Genomic Analysis of Antimicrobial Resistant *Escherichia coli*, *Enterococcus* and *Salmonella* Species Isolated from Humans and Broiler Chickens in Lusaka and Copperbelt Provinces in Zambia

By

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DECLARATION

I, **Kaunda Yamba**, do hereby declare that the contents of the thesis being submitted herein are my original work, and they have not been previously submitted to any University for the award of a degree or any other qualification.

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ABSTRACT

Antimicrobial resistance (AMR) is a global public health and economic threat involving humans, animals and the environment. Globally, AMR threatens historic gains attained in the treatment of infectious diseases. *Escherichia coli* and Enterococcus species initially considered harmless members of the gut resident biota, are now important human pathogens associated with a wide range of clinical syndromes, including urinary tract infections (UTIs) and bloodstream infections (BSIs). *Salmonellae* are the leading cause of community acquired BSIs in low- and middle-income countries (LMIC). In order to understand AMR, the distribution and the genomic relatedness of these pathogens, a holistic approach in the three sectors is of critical importance. The aim of this study was to determine the phenotypic and genotypic characteristics of *E. coli, Enterococcus* and *Salmonella* species and establish genotypic relatedness of *E. coli* and *Enterococcus* species isolated from humans (clinical and carriage) and poultry in Lusaka and Copperbelt provinces of Zambia.

This was a two-tier cross-sectional study comprised of human and poultry bacterial isolates. The human study had two components: hospitalized patients and the healthy community. *E. coli* and *Enterococcus* species were isolated from hospitalised patients with UTIs and BSIs, healthy pregnant women and children < 5 years and from market-ready-chickens while *Salmonella* species was isolated from hospitalised patients with BSIs only. Antimicrobial susceptibility patterns were determined by Kirby-Bauer disc diffusion method and the Vitek 2 compact automated machine. AMR data was entered into Excel spreadsheets and WHOnet 2020, then analysed in STATA version 14. Subsets of *E. coli*, *Enterococcus* and *Salmonella* species were selected for whole genome sequencing (WGS) using Illumina short read sequencing. The raw sequence data of the three pathogens were uploaded and investigated on different platforms such as EnteroBase and SnapperDB. Assembled sequence data for all three pathogens were analysed using online bioinformatics pipelines at the Centre for Genomic Epidemiology (GCE). Phylogenetic analysis of *E. coli* and *Salmonella enterica* were carried out on Enterobase and visualised using Microreact.

High levels of resistance to a number of antibiotic classes were recorded in all the three studied pathogens. Notably, resistance to third generation cephalosporins and extended spectrum beta-lactamases in *E. coli* clinical isolates was 62% and 89% respectively. It was interesting to note the presence of similar resistance gene determinants and incompatibilty (Inc) plasmid replicons FII, FIB, FIA and I1 in both clinical and non-clinical *E. coli* strains. The most prevalent sequence type (ST) was *E. coli*-ST131(79%), most of which were clinical isolates (97%).

Notable was the wide diversity of other STs in the healthy community and poultry, most of which were phylogenetically unrelated but carried similar resistance genes on similar plasmids. *E. faecalis* (52%) was the most prevalent *Enterococcus* species with emerging resistance to ampicillin/penicillin (26%/34%), high-level gentamicin (12%) and quinupristin-dalfopristin (1%) in clinical isolates. *Salmonella* infections were mostly caused by genomic diverse *S.* Typhi (86%) with an emergence of *S.* Typhimurium ST313 (3%). *Salmonella* infections recorded an increase in resistance to ciprofloxacin (1% to 20%) and ceftriaxone (1% to 11%) in 2020/2021 compared to 2018/2019. All the three pathogens had phylogroups/STs that are known to be commensals or less virulent strains but associated with clinical infections.

The occurrence of strains known to be commensals as causative agents of BSIs/UTIs, the distribution of similar antimicrobial resistance profiles and resistance gene determinants on the same type of transferable plasmids in all the three interfaces, supports the possibility of cross transmission across species. It further supports the ability of previously non-pathogenic strains to acquire traits that allow them to become pathogenic and further spread AMR. These findings warrant for adoption of policies and regulations that address the prevention and containment of AMR using the one health approach.

DEDICATION

I dedicate this work to my children, Kondwani and Kafula, who had to endure my absence during the time of pursuing this PhD.

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ABBREVIATIONS AND ACRONYMS

AMR	Antimicrobial Resistance
ADCH	Arthur Davison's Children's Hospital
ASM	American Society for Microbiology
BSI	Blood Stream Infections
CLSI	Clinical Laboratory Standard Institute
E. coli	*Escherichia coli*
ExPEC	Extra Intestinal Pathogenic *E. Coli*
ESBL	Extended Spectrum Beta-lactamases
ESBL-Ec	Extended Spectrum Beta-lactamases *E. coli*
FAO	Food and Agriculture Organisation
H_2S	Hydrogen Sulphide
LIA	Lysine Iron Agar
NHRA	National Health Research Authority
NTH	Ndola Teaching Hospital
MDR	Multi Drug Resistant
MDRO	Multi Drug Resistant Organism
MLST	Multi-locus Sequence Type
PCR	Polymerase Chain Reaction
UTH	University Teaching Hospital
UTI	Urinary Tract Infection
SNP	Single Nucleotide Polymorphisms
SIM	Sulphide Indole Motility
TSI	Triple Sugar Iron
TSB	Thiosulphate Broth
VRE	Vancomycin Resistant Enterococcus
WGS	Whole Genome Sequencing
WHO	World Health Organisation
WGS	Whole Genome Sequencing

DEFINITIONS

In this study, the following definitions were applied to the key in terms:

i. BSIs are infectious diseases defined by the presence of viable bacterial or fungal microragnsims in the bloodstream that elicits an inflammatory response characterised by the alteration of clinical, laboratory and hemodynamic parameters (Viscoli, 2016).

ii. UTIs are an infection of any part of the urinary system which includes the kidneys, ureters, bladder and urethra (Kumar, 2019).

iii. Clinical isolates were considered pathogens that were isolated from patients that showed clinical symptoms and signs of bloodstream and urinary tract infection (Del Bono and Giacobbe, 2016).

iv. Non-clinical isolates were considered as bacteria (normal flora) isolated from participants that had no symptoms or signs of infection (Todar, 2012).

v. MDR isolates were defined as resistance to at least one agent in three or more antibiotic classes, XDR as resistance to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories), and PDR was defined as resistance to all agents in all antimicrobial categories (Magiorakos et al., 2012).

vi. 'Susceptible' was defined as isolates inhibited by the usually achievable concentrations of an antimicrobial agent when the recommended dosage is used for the site of infection; 'resistant' as isolates with zone diameters that fall in the range where specific microbial resistance mechanisms are likely not inhibited by the usually achievable concentrations of the antimicrobial agents with normal dosage schedules and, 'intermediate' as isolates with antimicrobial minimum inhibitory concentrations (MICs) that approach usually attainable blood and tissue levels and for which response rates may be lower than for the susceptible isolates (Clinical and Laboratory Standards Institute, 2022).

vii. Molecular profiles included speciation, resistance gene determinants and sequence types (Adzitey et al., 2013).

viii. Phylogenetic relatedness based on the number of nodes separating strains and having a more recent common ancestor (Johnson et al., 2011)

ix. Genetic diversity, the range of different inherited traits within a species with a wide variety of different traits (Johnson et al., 2011)

CHAPTER ONE: INTRODUCTION

1.1 Background information

Antimicrobial resistance (AMR) is a global challenge concerning human and animal health and the environment (Woolhouse et al., 2015). Resistant microbes are considered a major global public health and economic challenge, threatening historic gains attained in the fight against treatment of infectious diseases. This is because AMR hinders the successful treatment of infectious diseases and may lead to a higher disease burden, prolonged recovery periods, and increased cost of disease management (Aidara-Kane et al., 2018). The global burden of AMR is on the rise because the rate at which microbes are becoming resistant to antimicrobials is not equivalent to the innovations in new antimicrobial discovery (Jasovský et al., 2016). Given the present AMR challenge, it is important to improve AMR surveillance and design intervention strategies to prevent the spread of resistant bacteria and related genetic materials.

According to the 2019 Global burden of bacterial antimicrobial resistance (GRAM), 1·27 million deaths were directly attributable to resistance and 4·95 million deaths were associated with bacterial AMR, most of which were in sub-Saharan Africa (SSA) (Murray et al., 2022a). A study done at UTH found 100% ESBL production in *E. coli* and *K. pneumoniae* isolates (Kabwe et al., 2016; Mumbula et al., 2015). Another study conducted between 2015 to 2017 at the same hospital analysed revealed high levels of antibiotic resistance among GNB such as *E. coli* and *K. pneumoniae* with the highest being to ceftriaxone and ciprofloxacin (Roth et al., 2021). A study in Southern province of Zambia in 2019 to 2021 found *E. coli* to be second highest pathogen isolated with prevalent MDR pathogens and resistance to third generation cephalosporins at 60% (Mwansa et al., 2022a). A study that investigated AMR in *Enterococcus* species from BSI found high resistance to penicillins, ciprofloxacin and reduced susceptibility to vancomycin (Mutalange et al., 2021). A *Salmonella* study during the 2010-2012 outbreak in Zambia revealed high prevalence of MDR (> 80%) and an emergence of fluoroquinolone resistance (Kalonda et al., 2015a).

Investigation of AMR in different spheres, such as humans, food-producing animals and the environment, can pose a challenge as many pathogens are specific to a particular sphere. Commensal bacteria such as *Escherichia coli (E. coli)* and *Enterococcus* species are suitable indicators of AMR in these spheres. This is because they can be isolated from different hosts and

the environment and provide information on resistance in a population (EFSA, 2012; Nhung et al., 2017). Both pathogens have the potential for human-animal transmission (Klare et al., 2003), can be found in the environment and have both been used as sentinel organisms for AMR One Health studies (EFSA, 2012). As commensals, *E. coli* and *Enterococcus* species both colonise the gut of humans and animals and are also ubiquitous in soil and water (Rousham et al., 2018; van den Bogaard et al., 2002). *E. coli* and *Enterococcus* species were initially considered harmless members of the colon resident biota but are now recognised as important human pathogens associated with a wide range of clinical syndromes such as urinary tract infections (UTI) and bloodstream infections (BSI) (Gold, 2001; Partridge, 2015).

Salmonella enterica is a leading cause of community-acquired BSIs in low- and middle-income countries and is an important cause of typhoid and paratyphoid fever in crowded, underprivileged populations with poor sanitation and lack of access to safe food and clean drinking water (Parry et al., 2015; Crump et al., 2015). Today, most of the disease burden occurs in developing countries where the pace of investment in water and sanitation infrastructure has not met the growing need and increasing population (Bennett et al., 2018). *Salmonella* mainly affects children, leading to profound effects on intestinal absorption, nutrition, and childhood development and is a significant contributor to global morbidity and mortality (Crump and Mintz, 2010; Kabwama et al., 2017).

All three pathogens are known to cause invasive infections with sequelae; the rise in AMR in these pathogens complicates the treatment outcome, thereby increasing morbidity and mortality. *E. coli* is known to be the leading causative agent of community-acquired and healthcare-associated bloodstream and urinary tract infections; and AMR in *E. coli* complicate the treatment of patients with uncomplicated UTIs that are supposed to be managed as outpatients. This is because most pathogens have become resistant to oral treatment options (Singer, 2015). AMR in *Salmonella* can lead to complications such as gastrointestinal bleeding, intestinal perforation, hepatitis and typhoid encephalopathy, all of which are closely associated with risk for death (Crump et al., 2015). *Enterococcus* species are known to cause infective endocarditis that requires prolonged treatment with multiple antibiotics. AMR in *Enterococcus* species can further complicate treatment and lead to mortality (Beganovic et al., 2018).

AMR in bacteria is attributed to presence of resistance genes. These AMR genes can be transferred among the bacterial population by chance during recombination and on mobile genetic elements such as plasmids, integrons, or transposons (Partridge, 2015). This is enhanced by the presence of

sufficient quantities of antibiotics or their metabolites as residues from their various sources to cause further selection pressure for recombinants and mutants (Prestinaci et al., 2015). This is how the currently most feared highly resistant bacteria, such as Extended Spectrum Beta-Lactamases (ESBLs), Carbapenem-resistant Enterobacterales (CRE), Vancomycin-resistant Enterococci (VRE), and Fluoroquinolone-resistant *Salmonella* have emerged (EFSA, 2012; Gold, 2001).

In low-or middle-income countries (LMICs), beta-lactams are widely prescribed as empiric treatment, with third-generation cephalosporins (3GCs) being the most commonly used antibiotics (Wen et al., 2021). A 2016 study that looked at aetiology, antibiotic resistance and risk factors associated with neonatal sepsis at the University Teaching Hospital (UTH) in Lusaka, Zambia, found 3GC resistance to be above 95% (Kabwe et al., 2016). However, a point prevalence survey conducted at the same hospital in 2018 still found 3GCs to be the most commonly prescribed antibiotics in hospitalized patients and prescription of 3GCs was at 57.9% (Masich et al., 2020). Another study that assessesed antibiotic use and stewardship indicators in the First- and Second-Level Hospitals in 10 provinces in Zambia found the prevalence of antibiotic use among the in-patients to be 59%, with a high rate of empiric prescribing of ceftriaxone at 36% of all antibiotics prescribed (193/534) (Kalungia et al., 2022). Compliance with the national standard treatment guidelines (STGs) was also low at only 27.0% and the use of microbiology to guide treatment was low at 3% (Kalungia et al., 2022). This confirms the inappropriate use of antibiotics most of which are used as empiric treatment.

The emergence and spread of resistant bacteria and resistance determinants are driven by several factors, including the use and misuse of antimicrobials in humans, animals, and environmental sectors (Hammerum et al., 2010). The AMR bacteria in a host belonging to one sector have the potential to spread within or to hosts in another sector. The use of medically important antimicrobials in food-producing animals for growth promotion and disease treatment or prevention is also a significant contributor to AMR (Prestinaci et al., 2015). Equally, the environment plays an important role in sustaining AMR in populations. This is because antimicrobials used in human and animal sectors, in most cases, are disposed off in the environment (Lekshmi et al., 2017). For example, the effluent with antimicrobial residues from hospitals, abattoirs, residential areas and farms can spill into the environment and propagate the emergence and spread of AMR. The environment is a reservoir of bacteria carrying numerous natural resistance genes in their chromosomes and bacteria with different levels of resistance genes from human and animal waste (Prestinaci et al., 2015).

In order to understand the dynamics, epidemiology and transmission of AMR, a holistic approach to management in these three sectors, namely humans, food-producing animals, and the environment, is of critical importance. Therefore, to address the AMR problem, it is reasonable to take the One Health approach given the important and co-dependent human, animal, and environmental scopes of AMR (McEwen and Collignon, 2018). AMR is recognised as a One Health challenge that requires a multidisciplinary approach. This is attributed to the rapid emergence and dissemination of resistant bacteria and genes among humans, animals and the environment (Rousham et al., 2018). By definition, One Health is the collaborative effort of multiple health science professions to attain optimal health for humans, animals and the environment (Hammerum et al., 2010). Integrating all three components of the One Health spectrum to understand the dynamics of AMR is of great importance (Aidara-Kane, 2012). A concept that has been embraced in Zambia (Republic of Zambia NAP on AMR, 2017).

