV-2-induced cytokine storm. *Life, 12*(4), Article 478. https://doi.org/10.3390/life12040478

16. Rangila, T., Zondo, A., Mtshali, A., Tar, N. I. S., Dada, U. S., **Ayukafangha, E.,** & Abia, A. L. K. (2026). Antibiotic resistance patterns of *Escherichia coli* from children's sandpits in Durban, South Africa: A point prevalence study. *Hygiene, 6*(1), Article 3. https://doi.org/10.3390/hygiene6010003

17. Sharma, P., Chowdhury, K., Kumar, S., Bhatt, R., Hirani, T., Duseja, S., Haque, M., Lutfor, A. B., **Etando, A.,** Škrbić, R., Shahwan, M., Jairoun, A. A., & Godman, B. (2022). A pilot study regarding the consequences of the COVID-19 pandemic on healthcare education in India and the implications. *Advances in Human Biology, 12*(2), 180-189. https://doi.org/10.4103/aihb.aihb_34_22

Appendix 6: Evidence of Submission of Manuscritps for Publication

Manuscript 1 - Submitted to Evironmentl Sciences and Pollution Research

Gmail [redacted]

Environmental Science and Pollution Research - Submission Notification to co-author - [EMID:3c422293bc53dee6]

1 message

Environmental Science and Pollution Research <em@editorialmanager.com> 22 May 2026 at 19:44
Reply-To: Environmental Science and Pollution Research <carmina.cayago@springer.com>
To: [redacted]

Re: "Can surface water be a good proxy for phenotypic antimicrobial resistance in ESBL-producing Escherichia coli from healthcare facilities and healthy informal settlement residents? A triangulation study"
Full author list: Etando Ayukafangha; Joshua Mbanga; Sabiha Y. Essack; Akebe Luther King Abia

Dear Mr Etando Ayukafangha,

We have received the submission entitled: "Can surface water be a good proxy for phenotypic antimicrobial resistance in ESBL-producing Escherichia coli from healthcare facilities and healthy informal settlement residents? A triangulation study" for possible publication in Environmental Science and Pollution Research, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. Professor Akebe Luther King Abia who will be able to track the status of the paper through his/her login.

If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

Springer Journals Editorial Office
Environmental Science and Pollution Research

This letter contains confidential information, is for your own use, and should not be forwarded to third parties.

Recipients of this email are registered users within the Editorial Manager database for this journal. We will keep your information on file to use in the process of submitting, evaluating and publishing a manuscript. For more information on how we use your personal details please see our privacy policy at https://www.springernature.com/production-privacy-policy. If you no longer wish to receive messages from this journal or you have questions regarding database management, please contact the Publication Office at the link below.

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/esom/login.asp?a=r). Please contact the publication office if you have any questions.

Manuscript 2 - Submitted to Environmnetal Monitoring and Assessment

Gmail [redacted]

Environmental Monitoring and Assessment - Receipt of Manuscript 'Monitoring Extended-spectrum-β-lactamases-producing Klebsiella...'

1 message

Springer Nature <do-not-reply@springernature.com> 11 May 2026 at 17:48
To: [redacted]

Ref: Submission ID daab4231-0147-49dd-bb9a-7b9428fe3332

Dear Dr Ayukafangha,

Thank you for submitting your manuscript to Environmental Monitoring and Assessment.

Your manuscript is now at our initial Technical Check stage, where we look for adherence to the journal's submission guidelines, including any relevant editorial and publishing policies. If there are any points that need to be addressed prior to progressing we will send you a detailed email. Otherwise, your manuscript will proceed into peer review.

You can check on the status of your submission at any time by using the link below and logging in with the account you created for this submission:

https://submission.springernature.com/submission-details/daab4231-0147-49dd-bb9a-7b9428fe3332?utm_source=submissions&utm_medium=email&utm_campaign=confirmation-email&journal_id=10661

Kind regards,

Editorial Assistant
Environmental Monitoring and Assessment

Environmental Monitoring and Assessment is a hybrid journal. This means when the journal accepts research for publication, the article may be published using either immediate gold open access or the subscription publishing route. For further information please visit https://www.springernature.com/gp/open-research/about/green-or-gold-routes-to-OA/hybrid-options

Manuscript 3 - Submitted to Environmental Research

Gmail [redacted]

Confirm co-authorship of submission to Environmental Research

1 message

Environmental Research <em@editorialmanager.com> 30 May 2026 at 09:41
Reply-To: Environmental Research <support@elsevier.com>
To: [redacted]

"This is an automated message."

Journal: Environmental Research
Title: Does AMR surveillance of surface water reflect AMR in humans? A genomic comparison of ESBL-producing Escherichia coli from surface water, healthy residents, and a clinical facility
Corresponding Author: Dr. Akebe Luther King Abia
Co-Authors: Etando Ayukafangha; Joshua Mbanga; Arshad Ismail; Sabiha Y. Essack
Manuscript Number: **ER-26-8381**

Dear Etando Ayukafangha,

The corresponding author Dr. Akebe Luther King Abia has listed you as a contributing author of the following submission via Elsevier's online submission system for Environmental Research.

Submission Title: Does AMR surveillance of surface water reflect AMR in humans? A genomic comparison of ESBL-producing Escherichia coli from surface water, healthy residents, and a clinical facility

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