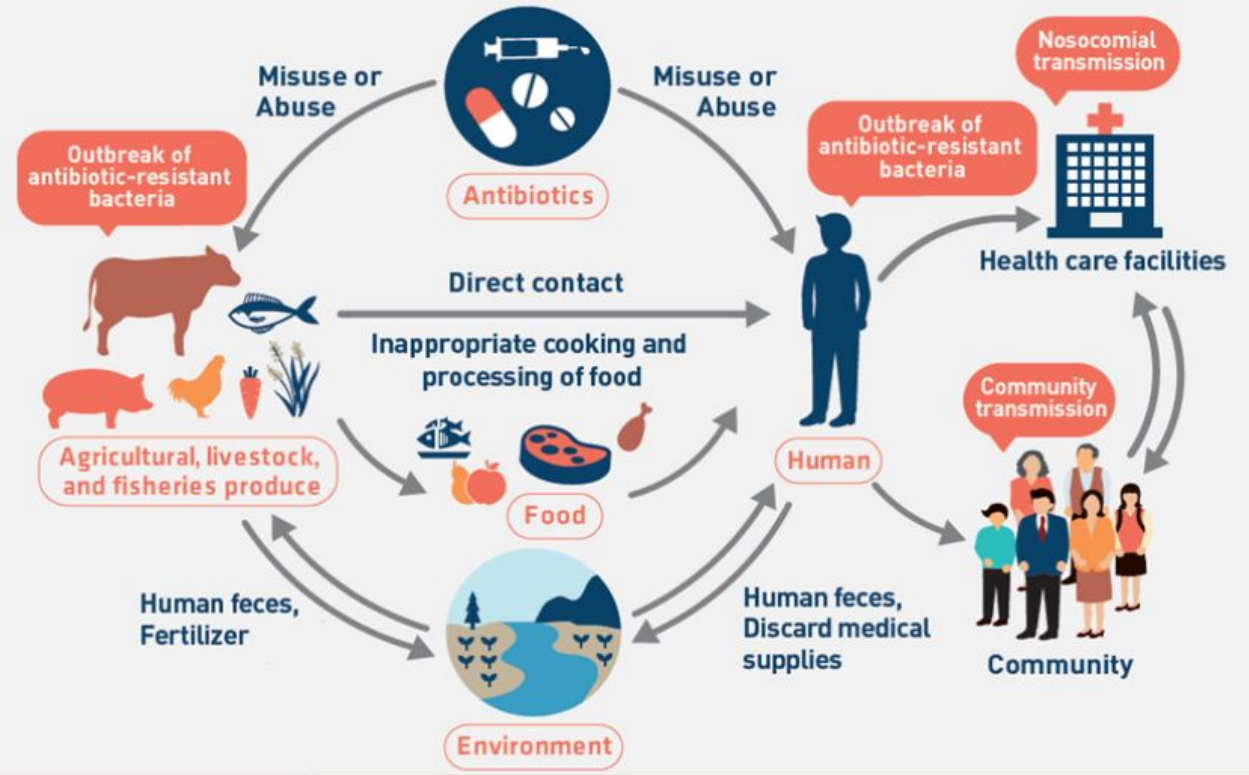

Figure 1: Factors involved in the spread of AMR in the three different sectors: Misuse of antibiotics in humans and food-producing animals contributes to the presence of resistant bacteria in the environment.
Picture from https://nih.go.kr/menu.es?mid=a50301040000

1.2 Problem statement

E. coli, *Enterococcus* and *Salmonella* species are on the WHO priority list of pathohgens and have been known to cause hospital and community-acquired infections, with *E. coli* being more prevalent and the leading pathogen for deaths associated with AMR (Murray et al., 2022a). Previous studies carried out in Zambia have found a growing problem of AMR in all three pathogens (Bumbangi et al., 2022; Hendriksen et al., 2015; Kabwe et al., 2016; Mutalange et al., 2021; Yamba et al., 2022). Zambia is one of the developing countries burdened by infectious diseases, and in most cases, treatment relies on empirical antimicrobials. The use of empiric treatment, irrational use of antimicrobials, lack of diagnostic capacity in primary healthcare facilities and lack of strict regulations that forbid the purchase of antimicrobials without prescription are some of the drivers of AMR that have led to resistance to affordable first-line antibiotics such as beta-lactams and fluoroquinolones (Andrew et al., 2014; Chibwe et al., 2017; Muchemwa et al., 2016). The University Teaching Hospital (UTH), the largest referral hospital in Zambia, has been detecting multidrug-resistant (MDR) pathogens resistant to the first, second, and third-line antimicrobials (Kabwe et al., 2016). A study by Kabwe et al (2016) documented AMR ranging between 92-99%, with all the *E. coli* isolates being ESBL producers and a mortality rate of 72%. This further causes challenges in therapeutic options as most of the tertiary hospitals rely on beta-lactam antibiotics for treatment (Kalungia et al., 2022).

Another study at UTH that determined outcomes of patients that had BSIs caused by Gram negative bacteria (GNB) revealed that 68% of the patients that received 3GCs as empiric treatment were later confirmed to have BSI caused by 3GC resistant GNB, suggesting inappropriate empiric treatment and lack of adherence to treatment guidelines (Yamba et al., 2023). In comparison to *E. coli* as a causative agent of BSI, the odds of death were significantly higher among patients infected with *Acinetobacter baumannii* (OR = 3.8) than *Klebsiella pneumoniae* (OR = 0.9) or other causative agents (OR = 1.1) (Yamba et al., 2023). The odds of death were significantly higher among patients receiving 3GCs (OR = 13.4) or other types of antibiotics (OR = 3.1) than those receiving 4GCs as empiric treatment (Yamba et al., 2023).

The AMR problem is not only limited to patients admitted to hospitals but also healthy individuals in the community and those attending outpatient departments have been found to carry AMR bacteria. This was found to be true in a prevalence study conducted in a rural area in the Eastern Province of Zambia (Nagelkerke et al., 2017) and another study conducted in Lusaka and Ndola districts (Bumbangi et al., 2022). Nagelkerke et al. found a high colonisation rate of ceftriaxone and ciprofloxacin-resistant Enterobacterales, and all ceftriaxone-resistant isolates were ESBL-producers (Nagelkerke et al., 2017). The study by Bumbangi et al. that collected rectal swabs from

children documented high levels of AMR *E. coli* with over 70% resistance to ampicillin and co-trimoxazole and 50% and 38%, resistance to ceftriaxone and ciprofloxacin, respectively (Bumbangi et al., 2022). Carriage of AMR genes in healthy individuals in the community not only increases the chance of human-to-human transmission but further limits the treatment choices in the event that these individuals become clinically ill (Sharma et al., 2022; Singh et al., 2020).

Additionally, the use of antibiotics as growth promotors and treatment in food animals such as chickens has given rise to the carriage and spread of AMR bacteria in food-producing animals. A few studies that screened for AMR in chickens and cattle in Zambia have also recorded high levels of resistance in antimicrobials that are used for the treatment of human infections (Mainda et al., 2015a; Mubita et al., 2008; Muligisa-Muonga et al., 2021; Phiri et al., 2020). This situation could affect food safety and contribute to environmental contamination of AMR genes (Woolhouse et al., 2015). AMR bacteria have the potential to move between humans and food-producing animals by direct exposure through the food chain or the environment (World Oragnization for Animal Health, 2016). This has created a multi-sectorial problem affecting the interface between humans and food-producing animals. Based on the foregoing, the key research questions that this study wished to investigate were:

(1) Are there AMR-resistant *E. coli*, *Enterococcus* and *Salmonella* species circulating in humans and poultry that are of public health significance?

(2) If such exist, are they shared between humans and poultry?

1.3 Study Justification

The rise in AMR in human and food-producing animals has resulted in global initiatives to improve One Health AMR surveillance in bacteria found in both sectors (AGISAR, 2017; Hashim et al., 2022a). Addressing AMR requires a One Health-holistic and multi-sectoral approach, as resistant bacteria could spread from one sector to another, consequently leading to more significant threats (Hashim et al., 2022a). The surging of resistant infections has led to difficult-to-treat infections, worsening clinical outcomes and causing economic loss (James A. Ayukekbong et al., 2017). In order to have a structured One Health study, this study adopted the WHO Tricycle project concept minus the environmental sector (Hashim et al., 2022a). Several AMR studies have been done in the human and animal sectors, but very few have adopted the holistic One Health concept to study the relatedness of strains and AMR genes found in the two sectors.

E. coli and *Enterococcus* species are diverse and natural inhabitants of the intestinal tracts of humans and animals, with some strain variants having the potential to cause disease (Rousham et

al., 2018; van den Bogaard et al., 2002). *E. coli* and Enterococcus species have been found to be reservoirs of resistant genes that can be transferred to other pathogens transiting the intestinal tract. It is for this reason that *E. coli* and Enterococcus species serve as indicator organisms for monitoring resistance genes and providing information on the movement of Gram-negative (GN) and Gram-positive (GP) resistance traits, respectively, in humans, livestock and food (AGISAR, 2017; Karp et al., 2017; Lekshmi et al., 2017).

Further, *Salmonella* species are a priority for inclusion in integrated surveillance of AMR in foodborne bacteria; this is because *Salmonella* serovars which are widely distributed globally, mainly result in outbreaks commonly linked to the consumption of poultry such as chickens (Ramatla et al., 2022). In Zambia, just like in many other African countries, chicken is the most consumed meat, and the primary source of protein (AgriProFocus, 2015; Ramatla et al., 2022). This is evidenced by growth in the poultry industry at a rate of 8%-10% annually, a phenomenal growth attributed to demand and supply side factors (AgriProFocus, 2015). In order to meet the demand and make profits from the significant rise in poultry meat consumption, farmers have resorted to using antimicrobials as growth promotors in poultry production, most of which are obtained without prescriptions (Mudenda et al., 2022c). This has led to the carriage of AMR bacteria in food-producing animals (Mudenda et al., 2022c; Muligisa-Muonga et al., 2021; Phiri et al., 2020).

1.4 General objective

In order to gain insight into the clinical and public health significance of *E. coli, Enterococcus and Salmonella* species isolated from humans and poultry in Lusaka and Copperbelt provinces in Zambia, the general objective of this study to generate knowledge on the genomic and AMR profiles of *E. coli*, Enterococci and Salmonella species isolated from humans and poultry.

1.5 Specific objectives

1. To detect the phenotypic AMR profiles of isolated *E. coli*, *Enterococci* and *Salmonella* species in relation to prescription patterns;
2. To characterise the molecular profile of AMR *E. coli* and *Enterococcus* species from humans and poultry;
3. To determine the phylogenetic relatedness of *E. coli* isolated from humans and poultry; and,
4. To identify the diverse *Salmonella* serovars causing bloodstream infections.

1.6. The organisation of the thesis

This thesis is organised into six chapters. Chapter One provides the background to the study and highlights the problem statement and justification. Chapter Two reviews the available literature on the clinical and public health significance of *E. coli*, *Enterococcus* and *Salmonella* species, and AMR in all three pathogens based on the One Health concept. The third chapter explains the study designs and materials used in the study, and describes the different laboratory and data analysis methods used. This chapter also describes ethical considerations observed in undertaking the study and study limitations. Chapter Four provides a detailed description of the study findings, while Chapter Five discusses the study findings according to the study objectives and highlights the study limitations. Finally, Chapter Six draws conclusions and recommendations from the findings.

CHAPTER TWO: LITERATURE REVIEW

2.1 General overview of antimicrobial resistance

Antimicrobials have transformed the practice of medicine in many aspects and saved numerous lives; their discovery and innovation were turning points in human history (Reygaert, 2018). Unfortunately, the use of these wonder drugs has been accompanied by the rapid emergence of resistant strains that may cause us to return to the negative effects of the pre-antibiotic era (Reygaert, 2018). AMR is the ability of pathogenic bacteria to withstand the action of antimicrobial drugs (Nolte, 2014). In microbiology, AMR is determined by measuring the susceptibility of microorganisms in vitro in the presence of antimicrobials (CLSI 2017). The use of antimicrobials when microorganisms are resistant to a particular drug may foster selection pressure hence developing resistance against another antibiotic; this has resulted in bacteria acquiring more resistant traits and ending up as multi-resistant organisms (MDRO). The use of one antibiotic or antibiotic class might select for resistance against several antibiotics/antibiotic classes if the AMR genes are on the same mobile genetic element (Nolte, 2014).

2.1.1 Antimicrobial Resistance as a Global and Regional Challenge

2.1.1.1 AMR Global Perspective

In May, 2015 at the 68th World Health Assembly (WHA), a global action plan (GAP) on AMR was adopted with a main goal of ensuring treatment and prevention of infectious diseases with quality-assured, safe and effective medicines (WHO Geneva, 2017). The five objectives outlined in the GAP are; 1) to improve awareness and understanding of AMR through effective communication, education and training; 2) to strengthen the knowledge and evidence base through surveillance and research; 3) to reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures; 4) to optimize the use of antimicrobial medicines in human and animal health; and 5) to develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other interventions (WHO Geneva, 2017).

In order to address the fourth objective in the GAP and support antibiotic stewardship at local, national and global levels, the WHO Expert committee developed the Aware classification of antibiotics, to guide the selection and use of essential medicines (WHO, 2019). Antibiotics were classified into three groups, Access, Watch and Reserve. This classification took into account the

impact of different antibiotics and antibiotic classes on AMR. This tool is beneficial for monitoring antibiotic consumption, defining targets and monitoring the effects of stewardship policies that aim to optimize antibiotic use and limit AMR(WHO, 2019).

The Food and Agriculture Organization (FAO) has emphasized the significance and earnestness of addressing the growing global threat of AMR in all countries through a coordinated, multisectoral, One Health approach in the context of the 2030 Agenda for Sustainable Development (FAO, 2021). Building resilient systems whose responsibility should be shared among farmers, herders, growers, fishers, prescribers and policy-makers in food and agriculture might limit the emergence and spread of AMR (FAO, 2021). Preventive actions could provide an economic benefit, especially when compared to the considerable percent of GDP expected to be lost if AMR is permitted to develop into a global emergency through the widespread failure of medicines (FAO, 2021). FAO's 2021-2025 Action plan has five key objectives that have been adopted to help focus efforts and accelerate progress and these are: 1) increasing stakeholder awareness and engagement, 2) strengthening surveillance and research, 3) enabling good practices, 4) promoting responsible use of antimicrobials and 5) strengthening governance and allocating resources sustainably (FAO, 2021).

AMR has become a public health concern, not only in terms of limited treatment options but also due to its economic burden (Nolte, 2014). AMR has affected the Global Gross Domestic Product (GDP), and a decrease of US$ 100 trillion is expected by 2050, with the cost being more than 50 times the expected economic output of sub-Saharan Africa (SSA) (Food and Agriculture Organization of the United Nations, 2016). In 2014, deaths from drug-resistant infections were at 700,000 and it was estimated that by 2050, AMR would be responsible for up to 10 million deaths annually if nothing was done to contain and prevent its spread, with about 4,150,000 deaths occurring in Africa ("WHO | World Health Assembly addresses antimicrobial resistance, immunization gaps and malnutrition," 2016). The recent findings in the Global burden of bacterial antimicrobial resistance (GRAM) study indicate a rise in estimated deaths from 700,000 in 2014 to 4·95 million deaths associated with bacterial AMR globally, including 1·27 million deaths attributable to bacterial AMR (Murray et al., 2022a). It has been reported that South Asia had one of the highest mortality records attributable to AMR at 21.5 deaths per 100,000 (Murray et al., 2022a).

The GRAM study provided a comprehensive assessment of the global burden of AMR and an evaluation of the availability of data from different countries/regions (Murray et al., 2022b). Estimating the burden of AMR and the leading pathogen–drug combinations contributing to it is crucial for making informed and country-specific policy decisions that address the objectives highlighted in the GAP (Murray et al., 2022b; WHO Geneva, 2017). There are serious data gaps in many low-income settings, where the burden is highest and the need to expand microbiology laboratory capacity and develop data collection systems is of utmost importance (Murray et al., 2022b). Country specific AMR data has helped countries develop and implement measures to prevent and control AMR, thereby reducing AMR and its clinical and economic negative effects (D'agata et al., 2018).

Measures that have been put in place to curb AMR include continuous surveillance of AMR trends and the causative pathogens, the introduction of a functional Antimicrobial Stewardship Programme (ASP), and strict regulation that restrict the purchase of antimicrobials over the counter (D'agata et al., 2018; Lopes-Júnior et al., 2015; Moura et al., 2015; Nathwani et al., 2019; Santa-Ana-Tellez et al., 2013). Sweden is one of the countries that has been successful in reducing the burden of AMR to relatively low use of antibiotics per capita (Public and Agency, 2010). Antibiotic consumption in Sweden has decreased substantially since the mid-1990s, a change that has been influenced by factors such as intentional work on both regional and national level, utilising lengthy practise of evidence-based guidelines and strong local commitment (Public and Agency, 2010). Many other countries have banned the use of antimicrobials as feed additives to control the scourge of AMR (Cha et al., 2018; Maron et al., 2013; McEwen and Fedorka-Cray, 2002).

2.1.1.2 Regional perspective

According to the GRAM study, SSA had the highest mortality attributable to AMR at 23.5 deaths per 100,000 and globally, Western SSA had the highest death rate at 27.3 deaths per 100,000, followed by Eastern, Central, and Southern SSA (Murray et al., 2022a). In contrast to high-income countries, the high death rate attributable to AMR in SSA can be attributed to the numerous challenges in implementing effective and sustainable AMR surveillance programmes. These challenges are possibly due to inadequate safe water, sanitation, and hygiene (WASH) infrastructure, abundant insect vectors of disease, inefficient infection prevention and control, poor waste disposal, poor regulation of antimicrobial use, and constrained healthcare systems (Elton et al., 2020; Kariuki et al., 2022; Musoke et al., 2021).

Despite the long-standing recognition of AMR in most of the low- and middle-income countries (LMICs), there is still remarkably poor data on geographical distribution, trends, prevalence and incidence over time, making the assessment of the health burden attributable to AMR difficult and lessening the evidence base to drive forward research and policy agendas to combat AMR (Hay et al., 2018). Surveillance data is inadequate in both the human and livestock sectors, but the latter tend to face more challenges. In resource limited countries in SSA, there is need to optimise use of resources allocated to the fight against AMR by using a One Health approach, and sharing experiences between countries (Elton et al., 2020). However, the main challenge of adopting the one health in AMR surveillance has been the need for more data on the actual burden of resistance, particularly in regions where surveillance is minimal and data are sparse (Elton et al., 2020). Even in situations where data exists, there are poorly developed mechanism for sharing data both at national and regional levels (Kariuki et al., 2022).

The main AMR data gaps in Africa include; the actual burden of AMR in the community, hospital settings, animals and the environment, as well as the microbial acquisition of AMR, transmission patterns, genotypic evolution of antimicrobial resistance mechanisms, clonal spread and asymptomatic carriage (Elton et al., 2020).

2.1.2 The Evolution of AMR in bacteria

2.1.2.1 Historic Account

In 1907, salvarsan was the first antimicrobial drug developed to treat syphilis, and in the same year, resistance to salvarsan was noted in mice (Zaffiri et al., 2012). Alexander Fleming discovered penicillin in 1928 (Stekel, 2018). The first sign of antibiotic resistance became apparent soon after the discovery of penicillin (Lobanovska and Pilla, 2017a). It was noted that even before the extensive use of penicillin, as far back as 1940, researchers had already observed the possibility of bacteria destroying penicillin by enzymatic degradation; in the same year, it was also assumed that AMR was also being caused by mutation and selection (Abraham and Chain, 1988; Lobanovska and Pilla, 2017b). By 1948, penicillin resistant Staphylococcus was found to be spreading, after which methicillin antibiotics were developed to combat penicillin-resistant Staphylococcus, unfortunately, one year later, the first strains of methicillin-resistant *Staphylococcus aureus* (MRSA) appeared (Zaffiri et al., 2012). Sulphonamide drugs were released for use in 1935, and the first reports of sulphonamide resistance in patients treated for gonorrhoea were recorded three years later in 1938 (Zaffiri et al., 2012). Antibiotics were introduced into

animal feed to promote growth in 1950, and in the same year, new modes of genetic exchange were found between bacteria causing AMR (Castanon, 2007). By 1970, numerous bacterial strains were found to be resistant to multiple antibiotics. Unfortunately, this finding was followed by a sharp drop in the development of new antibiotics in the 1990s (Fair and Tor, 2014). What is surprising is that AMR was only declared an emergency public health problem causing 700,000 deaths globally each year in 2014 (O'Neill, 2016; WHO, 2014).

2.1.3 Methods of AMR Detection

2.1.3.1 Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing (AST) helps determine whether the antimicrobials will be effective or not for the therapy of an individual patient against infectious diseases (Martinez, 2014). The commonly used guideline for the interpretation of AST results is the Clinical and Laboratory Standards Institute (CLSI), which is the most popular guideline and is based on pharmacokinetic–pharmacodynamics (PK-PD) properties and mechanisms of resistance (Kassim et al., 2016). The other guidelines used commonly, more so in European countries, uses interpretations based on PK-PD properties and the epidemiological minimum inhibitory concentration (MIC) cut-offs (ECOFFS) as determined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Kassim et al., 2016). Although some of the cut-offs in the CLSI and EUCAST guidelines are different, the results show comparable antimicrobial susceptibility patterns between CLSI and EUCAST breakpoints; given that EUCAST guidelines are freely available, it makes it easier for laboratories in resource-poor settings to have updated and readily available reference for interpreting antimicrobial susceptibilities (Kassim et al., 2016). Prompt AMR profiling is essential for the well-timed objective of treating infected patients and public-health surveillance (Yang and Rothman, 2004).

2.1.3.2 Phenotypic testing

The disc diffusion method is the gold standard for confirming susceptibility of bacteria (Balouiri et al., 2016) . Standardized disc diffusion was introduced by Bauer and Kirby's experiments in 1956, after finalizing all aspects of optimization by changing physical conditions (Balouiri et al., 2016). The advantages of using the Kirby-Bauer method are that it does not require special equipment to perform, it is easily interpreted and affordable, while disadvantages are the long time it takes to obtain results and the fact that it only gives qualitative results as susceptible, intermediate, or resistant hence unable to quantify the minimum concentration of antimicrobial needed to inhibit the apparent growth (Balouiri et al., 2016). Conventional AST methods are

limited by prolonged culturing time (48–72 h) and poor accuracy due to inconsistency in inoculum size and culturing conditions (Yang and Rothman, 2004)

2.1.3.3 Automated machine for identification and AST determination

Several automated machines are being used to identify organisms and determine AST. A study in China evaluated the performance of five commonly used automated AST systems, namely Vitek 2 (bioMérieux, Marcy-l'Étoile, France), Phoenix (Becton Dickinson Diagnostics, Sparks, MD, USA), MicroScan (Beckman Coulter, Brea, CA, USA), Tiandiren (Mindray TianDiRen, Changsha, China), and Dier (Zhuhai DL, Zhuhai, China) (Zhou et al., 2018). This study revealed that none of the five automated systems met the criteria for acceptable AST performance, but Vitek 2 provided a relatively accurate and conservative performance for most of the antimicrobials (Zhou et al., 2018).

The advantages of the Vitek 2 compact machine are the ability to provide rapid test results, less labour intensive, and its ability to determine the MIC, thus providing accurate, reproducible quantitative results, while the disadvantages are the high cost and a complicated pre-analytical process (Balouiri et al., 2016; Khan et al., 2019). MIC defines in vitro levels of susceptibility or resistance of specific bacterial strains to the tested antimicrobials. Reliable quantitative MIC results significantly impact the choice of a therapeutic strategy, which affects the efficiency of an infection therapy (Kowalska-Krochmal and Dudek-Wicher, 2021).

2.1.3.4 Molecular methods for identification and genotyping of pathogens

In order to interpret physiological characteristics of microorganisms, various culture dependent classical techniques such as pour plate and spread plate methods followed by Gram's staining and biochemical tests can be used (Das et al., 2014). The use of colony morphology on growth medium, microscopic observation and biochemical tests can assign bacteria to specific genera. However, these techniques are time consuming as well as dependent upon many environmental factors (Das et al., 2014). As a result, advanced techniques like sequence based, gel based and protein-based systems have become beneficial due to their fast reactions, high specificity and less chance of error and development (Cohan, 2002). These advanced techniques have changed drastically the definition of microorganisms as in many cases sequence diversity within a species reveals multiple sequence clusters that are ecologically distinct (Cohan, 2002). The prevalent conventional techniques are not sufficient to provide a complete draft for microbial taxonomy as these conventional techniques describe only shape, colour, size, staining properties, motility, host-range,

pathogenicity and assimilation of carbon sources, however, a comprehensive approach is required to provide descent of a microbial lineage (Prakash et al., 2007).

Genotyping of pathogens is very imperative in evaluating the global evolution and studying their genetic relatedness, data that can be used to determine their point source during epidemiological investigations (Sharma-Kuinkel et al., 2016). The introduction of molecular biology techniques enabled several identification and detection techniques of pathogens by amplifying specific conserved DNA sequences (Gohil et al., 2019). There have been remarkable modifications in the development of rapid and low-cost methods of identifying and genotyping pathogens, with the most recent being Whole genome sequencing (WGS) (Gohil et al., 2019). Although the cost of WGS has dramatically reduced, the cost is still relatively expensive, especially for LMICs like Zambia. A wide range of molecular techniques such as polymerase chain reaction (PCR), multiplex polymerase chain reaction, pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), restriction fragment length polymorphism (RFLP), Multilocus VNTR (Variable Number Tandem Repeat) analysis (MLVA), and whole-genome DNA sequence typing have been used to detect, speciate, type, classify and characterise pathogens (Adzitey et al., 2013; Sharma-Kuinkel et al., 2016).

i. Polymerase Chain Reaction (PCR)

PCR is one of the most efficient and rapid molecular tools for quantifying and profiling bacterially infectious genes. PCR is an in-situ DNA replication process that allows for the exponential amplification of target DNA in the presence of synthetic oligonucleotide primers and a thermostable DNA polymerase and may include the use of one primer (single PCR) or multiple primers (multiplex PCR) to detect bacterial isolates (Adzitey et al., 2013) . Other commonly used forms of PCR are real-time PCR, nested PCR and reverse-transcription PCR (Mo et al., 2012). A wide range of different concentrations or units of DNA templates (5–25 ng), Taq DNA polymerase (0.6–1.25 U), primers (0.11–10 μM), and temperature cycles (45–95.8 °C and 30–40 cycles) have been employed to detect or confirm bacteria (Lorenz, 2012). Deoxyribonucleotide triphosphates (dNTPs), magnesium (Mg^{2+}) and buffer solutions used at different concentrations to increase detection limits are other components of a PCR reaction (Adzitey et al., 2013).

Specific genotypic markers are essential for differentiating phylogenetic lineages for correlation within genomes of microorganisms. Varieties of house-keeping genes are targeted in different microbial entities to confer precise genetic fingerprints (Das et al., 2014). There are several genetic markers for rapid classification of gene families having conserved sequences that fall within

definite microbial clades. Different genetic targets for microbial identification are 16S rDNA, gyrB, rpoA, rpoB, rpoC, rpoD and 16S rRNA genes is the primary gene target for identification of bacteria as the gene sequences contain conserved, variable and hypervariable regions (Clarridge, 2004). 16S rRNA gene sequence analysis can better identify poorly described, rarely isolated, or phenotypically unusual strains and can lead to the recognition of novel pathogens and non-cultured bacteria (Clarridge, 2004). Some limitations of 16S rRNA are; inaccurate sequences in some databases, the lack of consensus quantitative definition of genus or species based on 16S rRNA gene sequence data, the increase of species names based on minimal genetic and phenotypic differences that raise communication difficulties, and microheterogeneity in 16S rRNA gene sequence within a species is common (Poretsky et al., 2014).

ii. Pulsed Field Gel Electrophoresis (PFGE)

PFGE was first developed by Schwartz et al. in yeast (Schwartz and Ft Cantor, 1964) and was previously considered as a gold standard due to its discriminatory power, reproducibility, ease of execution, data interpretation, cost, and availability (Sharma-Kuinkel et al., 2016). It is a powerful genotyping technique used for the separation of large DNA molecules (entire genomic DNA) after digesting with unique restriction enzyme and applying to a gel matrix under the electric field that periodically changes direction between spatially distinct pairs of electrodes and facilitates mega base (mb) size DNAs to reorient and migrate at different speeds through the gel pores towards the anode in a size dependent manner (Reed et al., 2007; Sharma-Kuinkel et al., 2016). The time required for reorientation is also inversely proportional to the size of DNA fragment. PFGE provides a good representation of the entire bacterial chromosome in a single gel with a highly reproducible restriction profile, providing clearly distinct and well-resolved DNA fragments (Sharma-Kuinkel et al., 2016). The obtained gel images are normalized and patterns of the DNA fragments analysed by BioNumerics Software following the criteria to interpret PFGE patterns developed by Tenover et al. These patterns serve a virtual barcode that determines the strain types and pathogen relatedness (Tenover et al., 1995).

iii. Multilocus sequence typing (MLST)

MLST was proposed in 1998 as a portable sequence-based method for identifying clonal relationships among bacteria (Maiden et al., 2013). It is considered to be the “gold standard” for typing of bacterial and fungal species and the MLST scheme identifies internal nucleotide sequences of approximately 400 to 500 bp of six or seven well-conserved, house-keeping genes or loci within the bacterial genome (Das et al., 2014). MLST was designed to accommodate the

conflicting signals of vertical and horizontal genetic transfer that are present in bacterial populations by examining the genome at multiple 'housekeeping' gene loci (Maiden et al., 2013). Unique sequences (alleles) are assigned as a random integer number and a unique combination of alleles at each locus. Allelic variation at each locus is catalogued, and a sequence type or lineage is assigned by comparing the set of alleles to other isolate profiles in the database (Larsen et al., 2012) . Although MLST is an important tool for understanding the molecular evolution of bacteria, it does not typically have the necessary resolution for outbreak investigations and in these cases MLST can be supplemented with additional typing schemes that index more variable loci, such as antigen genes or variable-number tandem repeats (VNTRs) (Adair et al., 2000; Dingle et al., 2008).

iv. Multilocus variable-number tandem repeat (VNTR) analysis (MLVA)

VNTR/MLVA is a fast, portable method that analyses multiple VNTR loci by targeting short tandem repeats (TRs), which are areas of the bacterial genome that evolve rapidly. Targeting of these elements, which often vary in number among different strains of the same species (the definition of a variable-number TR [VNTR]), has successfully been used to discriminate between strains of prokaryotes (Noller et al., 2003). MLVA was performed by comparing the number of tandem repeats at seven loci thereby providing a powerful tool for assessing the genetic relationships between bacterial strains of the same species (Noller et al., 2003).

v. Restriction fragment length polymorphism (RFLP)

RFLP refers to differences (or variations) among microorganisms in their DNA sequences at sites recognized by restriction enzymes. The variation results in different sized (or length) DNA fragments produced by digesting the DNA with a restriction enzyme is one of the easiest ways to study the diversity of the microbes. This technique uses the simple restriction digestion of purified DNA from bacteria, and variation in the banding pattern in the digestion reveals the genetic diversity (Das and Dash, 2019).

vi. Whole Genome Sequencing (WGS)

The recent improvements in WGS sequencing technologies and analysis tools (expansion of the targets in various databases) have reduced the overall costs and increased the output, thereby paving way for its use in healthcare delivery to diagnose, monitor, treat, predict, prevent disease and provide a more comprehensive range of ARGs for identification (Bilkey et al., 2019; Quainoo et al., 2017; Waseem et al., 2019). The use of genomic investigation in collaboration with clinical and epidemiological data has transformed clinical practice and public health by providing a deeper

understanding of pathogens, their genetic evolution and circulation in communities/populations (*Global genomic surveillance strategy*, 2022). WGS of pathogens enables the sources and patterns of transmission to be identified; this can be in a hospital or community setting, as well as in an outbreak (Bilkey et al., 2019).

When WGS is combined with phylogenetic analysis, it provides new insight into disease spread and transmission by determining mechanisms and possible modes of inter-species and/or cross-species transmission, the population at risk and patient-to-patient transmission (Sintchenko and Holmes, 2015). Continuous structured and systematic genomic surveillance can enhance strategies for disease control by identifying determinants of transmission, monitoring pathogen evolution and adaptation, and ensuring precise and timely diagnosis of infections, especially those with epidemic potential (Sintchenko and Holmes, 2015).

The use of molecular/genomic methods plays an essential role in monitoring the spread of resistant organisms or resistance genes throughout the hospital and community (Fluit et al., 2001). WGS provides information on the genetic basis of resistance mechanisms, as well as AMR evolution in the different pathogens. WGS can be a valuable addition to phenotypic surveillance of AMR in LMICs where the burden is high, and there is a lack of access to alternative antibiotics (Vegyari et al., 2020). Currently, only high-income countries (HICs) widely use WGS, while LMICs have been disadvantaged due to its high cost and complexities, such as lack of comprehensive national policies, a limited number of well-trained genomic scientists and poor research infrastructure (Tekola-Ayele and Rotimi, 2015). In order to produce an accurate global picture of AMR and given its potential to inform national and international action plans against AMR, establishing WGS as a surveillance tool in LMICs is of utmost importance (Vegyari et al., 2020). A robust genomic surveillance of AMR infections is key to inform policy and heighten public health interventions to prevent and control AMR.

Although molecular/genomic methods are being advanced for identifying genetic mechanisms of antimicrobial resistance genes (ARGs) and clinical applicability of resistance profiling, they are incapable of defining MIC; some ARGs could be missed in terms of sensitivity and coverage since molecular/genomic methods can only detect resistance that is searched for and has previously been included in the databases and not the newly developed (Kaprou et al., 2021). The massive diversity of different ARGs poses a challenge in assay development due to the cost involved; thus competing with phenotypic assays is sometimes difficult (Kaprou et al., 2021).

2.2 The Challenges of Antimicrobial Resistance in the Human health sector

AMR challenges have been observed in both clinical conditions and community carriage. In clinical settings, commonly identified infectious agents have developed resistance to readily available antimicrobials (Pokharel et al., 2019). The several factors that promote the rise in AMR can be grouped into political, economic, technological, sociological and industry (Pokharel et al., 2019). Weak governance often leads to lack of attention to health systems and weakened regulations for antimicrobial stewardship (AMS) (Raut and Adhikari, 2016). Poor AMS and inappropriate antimicrobial use often in substandard doses, challenge the efforts to contain the emergence and spread of AMR (Pokharel et al., 2019). Further budgetary constraints limit the prioritisation of AMR surveillance, a necessity to inform policies and to respond to both the emerging threats and the long-term trends in resistance (Raut and Adhikari, 2016).

The lack of infrastructure due to poor economy, corruption and low preparedness in many LMICs has led to inadequate attention to preventive measures, such as water, sanitation and hygiene, leading to high burden of infectious diseases (Willis and Chandler, 2019). Health systems in LMICs often lack resources (functional and infrastructural) to reach a large population, especially in rural areas, universal access to primary healthcare services becomes a major challenge (Raut and Adhikari, 2016).

The lack of diagnostic capacity and stock outs of reagents to inform the appropriate prescription of antimicrobials at the point of care, easy access to over the counter antimicrobials and the treatment of infections empirically are common occurrences in LMICs (Pokharel et al., 2019). These social factors and cultural practices, combined with poverty, further leads people to self-medicate against common infections and buy medications from unregulated drug dispensaries, visit traditional practitioners and borrow medicines from their neighbours (Green et al., 2023). Practices that further intensify AMR in the communities. The diminished production of newer antibiotics by pharmaceutical industries and growing AMR has resulted in very minimal options for treatment of infections caused by multidrug resistant (MDR) pathogens and with only expensive and are unaffordable antibiotics for many LMICs (O'Neill, 2016).

AMR in Clinical Conditions

There are several clinical conditions demanding management using antimicrobials. Among those posing challenges are BSI, UTI, and diarrhoea. A major concern has been the emergence and global spread MDRO, including methicillin resistant *Staphylococcus aureus* (MRSA),

vancomycin-resistant *Enterococcus* spp. (VRE), and MDR Gram-negative bacilli (GNB) (including extended-spectrum-β-lactamase [ESBL] producers), carbapenem-resistant *Enterobacteriaceae* (CRE), and MDR non-lactose fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter* species (Daniel J Diekema et al., 2019). Several studies have demonstrated the high mortality attributable to BSI due to these MDR organisms (Goto and Al-Hasan, 2013; Hurley et al., 2003; Matthew E. Falagas et al., 2014; Verway et al., 2022).

UTIs are one of the most common infections and the second most common clinical indication for empirical antimicrobial treatment in most primary and secondary healthcare facilities (McCowan et al., 2022a). Frequently, antimicrobial therapy for UTI are initiated empirically before culture results are available due to limited or poor diagnostic capacity, mostly in LMICs (Sharma et al., 2022). Most prescriptions are considered to be inappropriate and regularly consist of broad-spectrum antimicrobials with amoxicillin, ciprofloxacin, amoxicillin/clavulanic acid, nitrofurantoin, trimethoprim and sometimes third generation cephalosporins being the commonly prescribed (Aubrey C Kalungia et al., 2022; Mudenda et al., 2022a). AMR has been recognised as a concern for the future treatment of infections, leading to repeat prescriptions, continued symptoms, complications, increased use of broad-spectrum antimicrobials and mortality (McCowan et al., 2022a).

Other clinical conditions that are frequently treated with antibiotics inappropriately at primary healthcare facilities are diarrhoea and respiratory tract infections (RTIs). Diarrhoeal diseases being the second most common cause of death amongst children, and claiming 1.1 million lives each year impose a substantial burden on LMICs (Wall, 2019). Sixty percent of these deaths are associated with inadequate access to safe water and sanitation (Wall, 2019). Antibiotics are often administered to treat diarrhoea and acute respiratory infections, although in many cases these conditions are viral and do not need antibiotics (Frost et al., 2018). Inappropriate antibiotic prescribing in children in LMICs has increased from 42% before 1990 to 72% in 2006–2009 for viral upper respiratory tract infections (Holloway et al., 2014).

In LMICs, diagnostic tests are often not affordable, accessible, or reliable, and human resources and laboratory infrastructure may not be available (Frost et al., 2018). In most cases where diagnostic capacity are unavailable and first-line antibiotics are cheap and probably substandard or falsified, it can seem more cost-efficient to treat empirically (James A Ayukekbong et al., 2017;

Frost et al., 2018). A practice that worsens the emergence and spread of AMR and further limits treatment options for more invasive infections (James A Ayukekbong et al., 2017).

2.2.1 Bloodstream infections (BSIs) –Bacteraemia

BSIs are demonstrated by the positivity of one or more blood cultures (Viscoli, 2016). Primary bacteraemia is defined as bacteraemia without a documented source of infection, while secondary bacteraemia develops from an evident area of infection as the source; an example of such is a UTI with subsequent bacteraemia (Álvarez-Moreno et al., 2016). BSI can be either community or hospital acquired infections (HAI), also referred to as nosocomial or health-care-associated infections (HCAI) (Jennifer Cheung, 2021). HCAI/HAI are infectious diseases not present at admission but acquired in a healthcare facility after about 48 hours of admission (Jennifer Cheung, 2021). It has been estimated that approximately 30% of hospital-acquired BSIs are due to Gram-negative bacteria (GNB), most of which are caused by MDRO and linked to 15% to 29% higher mortality (Al-Hasan et al., 2012; Daniel J. Diekema et al., 2019). This is especially true for infections caused by GNB harbouring ESBLs or carbapenemases (Kern and Rieg, 2020).

BSIs are a severe and life-threatening illness that is still one of the most common causes of hospital admissions, exceeding that of acute myocardial infarction in the United States (Angus et al., 2001). BSIs are also a leading cause of morbidity and mortality among hospitalised patients, especially when preceded by severe sepsis or septic shock (Fleischmann et al., 2016; Timsit et al., 2020). The outcome of BSI is dependent on host-specific factors such as age, underlying comorbidities and immune status as well as characteristics of the causative agent such as the type of pathogen and its antibiotic susceptibility profile (Del Bono and Giacobbe, 2016). Co-morbidities such as human immunodeficiency virus (HIV) and malnutrition contribute to an increased risk of mortality and other adverse health outcomes (Fuseini et al., 2021). This situation is more pronounced in SSA where two-thirds of the estimated 38 million persons with HIV worldwide reside (Fuseini et al., 2021). Initiation of Antiretroviral, improving dietary provision and prompt initiation of appropriate antimicrobial therapy based on the pattern of local antibiotic resistance is key in significantly improving patient outcomes and reducing mortality rates in bacteraemic patients (Del Bono and Giacobbe, 2016; Fuseini et al., 2021)

A 20-year microbiology trend from the SENTRY Antimicrobial Surveillance Program that consecutively collected BSI organisms from over 200 medical centres in 45 nations between 1997 and 2016 found the most common pathogens to be *S. aureus* and *E. coli* (together accounting for over 40% of BSIs) (Daniel J Diekema et al., 2019). *Klebsiella pneumoniae*, *Pseudomonas*

aeruginosa, and *Enterococcus faecalis* were other commonly isolated pathogens (Daniel J Diekema et al., 2019). In the early years, *E. coli* was the second highest causative agent after *S. aureus* but subsequently the most common after 2005. The prevalence of VRE was stable after 2012, this was however different from the MDR *Enterobacteriaceae* prevalence, which increased from 6.2% in 1997 to 2000 to 15.8% from 2013 to 2016 (Daniel J Diekema et al., 2019). The study concluded that during this 20-year surveillance period*, E. coli* and *S. aureus* were the predominant causes of BSI worldwide (Daniel J Diekema et al., 2019). Important resistant phenotypes such as VRE were stable or declining, while the prevalence of MDR-GNB increased continuously, thereby causing treatment challenges among common bacterial BSI pathogens (Daniel J. Diekema et al., 2019). Another population-wide study of 252,343 blood culture episodes, and 22,935 BSIs among 19,326 patients assessed the prevalence and mortality of all BSI organisms and found *E. coli* to be most commonly isolated, followed by *Staphylococcus* species and *Streptococcus* species (Verway et al., 2022). This study also demonstrated the mortality associated with each organism and revealed that *E. coli*, *S. aureus*, and coagulase negative Staphylococcus (CoNS) were associated with the largest number of deaths (Verway et al., 2022).

Inappropriate management of BSI can lead to sepsis, a life-threatening condition resulting from a dysregulated immune response to infection that leads to organ dysfunction (Rhee et al., 2019). Sepsis disproportionately affects neonates, pregnant women, elderly patients with severe comorbidities and people living in LMICs (Cheng et al., 2008). Although sepsis is treatable, AMR has contributed to longer hospital stays, morbidity and mortality (Goldstein et al., 2019). The World Health Organization (WHO) acknowledged sepsis as a significant public health problem in 2017 and called all the United Nations (UN) member states to improve sepsis prevention, diagnosis and management (WHO, 2017). In 2020, WHO estimated that approximately 20% of all-cause global mortalities were due to sepsis, affecting 49 million people and causing 11 million deaths globally every year (WHO, 2017; World Health Organisation, 2020).

Understanding the true burden of sepsis is complicated by many factors, and in order to reduce the global burden of sepsis, comprehensive global data on incidence and mortality is essential. However, despite advances in care, existing epidemiologic studies suggest that sepsis remains a huge burden across all economic regions, more so in LMICs (Del Bono and Giacobbe, 2016; Fleischmann et al., 2016). Findings from a global systematic review found that studies on population-level incidence and case-fatality rates for sepsis and severe sepsis are scarce and none existent for LMICs (Fleischmann et al., 2016). Notably, sepsis incidence and mortality are highest

in LMICs where data are lacking and poorly described; this is despite the sepsis problem being assumed to be enormous in SSA due to high rates of other infectious diseases (Cheng et al., 2008; Lewis et al., 2019; World Health Organisation, 2020). The limited health access, financial constraints and the lack of diagnostic capacity in LMICs has led to insufficient data on sepsis aetiology and outcomes (Rudd et al., 2018).

There are a few BSI and sepsis studies that have been done in Zambia, all of which were conducted at the same hospital. One study determined the aetiology, AMR and risk factors associated with neonatal sepsis in a Large Referral Centre in Zambia in 2016 (Kabwe et al., 2016). The study found *Klebsiella* species to be the most prevalent, with antibiotic resistance ranging from 96%–99% for WHO-recommended first-line therapy (gentamicin and ampicillin/penicillin) to 94%–97% for third-generation cephalosporins (Kabwe et al., 2016). Mortality was at 93% for late-onset sepsis and 82% for early-onset sepsis. Another study in 2011 that evaluated clinical characteristics, management, and outcomes of sepsis in Lusaka, Zambia found overall in-hospital mortality at 40.4% and in-hospital mortality for severe sepsis at 54.9% (Chimese et al., 2011). A study in 2018 on preventing BSI and death in Zambian neonates only examined the impact of a low-cost infection control bundle (Hamer et al., 2019).

2.2.2 Urinary tract infections (UTIs)

Data from the Global Burden of Disease Study 2019 were analysed to describe the incidence, mortality, and disability-adjusted life years (DALYs) of UTIs in 204 countries and territories from 1990 to 2019 by socio-demographic status, nations, region, sex, and age (Yang et al., 2022). Globally, 404.61 million cases, 236,790 deaths, and 520,200 DALYs were estimated in 2019 and there was a 2.4 times growth in deaths from 1990 to 2019, along with an increasing age-standardized mortality rate (ASMR) from 2.77/100,000 to 3.13/100,000 (Yang et al., 2022). Age-standardized incidence rate (ASIR) was consistently pronounced in regions with higher socio-demographic index (SDI), which presented notable upward trends in ASMR and age-standardized DALY rate (ASDR) (Yang et al., 2022). UTIs are among the most common infections encountered in outpatient and inpatient settings (Claeys et al., 2022). Despite being common, appropriate diagnosis and management of UTIs remain a challenge. Patients with asymptomatic bacteriuria (ASB) are prescribed unnecessary antibiotic therapy; these are patients with positive urine cultures but without urinary symptoms (Flokas et al., 2017). Antibiotic treatment in ASB and self-medication increase the risk of inappropriate use and the selection of resistant bacteria (Grigoryan et al., 2007).

Uncomplicated UTI is a bacterial infection of the bladder and associated structures in patients with no structural abnormality and comorbidities, such as diabetes, immunosuppression, or pregnancy (Swamy et al., 2020). Uncomplicated UTI is also referred to as cystitis or lower UTI and is most common in women than men because the pathogenesis involves pathogenic bacteria ascending from the perineum and rectum, predisposing women to UTIs (Kumar, 2019). Women also have shorter urethras than men, which further contributes to their increased susceptibility to UTIs (Kumar, 2019). Factors that predispose one to a UTI are the presence of anatomical abnormalities of the urinary tract, urinary catheters, frequent manipulation of the urethra and pelvic exams, sexual intercourse and the use of spermicides and diaphragms (Barski and Otto, 2014). Symptoms range from increased micturation, urgency, suprapubic discomfort, and dysuria (Kumar, 2019).

Most cases of uncomplicated UTIs may resolve naturally, without antimicrobials, but many patients seek therapy or self-medicate for symptom relief (Grigoryan et al., 2007; Kumar, 2019). Antibiotic treatment is aimed at preventing complications such as the spread to the kidneys or developing into upper tract disease called pyelonephritis, which is a bacterial infection causing inflammation of the kidneys (Long and Koyfman, 2018). In most countries, *E. coli* is the most common causative agent in uncomplicated UTIs, causing 80–90% of all episodes of UTIs (Barski and Otto, 2014). UTI diagnosis is based on clinical history, symptoms and urinalysis, with confirmation by a urine culture from a properly collected urine sample. A good, clean catch of mid-stream urine is vital for accurate results without contamination. Urine should be sent to the laboratory immediately or refrigerated at 2-8°C because bacteria proliferate when the sample is left at room temperature, causing an overestimation of the infection's severity. This is because urine is an ideal medium for bacterial growth (Kumar, 2019).

Further, data from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), indicates 64.89 thousand deaths in 2019 were attributed to UTI caused by bacterial AMR in UTI while 260,000 thousand deaths could be associated with the infection (Li et al., 2022) . The all-age death rates were higher in southern Latin America, tropical Latin America, and Europe and lower in SSA (Li et al., 2022). *E. coli* and *Klebsiella pneumoniae* accounted for more than 50% of deaths attributable to and associated with AMR. Resistance was high among multiple types of antibiotic classes, including fluoroquinolones, carbapenems, and third-generation cephalosporins (Li et al., 2022). Two pathogen-drug combinations caused more than 6000 resistance-attributable deaths: third-generation cephalosporin-resistant *E. coli* and fluoroquinolone-resistant *E. coli* (Li et al., 2022).

A study done in Lebanon found that UTIs resulting from antibiotic-resistant *E. coli* had a high economic burden and health strain compared with susceptible isolates (Iskandar et al., 2021b). Despite the high AMR in developing countries, data are scarce and limited (Iskandar et al., 2021b). In 2020-2021, a cross-sectional study at two tertiary hospitals in Freetown, Sierra Leone (2017–21) found *E. coli* to be the most prevalent with high levels of AMR, especially for trimethoprim-sulfamethoxazole (47%), nalidixic acid (44%), nitrofurantoin (32%) and cefotaxime (36%). Fifty-five per cent bacterial isolates showed MDR, especially *E. coli* (58%) (Campbell et al., 2022). A study in central South Africa that analysed the causative pathogens and antibiotic resistance in community-acquired UTI found trends that reflect global trends, where *E. coli* was the most isolated (Fourie et al., 2021). The resistance to commonly prescribed oral antibiotics was 77.1% to amoxicillin, 15.6% to amoxicillin-clavulanate, 18.5% to ciprofloxacin, 4% to nitrofurantoin and 11% to trimethoprim-sulfamethoxazole (TMP-SMX), indicating that TMP-SMX and nitrofurantoin can be used safely as alternatives to first-line ciprofloxacin (Fourie et al., 2021).

A study at Levy Mwanawasa University Teaching Hospital (LMUTH) in Lusaka, Zambia, evaluated AMR among Pregnant Women attending the Antenatal clinic and presenting with UTI and observed a high prevalence of UTI at 60%, with the most isolated bacteria being *E. coli* (59%) and *Klebsiella* (21%) (Yeta et al., 2021). Resistance was highest in nalidixic acid (88.3%), ampicillin (77.8%), and norfloxacin (58.5%) (Yeta et al., 2021). Another Zambian study in a different province at Kitwe Central Hospital found a frequency of UTI at 44% with an overall sensitivity pattern indicating *E. coli* to be primarily sensitive to ciprofloxacin (70%), norfloxacin (64%) and cefotaxime (61%) and least sensitive to co-trimoxazole (13%) (Chisanga et al., 2017).

AMR is on the rise in both community, hospital acquired infections, and the vast majority of these prescriptions are issued in primary care where diagnostic facilities are lacking, rather than secondary or tertiary settings with functional diagnostic capacity. In order to curb the increasing burden of AMR, all countries need to implement effective AMS strategies that will tackle the overuse and misuse of antibiotics. Additional health care costs due to AMR are driven by a variety of factors such as prescription of ineffective antibiotics, delayed initiation of antimicrobial therapies, and the severity of resistant infections and the additional care they require (Fabiana Meijon Fadul, 2019). Therefore, it is important that AMS strategies focus on both community settings and target the relevant stakeholders providing and accessing community-based care and hospital settings.

2.2.3 Carriage of AMR genes in a healthy community: Non-clinical isolates

There is an abundance of commensal bacteria that colonise the gastrointestinal tract of humans and animals, however the abundance and ubiquitous nature of *E. coli* and *Enterococcus* have made them the mot studied pathogens in the One Health continuum (Silva et al., 2012). The composition of the microbial community in the intestine is thought to reflect the co-evolution of the host and bacteria to achieve a balanced, mutually beneficial state (Littman and Pamer, 2011). These commensal bacteria play important roles such as; (1) maintaining human nutrition and health by promoting nutrient supplies, (2) preventing pathogen colonisation and maintaining the homeostasis of the intestinal immune system (Wu and Wu, 2012). As a result, the immune system and the commensal bacteria form a symbiotic system in the intestine (Littman and Pamer, 2011; Tanoue et al., 2010). When pathogenic bacteria invade the host, the intestine's immune system can distinguish commensal bacteria from pathogenic ones, only attacking those hazardous to the host (Tanoue et al., 2010). Both *E. coli* and *Enterococcus* species are opportunistic pathogens that can cause urinary tract and bloodstream infections (Braz et al., 2020; Zhou et al., 2020a).

AMR in commensal bacteria could contribute to an increase in AMR among pathogenic bacteria through the horizontal transfer of resistance genes (Szmolka and Nagy, 2013). This cross-transmission of AMR from commensal bacteria to pathogenic bacteria and vice versa may later cause infections and lead to community or hospital-acquired infections caused by resistant pathogens (Shrestha et al., 2022). Awareness of resistant patterns and the burden of MDR among commensal bacteria could help predict the resistance profile of a subsequent clinical infection (Shrestha et al., 2022). Resistance genes have been found to be prevalent in the faecal *E. coli* strains from healthy individuals; hence, AMR surveillance programs have highlighted the importance of assessing resistance patterns in the commensal intestinal bacteria so as to estimate AMR trends in the communities (Li et al., 2014).

The escalating predominance of MDROs in the community and increasing incidence of community-associated AMR infections pose a significant threat to public health (van Duin and Paterson, 2016). A study that was done in Siem Reap, Cambodia, compared hospital-associated households, in which an index child age group 2–14 years had been hospitalised for at least 48 h in the preceding 2–4 weeks, with matched community households on the same street, in which no other child had a recent history of hospitalisation recorded (Shweta R. Singh et al., 2020). A high prevalence of ESBL-Enterobacterales was observed across both household types thereby suggesting that MDRO reservoirs are common in the community (Shweta R. Singh et al., 2020).

In this study the carriage of ESBL-producing *E. coli* was 92.8% while 44% ESBL-producing *K. pneumoniae* were co-colonised with ESBL-producing *E. coli* (Shweta R. Singh et al., 2020).

A systematic review in the Asia-Pacific region comparing young children aged 2–6 months with children aged 7–60 months identified antibiotic use within the past three months, day-care attendance and hospital admission within the past as significant risk factors for AMR bacterial carriage whilst breastfeeding and concurrent colonisation of *S. pneumoniae* were protective factors (Chan et al., 2022). Contrary to the finding in Cambodia, a study on the carriage of antimicrobial-resistant bacteria in a high-density informal settlement in Kenya identified unsanitary living conditions as a significant player in the transmission of resistant bacteria while antibiotic use had little explanatory power on the prevalence of AMR (Omulo et al., 2021).

A study in Sydney, Australia, found that commensal *E. coli* of healthy individuals represent an important reservoir for numerous antibiotic-resistance genes in many combinations (Bailey et al., 2010). Further, another study used systematic reviews and meta-analyses (PRISMA) guidelines to synthesise studies conducted from 1989 to 2020 to investigate the prevalence of antibiotic resistance in the top ten antibiotics commonly prescribed in LMICs (Nji et al., 2021). The study found a high prevalence of antibiotic resistance in commensal *E. coli* from healthy humans in the LMIC community settings, suggesting that commensal *E. coli* could be the main reservoir for spreading antibiotic resistance to other pathogenic enteric bacteria via Mobile Genetic Elements (MGEs) (Nji et al., 2021).

A prospective point-prevalence study in 2015-2016 conducted at a hospital in Eastern province in Zambia found high levels of resistance in outpatients that had no hospital contact or antibiotic use in the past two weeks (Nagelkerke et al., 2017). In this study, resistance to gentamicin (90%), ciprofloxacin (78%), ceftriaxone (60%), all of which were ESBL-producers was recorded (Nagelkerke et al., 2017). Another study that studied the evidence of community-wide spread MDR *E. coli* in young children in Lusaka and Ndola Districts in Zambia revealed high level (≥ 50%) of resistance to ampicillin, cefotaxime and co-trimoxazole (Bumbangi et al., 2022).

2.3 The Challenges of antimicrobial Resistance in Food Producing Animals: Poultry sector

Globally, the demand for protein from food-producing animals such as chickens for human consumption has been rising at unprecedented rates (Van Boeckel et al., 2015). This is also true in

LMICs now that there is some level of improved income (Tilman et al., 2011). The increased human demand for food, especially in countries with large populations, has placed pressure on farmers to meet the demands and has also introduced modern animal production practices that involve the use of antimicrobials as growth promoters or as a prophylactic or therapeutic measure (Rabello et al., 2020; Van Boeckel et al., 2015). A significant fraction of antimicrobials used in food-producing animals includes antimicrobials that are critical in the treatment of common infections, and as prophylaxis for major surgeries in human medicine (Aarestrup, 2015). Therefore, AMR in animals can lead to untreatable infections in both humans and animals (Laxminarayan et al., 2013; Scott et al., 2018, 2019a).

ESBL-producing *E. coli* (ESBL-Ec) emerging on farms and/or abattoirs can disseminate directly to occupationally exposed workers and indirectly through the food chain via contact with or consumption of contaminated food products (Wadepohl et al., 2020). This direct and indirect transmission of ESBL-Ec facilitates the likelihood of their subsequent entrance and spread into communities and hospitals (Founou et al., 2022). This is further exacerbated by international travel and the globalisation of trade in animals and food products (Hodges and Kimball, 2005).

In the United States, antimicrobial use in food animals is estimated to account for 80% of the nation's annual antimicrobial consumption (Van Boeckel et al., 2015). The widespread inappropriate use of antimicrobials in food-producing animals causes natural selective pressure, thereby giving rise to antimicrobial-resistant bacteria (ARB) (Manyi-Loh et al., 2018). ARB of animal origin can be transmitted to humans through the environment and food products (Manyi-Loh et al., 2018; Vieira et al., 2011). Although direct causality is difficult to establish due to the ecological nature of antibiotic selection pressure, studies have shown a close association between the prevalence of livestock-associated ARB in animals and in humans (Aarestrup, 2015, 2005; Vieira et al., 2011). This was partly demonstrated in an ecological study in Europe that revealed a high correlation between resistant *E. coli* isolates from food-producing animals (especially poultry and pigs) and humans, thereby supporting the hypothesis that a large proportion of resistant *E. coli* isolates causing BSIs in people may be derived from food sources (Vieira et al., 2011). Another study revealed a correlation between the levels of antimicrobial use in animals at a population level and the prevalence of ARB in animals and humans (Aarestrup, 2005; Vieira et al., 2011).

A study in seven European countries, namely; Norway, Sweden, Denmark, Austria, Switzerland, The Netherlands, and Belgium, showed a strong correlation between consumption levels for eight

classes of antimicrobials and the prevalence of antimicrobial-resistant commensal *E. coli* from different food-producing animals (Chantziaras et al., 2014). Furthermore, additional works suggested that repeated exposure to low doses of antimicrobial agents such as is the case in growth-promotion and prophylactic use, creates ideal conditions for the emergence and spread of ARB in animals (You and Silbergeld, 2014).

Findings on AMR in food-producing animals in LMIC countries, including India, northeast China, Kenya, Uruguay, and Brazil, confirmed a rise in resistance to commonly used antimicrobials (You and Silbergeld, 2014). An African perspective literature review on antimicrobial use and resistance in food-producing animals documented antimicrobial use in animal production ranging from 77.6% in Nigeria to 100% in Tanzania, Cameroon, Zambia, Ghana and Egypt (Kimera et al., 2020). The most used antibiotics were tetracycline, aminoglycoside and the penicillin group, and the prevalence of MDR isolates ranged from 20% in Nigeria to 100% in South Africa, Zimbabwe and Tunisia. Of note is that none of the countries documented national antimicrobial use and resistance surveillance system in animals. The high prevalence of AMR and MDR on the continent, coupled with weak AMR surveillance systems in the region, is a significant concern to human and animal health (Kimera et al., 2020). Data on ESBL-Ec in food-producing animals from different African countries shows variable results (Alonso et al., 2017).

The first study in Zambia to determine AMR in poultry revealed high levels of resistance to critically important antimicrobials, MDR and ESBL-Ec isolates, confirming that poultry can be one of the major and potential reservoirs for the antimicrobial-resistant genes which could spread into the food chain (Chishimba et al., 2016). Similarly, two studies that estimated antimicrobial resistance in *E. coli* and *Salmonella* isolated from broiler chickens at retail, farms, abattoirs, and open markets in four different provinces in Zambia revealed resistance to several antibiotics of both animal and human importance with similar patterns at all levels (Muligisa-Muonga et al., 2021; Phiri et al., 2020). The findings from these studies suggest inappropriate antimicrobial usage at all the different stages of animal food production (Ma et al., 2021).

2.4 One Health approach to the fight against AMR

The fight against AMR requires a “One Health” approach that involves research, surveillance, and interventions across all sectors; human, veterinary, agricultural and environmental sectors (Velazquez-Meza et al., 2022). Improved education and increased public awareness can reduce the

number of antimicrobials prescribed and/or abused (Harbarth et al., 2015). Surveillance systems monitoring infections need to be expanded to include antimicrobial use, as well as the emergence and spread of AMR within clinical and environmental samples (Velazquez-Meza et al., 2022). Adherence to practices to prevent and control the spread of infections should be mandatory to reduce the requirement of antimicrobials in general use and agriculture (Aarestrup, 2015).

Antibiotics need to be banned as growth promoters for farm animals in countries where it has not yet been done as AMS programmes in animal husbandry have proved to be efficient for minimising AMR, without compromising productivity (Harbarth et al., 2015). Reducing the misuse/overuse of antibiotics in humans, requires new tools with a good turn-around time to provide highly specific diagnosis of pathogens so as to decrease diagnostic uncertainty and improve clinical management (Harbarth et al., 2015; Wall, 2019). Finally, functional AMS programmes in all healthcare facilities and a 'One health' strategy to address regulation, independent monitoring and policing (Wall, 2019).

2.5 AMR in bacteria and target bacteria

2.5.1 Mechanisms of AMR development

Understanding antimicrobial agents' mode of action is important before exploring AMR development mechanisms. The mode of action of antimicrobial agents can be placed into five groups; (1) inhibition of cell wall synthesis; (2) depolarisation of the cell membrane; (3) inhibition of protein synthesis; (4) inhibition of nucleic acid synthesis; and (5) inhibition of metabolic pathways in bacteria (Kırmusaoğlu et al., 2019). Similarly, the AMR mechanisms occurs at the different sites of action and fall into four different categories: (1) limitation of drug uptake by inactivating the porin channel; (2) modification of drug targets; (3) neutralisation of antibiotic efficacy through enzymatic action (4) active drug efflux (Martinez, 2014; Reygaert, 2018).

AMR can be either intrinsic/natural or acquired. Intrinsic resistance is defined as characteristics shared universally within a bacterial species, not related to horizontal gene transfer but transmitted to progeny vertically and independent of previous antibiotic exposure (Partridge, 2015). On the other hand, acquired resistance occurs through mutations of a chromosomal gene or the acquisition of MGE (Cloeckaert et al., 2017; Cox and Wright, 2013). Acquisition of genetic material may be temporary or permanent. Resistance is conferred through the three main horizontal gene transfer (HGT) mechanisms: transformation by extracellular DNA, transduction through bacteriophages, and conjugation by plasmids (Lerminiaux and Cameron, 2019). The most common route of resistance gene acquisition is through plasmid-mediated transmission allowing genetic material to

jump between strains and species (Lerminiaux and Cameron, 2019). Additionally, the bacteria may experience mutations to its own chromosomal DNA (Reygaert, 2018). Mutations that aid in AMR usually occur in four types of genes; (1) those encoding drug targets, (2) those encoding drug transporters, (3) those encoding regulators that control drug transporters, and (4) those encoding antibiotic-modifying enzymes, (Martinez, 2014).

Identification and classification of plasmids is based on genetic traits that are contantly present. In the recent past, a number of incompatibility (Inc) groups have been identified with the most prevalent belonging to the IncI and IncF groups (Carattoli, 2009). The IncI and IncF plasmids producing type I pili and type F pili respectively (Carattoli, 2009). A number of major plasmid families have been found to occur in *Enterobacterales,* some of which are HI2, HI1, I1-γ, X, L/M, N, FIA, FIB, FIC, W, Y, P, A/C, T, K, B/O, FrepB and FIIA (Carattoli, 2009). Several plasmids, comprising those that carry AMR genes and genes encoding increased virulence, are transmissible by conjugation (Foley et al., 2021). Similarly, Inc plasmids have been found in both humans and food-producing animals (Zurfluh et al., 2014).

2.5.2 Priority bacteria commonly associated with infections

The growing numbers of AMR pathogens, which are increasingly associated with nosocomial infection, place a significant burden on healthcare systems and have important global economic costs (Santajit and Indrawattana, 2016). The most prominent effects of AMR include high morbidity and mortality rates and increased treatment costs (Dadgostar, 2019). A number of pathogens implicated in nosocomial infections are part of the ESKAPE group. ESKAPE is a group of bacteria, encompassing both Gram-positive and Gram-negative species and these include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species (Agyepong et al., 2018). These bacteria are common causes of life-threatening nosocomial infections amongst critically ill and immunocompromised individuals and are characterized by MDR, most of whose AMR genes are carried on the bacterial chromosome, plasmid, or transposons (Santajit and Indrawattana, 2016).

The WHO frequently publishes a list of bacteria for which new antibiotics are urgently needed. The list highlights the threat of GNB that are MDR, most of which have the ability to resist treatment and can easily pass on AMR genetic material to other bacteria (World Health Organization, 2017). The list is divided into three categories according to the urgency of need for new antibiotics: priority one: critical, priority two: high and priority three: medium (World Health

Organization, 2017). Pathogens in the critical group includes carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacterales* including ESBL-producing (World Health Organization, 2017). Priority two includes vancomycin-resistant *Enterococcus faecium*, *Staphylococcus aureus* (methicillin-resistant, vancomycin-intermediate and resistant)**,** clarithromycin-resistant *Helicobacter pylori***,** fluoroquinolone-resistant *Campylobacter* spp., fluoroquinolone-resistant *Salmonellae*, fluoroquinolone-resistant and cephalosporin-resistant *Neisseria gonorrhoeae* (World Health Organization, 2017). Lastly priority three includes penicillin-non-susceptible *Streptococcus pneumoniae*, ampicillin-resistant *Haemophilus influenzae* and fluoroquinolone-resistant *Shigella* species (World Health Organization, 2017).

2.6 Target Bacteria

The three target bacteria selected in this study, fall in the WHO priority one (*E. coli*) and priority two (*Enterococcus* and *Salmonella* species) lists respectively. The focus on the WHO priortity list is ESBL-producing or carbapenem resistant *E. coli*, vancomycin resistant *E. faecium* and fluoroquionolone resistant *Salmonella*.

2.6.1. *Escherichia coli*

Escherichia coli (E. coli) are Gram-negative bacterium of the genus *Escherichia* that belongs to the order *Enterobacterale,* family *Enterobacteriaceae*. In 1885, a German-Austrian paediatrician Theodor Escherich identified the Bacterium coli commune as the causative agent of childhood diarrhoea (Friedmann, 2014). Decades later, the bacterium was officially named after its discoverer, and the species name *Escherichia coli* was born (Friedmann, 2014). Remarkably, genome sequencing of the original strain of Dr Escherich's laboratory later revealed that the strain isolated was a non-threatening member of the gut microbiota that lacked pathogenicity islands and well-known virulence factors (Méric et al., 2016). Although most strains of *E. coli* are a harmless component of the gut microbiota, a plethora of pathotypes exist that can cause specific types of illnesses in both normal and immunocompromised hosts (Dale and Woodford, 2015).

Pathogenic *E. coli* strains differ from commensal organisms in that they produce virulence factors specific for each pathotype; these may be encoded on bacteriophages, on plasmids, or on stretches of the chromosome known as pathogenicity islands (Dale and Woodford, 2015). Extra-intestinal pathogenic *E. coli* (ExPEC) are strains that have the potential to cause infections outside the gut. *E. coli* strains isolated from extra-intestinal sites in immunocompromised individuals are less likely to resemble ExPEC and more likely to resemble strains isolated from faecal flora (Dale and

Woodford, 2017). This can be justified by the non-virulent original strain of the gut microbiota that was identified as the causative agent of childhood diarrhoea (Friedmann, 2014). ExPEC strains comprise many lineages, but only a subset is responsible for the vast majority of human infections and the leading cause of morbidity and mortality globally (Manges et al., 2019).

Genomic studies have greatly exapanded the availability of detailed data on *E. coli* sequence types (ST), clonotypes, phylogroups, serotypes, virotypes and resistomes. Clermont et al., 2013 used phylogenetic analysis to categorise *E. coli* into four phylogenetic groups (A, B1, B2, and D) based on a combination of two genes (*chuA* and *yjaA*) and anonymous DNA fragment *TSPE.C2* (Clermont et al., 2013a). Genomic analysis and the use of multilocus sequence (MLST) has advanced our understanding of *E. coli* by further recognising the eight phylogroups, of which seven (A, B1, B2, C, D, E, F) belong to *E. coli sensu stricto* and the eighth is the *Escherichia* cryptic clade I (Clermont et al., 2013b). Genomic analysis has been used to link commensal *E. coli* and pathogenic *E. coli* by comparing their genomic characteristics such as phylogenetic assignment by pathogenicity, serogroups, virulence and resistance traits (Founou et al., 2022). Phylogenetic groups A and B1 have been associated with commensal *E. coli* strains while groups B2 and D were associated with ExPEC strains that carry more virulence genes. Notably, most of the extra-intestinal infections are due to phylogroup B2 and, to a lesser extent, group D (Deku et al., 2022; Rezatofighi et al., 2021).

E. coli ST131 is a pandemic clone that is evolving rapidly with increasing levels of AMR. It is the most predominant clonal group of ExPEC with worldwide distribution. Belonging to phylogenetic group B2, ST131 includes both strains responsible for extra-intestinal infections and the strains rarely isolated from stool of asymptomatic humans (Donnenberg, 2002; Escobar-Páramo et al., 2004; Marin et al., 2022). ST131 has been reported to be virulent due to the types of virulence genes contained, widespread ESBLs, such as CTX-M-15, and resistance to fluoroquinolones (Nicolas-Chanoine et al., 2014a). *E. coli* ST131 strains cause both community and hospital-acquired UTI (cystitis and pyelonephritis) and bacteraemia worldwide (Nicolas-Chanoine et al., 2014a). They have also been reported to cause other types of infection such as intra-abdominal and soft tissue infections, meningitis, and septic shock. This diverse spectrum of infections, typical of ExPEC, has increased the degree of concern about ST131, which is already considered a major potential problem due to its multidrug resistance (Nicolas-Chanoine et al., 2014a).

Several virulence factors are associated with the pathogenicity of *E. coli* strains mainly either as adhesns or toxins. Initiation and colonisation of the key specific host cells in anatomical sites is as a result of fimbriae adhesins *fimA*, *sfa/foc*, and *yfcV* (Melican et al., 2011). Cytotoxic necrotising factor (*cnf1*) and vacuolating toxin (*vat*) have been implicated in reducing immune response by causing the dysfunction of local immune response and delaying neutrophil infiltration of the urinary tract in response to uropathogenic *E. coli* (UPEC); this is achieved by cleaving surface glycoproteins from leukocytes that are involved in neutrophil attraction and migration (Deku et al., 2022). The virulence factors affecting physiology have been identified as haemolysins (*hylA and hylF*), responsible for lysing red blood cells and human renal epithelial cells and siderophores (ferric aerobactin receptor (*iutA*), Yersiniabactin receptor (*fyuA*), responsible for sequestering iron from the host and *cnf1* that has also been implicated in tissue damage (Abdallah et al., 2011).

Resistance in *E. coli* against various antimicrobials, including cephalosporins, fluoroquinolones and even carbapenems, is increasing worldwide (Komatsu et al., 2018). Diverse resistance mechanisms such as ESBLs, pAmpC beta-lactamases (pAmpC) and carbapenemases are on the rise (Thomson, 2010). *E. coli* has been used as an indicator organism for AMR in GNB, and many surveillance programs have evaluated the antibiotic susceptibility profile of *E. coli* to determine resistance levels in a given location and time (AGISAR, 2017). It has been demonstrated that substantial resistance emerges in commensal bacteria, especially those present in the gastrointestinal tract where horizontal gene transfer prevails; this has facilitated the transfer of resistance within and between species and genera (Founou et al., 2016).

ESBLs are the leading causes of *E. coli* resistance to beta-lactams. These are plasmid-encoded enzymes that typically have the ability to hydrolyse penicillins, third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid (Alhashem et al., 2017). The most frequently encountered ESBLs belong to the $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} families. They are often encoded on MGEs and are transmissible to other pathogens (Hoepers et al., 2018). The main problem concerning ESBL-producing bacteria is that they usually acquire multiple antimicrobial resistance mechanisms, causing resistance to beta-lactams and other essential classes of antimicrobials such as aminoglycosides and fluoroquinolones thus further narrowing the choices of effective therapeutic agents (Alhashem et al., 2017).

2.6.1.1 Genomic *E. coli* studies

A systematic review and meta-analysis of 217 studies from 1995 to 2018, the objective of which was to examine the global contribution of all major ExPEC STs to extra-intestinal infections, revealed a diverse proportion (n=84) of STs with ST131 being the most prevalent with geographic variability in different regions for each geographic region compared to other major STs (Manges et al., 2019). Though not significamt, ST131 were higher in European (0.25; 95% CI, 0.21, 0.30) and Asian (0.23; 95% CI, 0.19, 0.28) studies and lower in North American (0.21; 95% CI, 0.13, 0.31) and African (0.19; 95% CI, 0.12, 0.28) studies (Manges et al., 2019). European and North American studies showed similar studies distributions of ExPEC STs. However, Asian and African studies diverged (Manges et al., 2019).

The global spread of ESBL-Ec is primarily attributed to the dissemination of *E. coli* strains carrying the *bla*$_{\text{CTX-M-15}}$ gene, especially *E. coli* O25b: H4-ST131 (Ludden et al., 2020). A study analysed the global distribution and dissemination of the complex clonal structure of ST131 by reconstructing the phylogeny of 794 isolates based on a core genome alignment containing 12518 Single Nucleotide Polymorphisms (SNPs) from nine countries, namely; UK –Ireland inclusive, USA, Australia, Canada, Spain, New Zealand, India, Korea Portugal from 1965 to 2015 (Ludden et al., 2020). This analysis allocated isolates to subclades based on alleles of the type 1 fimbriae adhesin *fimH* gene and revealed the following results, *H*41 belonged to Clade A, *H*22 to Clade B and *H*30 to Clade C. *FimH* allele type 30 (H30) was the most prevalent, followed by *H*22 and then *H*41 (Johnson et al., 2013).

Although *H*30, *H*22 and *H*41 were the most frequently identified, other alleles such as *fimH*35, *H*27, *H*31 and *H*94 have been observed in B subclades, B1–B5 (Stoesser et al., 2016a). Clade C has predominated since the 2000s and corresponds with the rapid dissemination of the bla$_{\textit{CTX-M-15}}$ allele, while Clade B also comprises of subclade B0, which differs phylogenetically from the remaining B isolates by carrying *fimH*27 that is considered ancestral to Clade C (Matsumura et al., 2017). Clade C consists of three subclades termed C0, C1 and C2. Clade C0 has been reported as ancestral and is composed of fluoroquinolone (FQ)-susceptible isolates, different from clades C1 (also known as *H30*R) and C2 (also known as *H30*Rx) that are characterised by a double mutation at the *gyrA* and *parC* genes conferring high-level resistance to FQ (Kallonen et al., 2017). Clade C2 is subdivided from C1 based on specific SNPs at *fimH*30 and is associated with the bla$_{\textit{CTX-M-15}}$ gene (Price et al., 2013a).

A study was done at one of the hospitals in South-Kivu Province, Democratic Republic of Congo, to characterise 21 MDR *E. coli* isolates using WGS, highlighting the distribution of *E. coli* sequence types (ST) with ST131 being the most prevalent, and all belonging to the O25b-ST131 pandemic clone (Irenge et al., 2019). Other STs were ST405, ST410, ST10, ST58, ST95, ST393, ST443, S617, ST648, and ST2450 (Irenge et al., 2019). ESBL-genes included the plasmid-mediated *bla*$_{\text{CTX-M-15}}$ in all isolates and the *bla*$_{\text{SHV-12}}$ allele. Other AMR genes included *bla*$_{\text{OXA-1}}$, *bla*$_{\text{TEM-1}}$, and genes encoding resistance against aminoglycosides, quinolones, chloramphenicol, rifampicin, tetracycline, sulphonamides and trimethoprim (Irenge et al., 2019).

A genomic study that analysed 94 isolates collected between 2006 and 2013 in southern Malawi confirmed the presence of ST131 and the *bla*$_{\text{CTX-M-15}}$ gene in the country. The results revealed the presence of five *E. coli* phylogroups A, B1, B2, D, F, and 43 multilocus STs, of which ST131 and ST12 were the most common. The most common ESBL gene was *bla*$_{\text{CTX-M-15}}$ and it was present in all five phylogroups and 11 STs, and most commonly detected in ST391, ST648 and ST131 isolates (Musicha et al., 2017). Another study at a tertiary care centre in central Malawi, that performed WGS of 58 clinical *E. coli* isolates collected during the period 2012 to 2018, highlighted a rise of ST131 from 15% to 33% and *bla*$_{\text{CTX-M-15}}$ gene from 21% increasing to 45% (Musicha et al., 2017; Tegha et al., 2021). Phylogenetics indicates that isolates are highly related between the central and southern geographic regions and confirmed that ST131 isolates were contained in a single group (Musicha et al., 2017; Tegha et al., 2021). All AMR genes, including *bla*$_{\text{CTX-M-15}}$, were widely distributed across sequence types. Notably was the increase in the number of ST410 isolates carrying a plasmid-located copy of *bla*$_{\text{CTX-M-15}}$ gene at a higher frequency than *bla*$_{\text{CTX-M-15}}$ in ST131 (Tegha et al., 2021). This study confirmed the expanding nature of ST131 and the wide distribution of the *bla*$_{\text{CTX-M-15}}$ gene in Malawi.

Genomic study of 25 *E. coli* isolates from Mwanza district in Tanzania identified eight STs (Musicha et al., 2017). In this study, ST131 was the most prevalent, followed by ST38 and lastly clonal complex 10 (CC10: ST-617, ST-44). The pathogenic phylogenetic group D was more prevalent than group B2 and *bla*$_{\text{CTX-M-15}}$ was found to be located in multiple plasmids, but most were chromosomally located (Musicha et al., 2017). Similarly, another study in Moshi, Tanzania that sequenced 38 *E. coli* isolates, found ST131 to be the most prevalent with over 80% belonging to serotype O25:H4 (Sonda et al., 2018) and 21 other sequence types (ST) were observed. Of note was the isolates belonged to ST10 clonal complex with 40% belonging to ST617: serotype O89:H10, 74% carried genes encoding beta-lactam resistance enzymes (Sonda et al., 2018).

Another study in Tanzania sequenced 143 ExPEC strains collected between March 2016 and October 2017, of which 128 were from pregnant women with UTIs and 15 were from children with BSI (Seni et al., 2021). ESBL production was predominantly due to the *bla*$_{\text{CTX-M-15}}$. The commonest STs/CCs were CC10 (28%), ST131 (18%) and ST38 (10%). The ST131 clades C1 and C2 were predominantly associated with fluoroquinolone resistance and ESBL production (Seni et al., 2021).

A study in rural Gambia performed WGS of *E. coli* from 66 asymptomatic children aged three-to-five years (Foster-Nyarko et al., 2021). This study revealed five completely novel STs (ST9274, ST9277, ST9278, ST9279 and ST9281) and eight main phylogroups: A (27%), B1 (32%), B2 (9%), D (15%), C and F (5% each), E (1%), and the cryptic Clade I (7%) with the majority belonging to phylogroups A and B1 (Foster-Nyarko et al., 2021). Another study in Southern Malaysia described the prevalence of ESBL-Ec in the community (carriage isolates) and hospital settings (clinical isolates) (Dwiyanto et al., 2022). Based on the types of plasmids, antibiotic resistance genes, and virulence factors, the community and clinical isolates revealed similar profiles thus suggesting the frequent exchange of genetic materials through horizontal gene transfer between the two settings (Dwiyanto et al., 2022). In terms of the virulence genes detected, the clinical isolates had a higher carriage of *iutA*, *iuc*, *sit*, and *hly* while, community isolates more frequently carried the *esp* gene (Dwiyanto et al., 2022). Despite these differences, 77% of these virulence genes were detected from isolates in both settings. The *bla*$_{\text{CTX-M-65}}$ was only observed in the community isolates while *bla*$_{\text{CTX-M-55}}$ was only observed in the clinical isolates (Dwiyanto et al., 2022). The other two *bla*$_{\text{CTX-M-27}}$ and *bla*$_{\text{CTX-M-15}}$ were found in both clinical and community isolates. Despite a one-year sampling gap between the community and clinical isolates, the similarity in profiles suggests the persistence and stable inheritance of these antibiotic resistance determinants (Dwiyanto et al., 2022).

2.6.1.2 One Health studies using *E. coli*

ESBL-Ec have been detected across the animal, human and environmental interface globally, with the emergence of specific clones able to acquire ARGs and virulence factors (VFs) via MGEs, such as plasmids, transposons, gene cassettes, and other integrative genetic elements (Smet et al., 2010). One genomic health surveillance of *E. coli* sequenced 431 *E. coli* isolates, including 155 ESBL-producing isolates from cross-sectional surveys of livestock farms and retail meat in the East of England and compared them with the genomes of 1,517 *E. coli* associated with BSIs in the UK(Ludden et al., 2019a). The phylogenetic core genome comparisons demonstrated that

livestock and patient isolates were genetically different (Ludden et al., 2019a). However, with similar ARGs, there was limited overlap in the MGEs carrying these genes, suggesting that *E. coli* causing serious human infection did not directly originate from livestock in their region (Ludden et al., 2019a).

A study done in India that sequenced 28 ESBL-producing *E. coli* comprising of 12 broiler chicken isolates, 11 free-range chicken isolates, and 5 human ExPEC showed a total of 13 ST from the poultry *E. coli* and these included globally successful ST such as ST117 (9%), ST131 (4%), and ST10 (4%) (Hussain et al., 2019). The most common ESBL gene detected in poultry *E. coli* genomes was $bla_{\text{CTX-M-15}}$ (17%) while FIB (73%) and FII (73%) were the most common plasmid replicons identified. Conjugation experiments demonstrated the ability of broiler, free-range, and human ExPEC *E. coli* to transfer their ESBL genes, proposing the likelihood of ARG transmission between isolates from different sources. The *in vitro* virulence-associated phenotypic tests found comparable characteristics in biofilm formation, resistance to serum bactericidal activity, adherence, and invasion in broiler, free-range, and human ExPEC isolates, suggesting certain *E. coli* clones from broiler-poultry may indeed have the potential to cause infection in humans (Hussain et al., 2019).

In Brazil, ST131, ST410 and ST354 were the most common STs associated with a human origin, while ST131 and ST648 were predominant in animal hosts (Fuga et al., 2022). The virulence factors such as *afaA*, *faeC*, *fimC*, *focX*, *hra*, *iha*, *pap*, and/or *sfa* genes involved in adherence and biofilm formation were remarkably shared, mainly by the international high-risk clones identified (Fuga et al., 2022). There was a predominance of $bla_{\text{CTX-M-15}}$, followed by $bla_{\text{CTX-M-2}}$, and $bla_{\text{CTX-M-8}}$ ESBL genes. Interestingly, food samples were identified as potential reservoirs associated with $bla_{\text{CTX-M}}$-type genes (Fuga et al., 2022). It is well recognised that food-producing animals play an important role as a reservoir of MDR pathogens thus consistent with this hypothesis, it has been suggested that the commercial chicken meat could be a reservoir of *E. coli* strains co-harbouring $bla_{\text{CTX-M}}$ and colistin resistance *mcr*-1 genes (Subramanya et al., 2020a).

The Tricycle One Health approach project in Malaysia used WGS to sequence 98 ESBL-Ec derived from humans, the food chain, and the environment (Hashim et al., 2022b). In this study, 25 isolates were from hospitalised patients, seven were from healthy communities in clinics, 46 isolates were from chicken representing the food chain, and 20 were from water samples. The strains isolated were diverse, with 51 different STs identified and ST45 being the predominant

type. However, ST131 was more widely distributed and detected among hospital strains and the food chain sector. Nineteen new clones were identified. SNP phylogenetic analysis of core genomes revealed that all organisms were interrelated. The majority of the isolates harboured ESBL *bla*$_{\text{CTX-M-33}}$ gene while some isolates also harboured carbapenemases resistant gene; *bla*$_{\text{OXA-1}}$, *bla*$_{\text{OXA2}}$ and colistin resistant gene; bla$_{\text{MCR1}}$ and bla$_{\text{MCR3}}$ (Hashim et al., 2022b).

Although ESBL-Ec has been found in humans and animals, the resistance genes are mainly carried on different STs. This was synthesised in findings from Tanzania, Egypt and Nigeria that utilised the one health concept (Alonso et al., 2017). It was noted that even though *bla*$_{\text{CTX-M-15}}$ was the predominant gene encoding ESBL enzyme from livestock, rarely was it associated with the human epidemic clone *E. coli* ST131 (Alonso et al., 2017). Mshana et al., 2013, pointed out the limited availability of AMR data in the region in a review. In this review, it was reported that there was limited data on ESBL producing *E. coli* in published and unpublished data on bacterial resistance in the one health space. This review included data from Democratic Republic of Congo, Mozambique, Tanzania and Zambia (Mshana et al., 2013).

A study that synthesised the clonal relationship between multidrug-resistant *E. coli* ST69 using phylogenetic analysis and hierarchical clustering from poultry and humans in Lusaka, Zambia showed a high degree of genetic relatedness between *E. coli* O17:H18-ST69 from poultry and humans (Shawa et al., 2022). The *E. coli* O17:H18-ST69 clone accounted for 20% poultry and 25% human-associated isolates that shared two plasmids harbouring 14 AMR genes. Although comparison analysis showed that the isolates also had other AMR plasmids distinct for each niche, the results suggested clonal transmission of MDR *E. coli* between poultry and humans, with the potential acquisition of niche-specific AMR plasmids (Shawa et al., 2022).

2.6.2 *Enterococci* species

The *Enterococcus* genus are ubiquitous Gram-positive and facultative anaerobic bacteria belonging to the *Enterococcaceae* family and consists of species that are commensals in human and animal gastrointestinal tracts (GIT), food and in various environments including plants and water (Lebreton et al., 2014). Enterococci were previously classified as group D streptococci until 1984, when *Streptococcus faecalis* and *Streptococcus faecium* were reclassified as *Enterococcus faecalis* and *Enterococcus faecium*, respectively (Monstein et al., 1998). The broad distribution of *Enterococcus* is attributed to their ability to tolerate high concentrations of salt (6.5% NaCl), wide range of temperature (from 10°C to 40°C) and pH (from 4.4 to 9.6), as well as their ability to

hydrolyse esculin in the presence of high quantities of bile salts (40%) (Hollenbeck and Rice, 2012; Lebreton et al., 2014). Little is known about the main mechanisms used by *Enterococci* to colonise GITs of either healthy individuals or hospitalised patients. However, the exposure to antibiotics in hospitalised patients and healthy individuals in the community results in significant adjustments of the gut microbiota, which then enable colonisation of the GIT by drug-resistant enterococci (Donskey et al., 2000).

Primarily, *Enterococci* were considered as organisms of little clinical importance but species such as *Enterococcus faecalis* and *Enterococcus faecium*, commonly identified in clinical specimens have been progressively associated with an increasing number of HAIs in both human and veterinary medicine (Gold, 2001; Heuer et al., 2006). *Enterococci* are associated with a wide range of infections including UTIs, bacteraemia, endocarditis, wound infections (burn wounds or surgical incisions), abdomen and biliary tract infections, and infection of catheters and medical implants (Geraldes et al., 2022). Other species such as *Enterococcus hirae*, *Enterococcus durans*, *Enterococcus gallinarium* and *Enterococcus casseliflavus* are common in veterinary medicine but also infrequently cause infections in humans although not commonly associated with HAI (Geraldes et al., 2022; Lebreton et al., 2014). *E. durans* occurs occasionally among the faecal flora of healthy adults while *E. gallinarium* and *E. casseliflavus* infections are of great concern because of their intrinsic resistance to vancomycin, an antibiotic used to treat the MDR enterococcal infections (Lebreton et al., 2014).

E. faecium is mainly associated with HAIs and is rarely encountered in community settings (García-Solache and Rice, 2019). These hospital-associated clones are characterised by the acquisition of adaptive genetic elements, including genes involved in metabolism, biofilm formation, and antibiotic resistance (García-Solache and Rice, 2019). Divergent to *E. faecium*, clones of *E. faecalis* isolated from hospitalised patients, including strains causing clinical infections, are not exclusively found in hospitals but are also present in healthy individuals and animals (Guzman Prieto et al., 2016). Additionally, virulence genes of *E. faecalis* are frequently not exceptional to clinical isolates, but are also found in strains that originate from commensal niches, therefore may not represent true virulence genes but may instead act as host-adaptive functions that are useful in a variety of intestinal environments (Comerlato et al., 2013).

Enterococcus species show intrinsic and acquired resistance to several commonly used antimicrobial agents (Miller et al., 2014). *E. faecalis* and *E. faecium* are both intrinsically resistant

to cephalosporins, aminoglycosides and co-trimoxazole. At the same time, *E. faecalis* is also intrinsically resistant to Quinupristin-dalfopristin (Miller et al., 2014). Resistance of enterococci to glycopeptides such as vancomycin, teicoplanin was first described in the late 1980s (Gold, 2001). Eight vancomycin-resistant phenotypes have been described in enterococci, with *Van*A and *Van*B phenotypes being the most frequently encountered (Gold, 2001). The *Van*A phenotype is inducible and carried on a transposon (Tn1546) and characterised by high-level resistance to vancomycin (MIC>32µg/ml) and teicoplanin (MIC>16µg/ml) (Biavasco et al., 2007). The *Van*B phenotype is chromosomally mediated and characterised by variable levels of resistance to vancomycin and susceptibility to teicoplanin (Biavasco et al., 2007).

A systematic review and meta-analysis of the WHO European Region, conducted from 1 January 2010 to 4 February 2020, found that *Enterococci* accounted for 6–18% HAIs with a pooled incidence ranging between 0.7 and 24.8 cases per 1,000 patients with different incidences in different wards (Brinkwirth et al., 2021). Among patients with hospital-acquired BSIs caused by *Enterococcus* spp., pooled all-cause mortality was 21.9%, while all-cause mortality attributable to VRE was 33.5% (Brinkwirth et al., 2021). In the United States, 18% of all BSIs were due to *Enterococci* (Ali et al., 2018) and approximately 14% of *E. faecalis* and 87% of *E. faecium* isolated from blood are vancomycin-resistant (Miller et al., 2014). The frequency of VRE isolates in Europe ranged from 5% to 30% in various countries (Santajit and Indrawattana, 2016). In Africa, the prevalence of *Enterococci* in different clinical samples was documented to be between 5.7% to 88.9% (Ali et al., 2018).

Approximately 90% of *Enterococci* infections are caused by *E. faecalis*, whereas 5–10% are caused by *E. faecium* (Jabbari et al., 2019). However, *E. faecium* BSIs have been found to have higher rates of antibiotic resistance and mortality compared *E. faecalis* BSI (Jabbari et al., 2019). In order to collate data on the global prevalence of antibiotic resistant *E. faecalis* and *E. faecium* isolated from BSI, a systematic review and meta-analysis was conducted between 2000 and 2018, including the following regions; America, Africa, Europe, South-East Asia, Western pacific and Eastern Mediterranean (Jabbari et al., 2019; Ulrich et al., 2017). Out of the 291 studies enrolled in the meta-analysis, American countries showed the lowest prevalence of resistance for linezolid in *Enterococcus faecalis* while Western Pacific, European, and American countries had the lowest level of vancomycin resistance, contrary to South-East Asia and Eastern Mediterranean countries that showed the highest level of vancomycin resistance (Jabbari et al., 2019; Ulrich et al., 2017). Linezolid and vancomycin are the last resort treatment options for MDR enterococcal BSIs.

Therefore, resistance in these antibiotic classes may lead to poor treatment outcome and increased morbidity and mortality (Jabbari et al., 2019; Ulrich et al., 2017). The African region had the lowest number of studies (less than two for both vancomycin susceptibility in *E. faecalis* and *E. faecium* BSIs), implying the low prevalence of VRE documented is not a true prevalence of what is prevailing.

A retrospective matched case-control study was performed to estimate the economic burden of nosocomial infections caused by VRE, this study included patients who acquired nosocomial infection with either VRE or vancomycin-susceptible enterococci (VSE) within a time period of three years (Puchter et al., 2018a). A total of 42 cases with VRE infections and 42 controls with VSE infections were matched for age, gender, admission and discharge within the same year, time at risk for infection, stay on intensive care units and non-intensive care units as well as for the type of infection (Puchter et al., 2018a). Costs were similar between cases and controls before onset of infection, but higher after onset of infection. The most significant differences in costs between cases and controls were pharmaceutical, nursing staff, medical products, and assistant medical technicians (Puchter et al., 2018a). Additionally, multivariate analysis revealed that costs were driven independently by vancomycin-resistance (1.4 fold; $p = 0.034$) (Puchter et al., 2018a).

A study that aimed to determine the rate of intestinal carriage of VRE in the healthy community in Casablanca, Morocco, analysed 113 faecal samples for the presence of enterococci (Hannaoui et al., 2016). There was slightly more *E. faecium* (55%) than *E. faecalis* (45%), and the resistance profile indicated 88% MDR strains. The rate of faecal carriage of VRE was 21% (n=21), of which eight (18%) were *E. faecalis* and 13 (24%) were *E. faecium.* PCR analysis revealed that all of the VRE strains possessed the *van*A gene (Hannaoui et al., 2016). Similarly, a study in Lagos, Nigeria found carriage of MDR *E. faecium* and *E. faecalis* among healthy humans. *E. faecium* was also more prevalent than *E. faecalis.* *E. faecium* exhibited resistance to erythromycin (89%), gentamicin (78%) and vancomycin (17%) while *E. faecalis* recorded vancomycin resistance (14%) (Solayide A Adesida et al., 2017). The emergence of VRE and the high rate of colonisation by MDR enterococci are alarming.

In Canada, a two-year surveillance of *Enterococcus* spp., isolated from humans and beef cattle, abattoirs, manured fields, natural streams, and wastewater from both urban and cattle feedlot sources revealed distinct species and AMR diversity across a One-health continuum (Zaheer et al., 2020). Of the 8430 isolates collected, *E. faecium* and *E. faecalis* were the main species in urban

wastewater (90%) and clinical human isolates (99%). At the same time, *E. hirae* predominated in cattle (92%), and feedlot catch-basins (60%) and natural streams harboured environmental *Enterococci* (Zaheer et al., 2020). Phenotypic resistance to tetracyclines and macrolides were prevalent among *Enterococcus* spp. regardless of source and were encoded by *tet (M)* and *erm (B)*. Resistance to β-lactams and quinolones in *E. faecium* were higher in human clinical isolates by 76% and 70% than in cattle by 3% and 8%, respectively (Zaheer et al., 2020). Notably, vancomycin-resistant *E. faecium* exhibited high rates of MDR, with resistance to all β-lactam, macrolides, and quinolones tested. The dissimilarities in the AMR profiles among isolates could be attributed to antimicrobial use practices in each sector of the One-health continuum (Zaheer et al., 2020).

In Switzerland, a study that aimed to establish VRE from healthy food-producing animals revealed frequent resistance to tetracycline and erythromycin, and VRE harbouring *vanA* genes were present (Wist et al., 2020). Additionally, in Russia, a study that screened for AMR in commensal *E. faecalis* and *E. faecium* from different food-producing animals revealed high-level resistance to antibiotics of clinical importance, including rifampicin (44-84%) from all animals, tetracycline (45-100%) from poultry and pigs, and erythromycin (60-100%), ciprofloxacin (23-100%), and trimethoprim-sulfamethoxazole (33-53%) from chickens, turkeys, and pigs (Makarov et al., 2022). There was no vancomycin-resistance recorded and most isolates were MDR. The differences in resistance between *Enterococci* from different farm animals indicate that antimicrobial usage is among the crucial factors determining the level of resistance (Makarov et al., 2022). A total of 71 *Enterococcus* species were isolated in a rural communal farming area in South Africa, with *E. faecium* being the dominant species among the *Enterococcus* isolates and resistance to enrofloxacin (55%) and amoxicillin (3%) (Mupfunya et al., 2021).

In Zambia, a 2018 to 2019 study that analysed 39 BSI *Enterococci* recorded high resistance to a number of antibiotics, including erythromycin (97%), tetracycline (85%), ampicillin and penicillin (80%) (Mutalange et al., 2021). There was no vancomycin resistance but intermediate vancomycin *Enterococci* (14%) with MICs ranging from 1µg/ml to 8µg/ml (Mutalange et al., 2021). In veterinary medicine, 83 faecal samples were collected randomly from apparently healthy pastoral cattle belonging to different independent owners living in the Kafue basin interface areas (Mubita et al., 2008). Resistance in *E. faecium* and *E. faecalis* to gentamicin was 97% and 85%, penicillin, 79% and 81%, erythromycin 72% and 56%, nitrofurantoin 65% and 50%, respectively, and no vancomycin resistance detected (Mubita et al., 2008).

2.6.3 *Salmonella* species

The *Salmonellae* are major human pathogens that have contributed significantly to the global public health problem, giving rise to social and economic burden worldwide (Ashton et al., 2016). Their increasing prevalence in the global food chain, virulence and adaptability have an enormous medical, public health and economic impact worldwide (Crump, 2014)(Kirk et al., 2015). In 2000, typhoid fever was estimated to cause approximately 21.7 million illnesses and 216,000 deaths, and paratyphoid fever caused 5.4 million illnesses (Crump et al., 2004). After adjusting for water-related risk in 2010, the estimated number of typhoid fever cases in LMICs was 11.9 million, with 129 000 deaths (Mogasale et al., 2014). These numbers suggest a higher incidence in Africa and a lower incidence in Asia than previously thought (Crump, 2014). The global burden of enteric fever is usually underestimated in most endemic areas due to a lack of well-established population-based national surveillance systems (Kariuki et al., 2010a; Mogasale et al., 2014).

Salmonella is Gram-negative, non-spore-forming bacilli that belong to the Enterobacterale family. The genus consists of two subspecies: Salmonella *enterica* and Salmonella *bongori*. There are six subspecies of S. *enterica*, of which subspecies I, Salmonella *enterica* subspecies *enterica* causes 99% of human and animal infections (Ashton et al., 2016). *S. enterica* consists of typhoidal and non-typhoidal *Salmonella* (NTS) associated with two main pathologies: gastroenteritis and typhoid fever. NTS causes the majority of gastroenteritis: *S.* Typhimurium and *S.* Enteritidis (Bharmoria and Behari Vaish, 2016), while the human host causes the typhoidal Salmonella restricted serovars: *Salmonella enterica* serotype Typhi (*S.* Typhi) and *Salmonella enterica* serotype paratyphyi (*S.* Paratyphi) A, B and C (Brenner et al., 2000). These organisms cause typhoid and paratyphoid fever collectively called Enteric fever (Parry et al., 2015).

NTS are not exclusively associated with localised gastroenteritis but are more promiscuous and zoonotic than typhoidal serovars, thus infecting multiple hosts (Makendi et al., 2016). There has been an alarming increase in invasive NTS (iNTS) that cause diseases such as bacteraemia and meningitis, with higher case-fatality rate estimates among children ≤ 5 years, the elderly ≥ 70 years, people with HIV infection, and those in areas of low sociodemographic development in SSA (Park et al., 2018; Stanaway et al., 2019). *S.* Typhimurium and *S. Enteritidis* are the most widely reported invasive serovars across SSA (Carden et al., 2015). *S.* Typhimurium multilocus sequence type 313 (ST313) have been linked to niche adaptation, with some traits observed in *S.* Typhi and *S.* Paratyphi A (Carden et al., 2015). More importantly, most *S.* Typhimurium isolates causing

invasive disease are MDR, thereby compromising the clinical treatment of the disease (Kingsley et al., 2009a). iNTS might be linked to a diverse host niche, including several animal reservoirs, indicating the need for a 'One-health' approach (Cuypers et al., 2018).

The sources of many outbreaks of human salmonellosis are challenging to trace and often remain unidentified. In cases where the source is known, some outbreaks have been associated with raw or undercooked meat, eggs, and vegetables or contamination occurring during processing (Gajraj et al., 2012). Notably, the frequency of outbreaks associated with particular serovars can vary based on source, incidence and geographical distribution (Makendi et al., 2016). In HICs, *Salmonella* outbreaks linked to food occurred in February 2022; the UK reported a cluster of cases with monophasic *Salmonella* Typhimurium sequence type 34 (EFSA, 2022).. Three months later, 324 cases had been reported in 12 European Union (EU) / European Economic Area (EEA) countries and the UK, including two distinct strains. Most cases were recorded in children below the age of ten years, of which 41% were hospitalised. The two strains were MDR, with some isolates carrying resistance to disinfectants based on quaternary ammonium compounds and hydrogen peroxide. The two stains were susceptible to azithromycin, ciprofloxacin, meropenem, and third-generation cephalosporins (EFSA, 2022). Epidemiological investigations suggested specific chocolate products processed in Belgium as the likely vehicles of infection (EFSA, 2022).

Between May 2018 and December 2020, 193 *Salmonella* Enteritidis sequence type (ST) 11 human cases were linked to non-ready-to-eat poultry products in EU/EEA countries such as Denmark (2), Finland (4), France (33), Germany (6), Ireland (12), the Netherlands (3), Poland (5), Sweden (6), and the UK (122). Fifty per cent of the cases were children ≤ 18 years. Epidemiological studies in the UK identified an increased risk of *S.* Enteritidis infection associated with the consumption of frozen breaded chicken products and five batches tested positive for *S.* Enteritidis matching the outbreak strain. The five positive batches were traced back to different meat suppliers, slaughterhouses, and/or farms in Poland. The WGS analysis of human and food *S.* Enteritidis isolates confirmed a cluster with 0-3 allelic differences through single linkage clustering. This, in combination with epidemiological data, suggests common sources in the food chain (EFSA, 2021).

Before 2018, there was limited data on the geographical distribution, incidence, and phylogenetics of MDR *Salmonella* Typhi in SSA. Park et al, studied the geographic distribution of *S.* Typhi in 11 SSA (South Africa, Madagascar, Tanzania, Kenya, Uganda, Ethiopia, Ghana, Burkina Faso, Guinea-Bissau, The Gambia, Senegal) isolated between 2008 to 2015. Notably, most of the

countries had less than 20 strains represented, with Ghana having the highest (101), followed by Kenya (59) and Uganda (30). Phylogenetic reconstruction of 249 whole genome sequenced existing *S.* Typhi, in the context of 2,057 global *S*. Typhi genomic revealed a broad genetic diversity with the majority of strains (90%) belonging to only three genotypes (Park et al., 2018). The most prevalent was 4.3.1 (H58) at 40%, followed by 3.1.1 (39%), and 2.3.2 (12%). Strains belonging to genotype 4.3.1 were found only in East Africa, while genotype 3.1.1 and 2.3.2 were found only in West African sites. MDR was found in over 50% of strains restricted within these dominant genotypes with high incidences of MDR *S.* Typhi in locations with a high burden of typhoid, specifically in children aged < 15 years (Park et al., 2018).

Additional studies have since been done, further confirming the burden in Africa and Asia to be predominantly in children (Meiring et al., 2021). Differences in geographical distribution and incidence has been recorded, with countries such as Tanzania, India and China having *S*. Typhi as the predominant causative agent of typhoid infections (Misra et al., 2016; Omari A. Msemo et al., 2019a; Qian et al., 2020a). Malawi, Kenya, Ghana, Burkina Faso and The Gambia had iNTS as the leading cause of *Salmonella* infections (Dekker et al., 2018a; Feasey et al., 2015; Guiraud et al., 2017; Kwambana-Adams et al., 2015; Muthumbi et al., 2015). These differences reflect the heterogeneity of the epidemiology of invasive salmonellosis, valuable data that can be used to advocate the introduction of typhoid conjugate vaccines (TCVs) MDR strains are responsible for several outbreaks of typhoid fever across the African and Asian continents (Browne et al., 2020; Park et al., 2018). Fluoroquinolones and cephalosporins are now the recommended drugs to treat of invasive *Salmonella*, but the last decade has seen a rise in resistance to fluoroquinolones (Hendriksen et al., 2015; Kariuki et al., 2015). Resistance to the recommended antibiotics is still relatively low in some high burden countries in Africa (Labi et al., 2014; Muthumbi et al., 2015).

During the 2010 to 2012 Typhoid fever outbreak in Zambia, 2,040 cases were identified from a population of 14.2 million with an estimated overall case fatality rate of 0.5%. The majority of cases occurred in children less than 15 years of age, with an even distribution by gender (Hendriksen et al., 2015). Ninety-four isolates were subjected to antimicrobial susceptibility testing, of which 83% exhibited resistance to five different classes of antimicrobials and were classified as MDR (Hendriksen et al., 2015). Due to the MDR outbreaks, fluoroquinolones such as ciprofloxacin began to be mainly used; this led to the emergence of nalidixic acid-resistant *S*. Typhi exhibiting reduced susceptibility to fluoroquinolones (Thuy Chau et al., 2007). Zambia has

not been spared from antimicrobial resistance and MDR in *S*. Typhi; this is evident by the findings during the 2010-2012 outbreak (Hendriksen et al., 2015).

The WHO has prequalified two Typhoid conjugate vaccines (TCVs), namely Typbar TCV and TYPHIBEV. TCVs have the potential to overcome many challenges that have delayed the uptake of earlier vaccines through increased efficacy, suitability for children under the age of two, and easier inclusion in routine immunisation programs. Three large (phase three) efficacy studies conducted in Nepal, Bangladesh, and Malawi showed that TCV prevented 78%, 85%, and 84% of typhoid cases in children 9 months to 16 years old, respectively (Patel et al., 2021; Qadri et al., 2021; Shakya et al., 2021). These results demonstrate that TCV is protective across diverse settings in Africa and Asia. The introduction of routine immunisation with TCV at age 9 months with a catch-up campaign up to age 15 years was predicted to avert 46–74% of all typhoid fever cases in 73 countries eligible for Gavi support (Birger et al., 2022). A further prediction to reduce the relative prevalence of AMR typhoid fever by 16%, avert 42·5 million cases and 506 000 deaths caused by fluoroquinolone-resistant typhoid fever, and 21·2 million cases and 342 000 deaths from MDR typhoid fever over 10 years following introduction (Birger et al., 2022).

The first programmatic use of TCV in Africa was in early 2019 in Zimbabwe. This was as a response to a typhoid fever outbreak where the Zimbabwe Ministry of Health carried out an emergency mass TCV campaign that resulted in decreased typhoid cases (Olaru et al., 2019). A sharp decrease in one of the worst affected communities in the proportion of confirmed and suspected typhoid cases among children, but not among adults was observed. In this community, 23 (21%) of 109 blood cultures from children were positive for *S* Typhi before vaccination compared with none of 24 after vaccination (Olaru et al., 2019). Similarly, in November 2019, an outbreak of extensively drug-resistant culture-confirmed *S.* Typhi in Pakistan prompted the country to became the first to introduce TCV into its routine immunization schedule (Yousafzai et al., 2021). Results from the outbreak response in Pakistan show TCV was 95% effective against blood-culture confirmed typhoid fever and 97% effective again XDR typhoid fever (Yousafzai et al., 2021).

2.7 AMR: The Zambian situation

A number of studies have been undertaken in Zambia in order to investigate and understand the growing problem of AMR and most of these studies have indicated AMR in humans and in animals (Chishimba et al., 2016; Kabwe et al., 2016; Mainda et al., 2015a; Roth et al., 2021).

In human studies, a study done in the neonatal unit at UTH found 100% ESBL production in *E. coli* and *K. pneumoniae* isolates (Kabwe et al., 2016; Mumbula et al., 2015). Another study at the same hospital analysed WHOnet data from 2015 to 2017 revealed high levels of antibiotic resistance among GNB such as *E. coli* and *K. pneumoniae* with the highest being to ceftriaxone and ciprofloxacin (Roth et al., 2021). Most studies have been undertaken in the capital city, Lusaka but one that was conducted in the Southern province of Zambia from 2019 to 2021 found *E. coli* to be second highest pathogen isolated and prevalent MDR pathogens with resistance to third generation cephalosporins at 60% (Mwansa et al., 2022a). A study that investigated AMR in *Enterococcus* species from BSI found high resistance to penicillins, ciprofloxacin and reduced susceptibility to vancomycin, the last resort treatment option available in Zambia (Mutalange et al., 2021). A *Salmonella* study during the 2010-2012 outbreak in Zambia revealed high prevalence of MDR (> 80%) and an emergence of fluoroquinolone resistance (Kalonda et al., 2015a).

Regarding AMR in the carriage, a study that analysed AMR in rectal swabs from outpatients in the Eastern province of Zambia revealed a high rectal colonisation rate of ESBL-producing *E. coli* (Nagelkerke et al., 2017). Similarly, another study by Bumbangi et al. revealed wide spread of MDR in *E. coli* carriage from young children in Lusaka and Ndola districts (Bumbangi et al., 2022). Further, a cross-sectional study conducted at two leading hospitals in Lusaka and Copperbelt provinces showed a high AMR level among potential pathogens isolated in healthcare facilities (Chibwe et al., 2017).

In animals, market-ready chickens were found to carry *E. coli* that were 20% ESBL-producers with increased resistance to commonly used antimicrobials in human medicine (Chishimba et al., 2016). Other studies that determined AMR in abattoirs and ready-to-sell chickens from supermarkets and open markets showed high levels of MDR and an emergence of resistance to third-generation cephalosporins (Muligisa-Muonga et al., 2021; Phiri et al., 2020).

Furthermore, a cross-sectional study in 2019 that reviewed antibiotic prescribing patterns in adult patients in five primary healthcare hospitals in Zambia revealed indiscriminate prescribing with a higher prevalence of antibiotic use (85%) than the less than 30% threshold (Masich et al., 2020; Mudenda et al., 2022a). The most prescribed antibiotic being ceftriaxone (20%), a watch group

antibiotic that is widely used as an empiric treatment in tertiary hospitals. (Masich et al., 2020; Mudenda et al., 2022a). Similarly in animal health; a study revealed uncontrolled use of antimicrobials in dairy farming in Zambia (Mainda et al., 2015b) while another study found high antibiotic usage (86%) in layer poultry production, most of which were accessed from agrovets and pharmacies without prescriptions (Mudenda et al., 2022c).

In Zambia, the problem of AMR still needs to be defined in both humans and animals. However, the current situation indicates that there is the uncontrolled use of antimicrobials in both veterinary and human medicine; self-medication and off-the-counter purchase of antimicrobials have been found to contribute to AMR in the communities.

Knowledge gap

There needs to be more information related to phenotypic, molecular and WGS data of pathogens, and the phylogenetic analysis of AMR pathogens isolated from humans and poultry has not been exhaustively studied in Zambia.

Several studies have been conducted in Zambia, nonetheless, human and animal health AMR studies have been independent of each other and limited to phenotypic analysis. Phenotypic data has confirmed similarities in the type of AMR circulating in both sectors. Although transmission between sectors is difficult to prove, WGS can give an in-depth understanding of the interface of AMR in humans and poultry and the consequences of these AMR similarities. Most of the WGS studies and sequenced data are from HICs, with very few from LMIC on the African continent. The confirmed geographic and incidence diversity of pathogens in different countries and regions highlights the importance of local genomic analysis and data.

Although the human studies conducted in a few districts in Zambia, found *E. coli* to be one of the common causative agents of BSI and UTI and, to a lesser extent *Enterococcus*, genomic analysis of both pathogens from clinical isolates has yet to be done. The *Salmonella* study that utilised WGS in Zambia was conducted ten years ago and was restricted to the capital city. The WGS data from this study envisaged to provide an update on the evolution of *Salmonella* serovars and sequence types in the same location, as well as the diversity of *Salmonella* serovars from Ndola, a district that has not previously been studied. The WGS data of all three pathogens, will not only provide the prevalence, evolution, emergence and transmission of new variants but will also provide information that can be used to improve patient management, reduce the spread of AMR

in both human and animal health. Where applicable, facilitate for the design for vaccines suitable for our setting based on the local genomic findings.

Equally, the prevalence of AMR and carriage of resistant genes in healthy individuals will give a comprehensive perception of the spread of resistance in the community, data that can be used to introduce the screening for AMR carriage in patients that are being admitted to hospitals. A practice that has commonly been adopted in developed countries and has been found to reduce the spread of resistant pathogens, such as carbapenem resistant *Enterobacterales* and VREs, between patients in the hospital setting, thereby reducing the length of hospital stay and improving patient outcomes.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was conducted in Lusaka and Copperbelt provinces in Zambia. The two provinces were selected because they are both centrally located and provide habitation for much of Zambia's population. Currently, Zambia's population is estimated at 19,606,454; Lusaka and Copperbelt provinces have the highest at 3,042,000 and 2,542,000, respectively (Zambia Statistics Agency, 2022).

3.2 Study design

This was a two-tier cross-sectional study comprising the following:

(i) Human study

(ii) Poultry study

3.2.1 The Human Study

3.2.1.1 Study sites

The study was undertaken in both Lusaka and Copperbelt provinces. Three hospitals were purposively selected for sample collection, namely, University Teaching Hospital (UTH), Ndola Teaching Hospital (NTH) and Arthur Davidson's Children Hospital (ADCH). These were selected because they provided the highest level of care in the Country, with fully functioning microbiology laboratories.

UTH

The UTH is a national tertiary and referral hospital based in the capital Lusaka. It has a bed capacity of about 1,665 and offers specialised care to patients from within the province and referral patients from all the other provinces in Zambia. It is a highly specialised facility comprised of five hospitals, namely: The Adult Hospital, Children's Hospital, Mother and New-born Hospital, Eye Hospital and Cancer Disease Hospital (CDH). The Microbiology laboratory at UTH has been submitting data to the Global Antimicrobial Resistance and use Surveillance System (GLASS) since 2016 and is also a surveillance site for the WHO Paediatric Bacterial Meningitis (PBM) program.

NTH

NTH is the second largest tertiary and referral hospital located in Ndola which is the provincial capital of the Copperbelt. It has a bed capacity of about 800 and offers specialised care to adult

patients of the Copperbelt province and other neighbouring provinces. The Microbiology laboratory at NTH was recently (2021) enrolled as one of the sentinel sites that submits data to GLASS.

ADCH

ADCH is a paediatric hospital located in Ndola district, with a bed capacity of 250, offering specialised care to the paediatric population of the Copperbelt and neighbouring provinces. It is the biggest Children hospital on the Copperbelt and receives referrals from different parts of the Country.

Primary health centres and first level hospitals

Lusaka

For the enrolment of healthy participants from the community, three primary health care facilities were identified in Lusaka province, namely Chilenje first level hospital, Kanyama first level hospitals and Kalingalinga health centre. Kanyama is the biggest compounds in Lusaka city, that is densely populated with approximately 169,253 Inhabitants and a population density of 5,636/km^2 (Central Statistical Office Zambia, 2018). It has inadequate water and sanitation infrastructure, which favour the spread of the disease and suffers most of the waterborne disease outbreaks. Kalingalinga and Chilenje are middle density areas with approximately 39,139 and 52,220 inhabitants, respectively while Kalingalinga and Chilenje have 3,771/km^2 and 4,769/km^2 population density, respectively (Central Statistical Office Zambia, 2018).

Ndola

Similarly, three primary health care facilities were identified in Ndola district in the Copperbelt province for the enrolment of healthy community participants. The three facilities were Lubuto, with a population of 22,915 and 7,695/km^2 population density, New Masala with 9,059 inhabitants and 10,391/km^2 population density and Mapalo urban health centres with 37,703 inhabitants and 7,769/km^2 (Central Statistical Office Zambia, 2018). Mapalo is one of the fastest growing high-density areas of Ndola.

3.2.1.2 Study design

The human study included two sub-components as follows:

i. A study involving hospitalised patients with bloodstream infections (BSI) and patients with urinary tract infections (UTIs), both hospitalised and outpatients.

ii. A study involving the healthy community - pregnant women and children below the age of five from different communities attending antenatal and under-five care at clinics and first-level hospitals in Lusaka and Ndola.

3.2.1.3 Human Study Part I: Study involving patients with bloodstream infections and urinary tract infections.

This study involved isolating *E. coli* and *Enterococcus* species from patients with BSIs.

(a) **The sample size for bloodstream infections**

The sample size was calculated based on specimen flow into the lab as documented in the UTH Microbiology 2017-2018 Reports

Attribute:

i. AMR *E. coli* in blood
ii. 58 *E. coli* isolates from 4007 sampled blood cultures = 1.4%
iii. 56 Enterococci isolates from 4007 sampled blood cultures = 1.4%
iv. Among the positive *E. coli,* how many are likely to be resistant to cefotaxime = 95%
v. The likelihood of detecting cefotaxime-resistant *E. coli* in the blood is 0.014% x 0.95 = 0.0126
vi. Based on empirical studies, how many isolates should one need to detect diversity among *E. coli* isolates?
    - A study by Nagelkerke et al. (2015) = 50 to 100 isolates should be able to find genetic diversity.

    How many blood samples do you need to screen to get 50 isolates, given that the prevalence of resistant isolates was 0.0126? This was assumed to be the likelihood of finding resistance isolates.

    P = 0.0126

    X = 50

    N =?

Using the Negative Binomial distribution, we calculate a sample size of 3,969 blood samples; which should give us a minimum of 50 *E. coli* and 50 *Enterococcus* isolates, respectively.

(b) The sample size for Salmonella isolates from BSIs.

Salmonella infection tends to be seasonal; therefore, historic isolates stored in the bio-bank at UTH for the 2018/2019 phase while in the 2020/2021 phase, isolates were colleted prospectively.

(c) The sample size for Urinary tract infections

Calculation based on sample flow as documented in the UTH Microbiology 2017-2018 Reports

Attribute:

i. Urine samples processed = 12, 531
ii. *E. coli* isolated = 685
iii. *Enterococcus* species isolated = 281
iv. Probability of isolating *Enterococcus* species = 44.6%
v. Vancomycin-resistant Enterococcus (VRE) based on a study done in South Africa (Tasting Foka *et al*., 2018) = 8/55 (14.5%)
vi. P=P1xP2= 0.446 x 0.145=0.06 (6%)
    - From 100 samples, 6 VRE can be recovered
    - In order to have 50 isolates of VRE, 855 urine samples should be screened

This should give us a minimum of 50 *E. coli* and 50 *Enterococcus* isolates, respectively.

Sample collection and processing

After obtaining consent, blood samples were drawn from patients presenting with fever before antibiotic treatment was commenced. Each culture bottle was inoculated with 8 – 10mls of blood from adult patients, while the volume of blood drawn from paediatric patients was guided by body weight as described by Kellogg et al. (Kellogg et al., 2000). The blood was inoculated in two (when available) or one automated aerobic blood culture bottle (BD), after which it was transported to the Microbiology Laboratory within the UTH. Collecting two or more blood culture samples from different sites helps to distinguish true bloodstream infections from contaminants. The blood culture bottles were incubated in the Bactec machine (BD Bactec FX, Wokingham Berkshire, United Kingdom) till they flagged positive or up to seven days for those that were negative. All blood culture samples that flagged positive had a Gram stain prepared and were sub-cultured on MacConkey (Oxoid, Basingstoke, UK), blood, and chocolate agar plates (Oxoid, Basingstoke, UK). Before sub-culturing, the blood culture bottle tops were disinfected with iodine to prevent the introduction of contaminants. MacConkey agar plates were incubated aerobically at 37 ℃ for 18- 24 hours, after which, the plates were examined for lactose fermenters suggestive of GNB. Blood and chocolate plates were incubated at 35-37℃ in a 5% carbon dioxide incubator, after which they were examined for greyish colonies suggestive of Gram-positive bacteria (GPB). Gram stain was performed to confirm that the isolates were GNB and GPB, respectively. Each Gram

staining kit was quality controlled with Gram negative bacteria using *E. coli* ATCC 25922 and Gram-positive bacteria using *E. faecalis* ATCC 29212 strains before use.

Urine samples were collected in two 2ml sterile containers; this was done following sterile aseptic procedures. One sample was used for urinalysis while the other was used for culture. This was done in order to prevent contamination. The urine was sub-cultured on blood and CLED agar plates (Oxoid, Basingstoke). CLED plates were incubated aerobically at 37 ℃ for 18-24 hours while blood agar plates were incubated and examined for GPB per the standard protocol documented for blood culture samples above. CLED plates were then examined for lactose-fermenters suggestive of Gram-negative bacteria. Gram stains were performed to confirm the Gram-negative morphology.

3.2.1.4 Human Study Part II: Healthy Community

This study was undertaken in order to obtain the carriage of *E. coli* and *Enterococcus* species in the community from healthy individuals. Rectal swabs were collected from pregnant women and children below the age of five from different communities attending antenatal and under-five care at health centres and first-level hospitals in Lusaka and Ndola.

(a) The sample size for Rectal swabs

The calculation was based on the (Nagelkerke et al., 2017) study:

i. *E. coli* recovery out of 100 rectal swab samples = 100%
ii. Presence of ESBLs among the *E. coli* = 100%
iii. Required number of samples to detect resistant *E. coli* = 50
iv. 100 rectal swabs from each hospital

Based on these assumptions, a minimum of 50 *E coli* and 50 *Enterococcus* isolates were to be collected from each of the six health centres and first level hospitals.

Exclusion criteria

Pregnant women and under-five children that were admitted to any hospital within the study area in the past one month.

Sample collection and processing

After obtaining consent and explaining the procedure to the participants, a sterile swab was removed from the package and moistened in 0.85% sterile saline before swabbing. It was gently introduced into the rectal sphincter to a maximum of 2.5 cm, rotated three times and then withdrew and placed into Amie's transport medium (Oxoid, Basingstoke, UK)**.** Upon receipt in the

laboratory, the swabs were immediately placed in alkaline peptone water (APW) and incubated aerobically at 35-37°C for 18-24 hours before sub-culturing.

3.2.2 Poultry Study

The second component of the study involved the investigation of *E. coli* historical isolates collected under previous studies conducted between 2018-2020 (Muligisa-Muonga et al., 2021; Phiri et al., 2020). During this study, we ultilised stored *E. coli* from chickens collected from abattoirs, modern retail supermarkets and open markets in Lusaka, Kabwe, Ndola and Kitwe. The isolates were stored at -80 ℃ in the bio-bank at the School of Veterinary Medicine, University of Zambia. These historical isolates were initially isolated by culturing cloacal swabs and peptone water that was used for carcass wash. Cloacal swabs were collected from chickens from the abattoirs and open markets whereas the caracass wash was from chickens from retail supermarkets. For the isolation of *Enterococcus* species, cloacal swabs were placed in 10 mL of buffered peptone water (Oxoid, Basingstoke, UK) as a pre-enrichment media and incubated at 35-37◦C for 18-24 hours. Aliquots from the pre-enrichment broth were sub-cultured onto blood agar plates (Oxoid, Basingstoke, UK) as described in section.

3.2.1.3. **Sampling from the isolate bank**

A minimum of 100 stored *E coli* isolates were sampled from the bio-bank. The selection for *E. coli* was based on resistance to 3GC and/or MDR.

3.3 Laboratory Analysis

The human samples from hospitalised and outpatients were processed in the Microbiology Laboratory at the UTH; the community samples were processed at the Veterinary Public Health Laboratory at the University of Zambia. Poultry isolates were obtained from the School of Veterinary Public Health Laboratory Bio-bank.

3.3.1 Identification and antimicrobial susceptibility testing (AST) of *Escherichia coli*

The samples were cultured onto MacConkey (Oxoid, Basingstoke, UK). The plates were then incubated aerobically and in 5% carbon dioxide incubators at 35◦C - 37◦C for 18 -24 hours. Bacteria growth on the plates was examined for colony morphology that is characteristic of *E. coli* (Batra, 2018). Gram stain was also performed to confirm Gram negative morphology.

i. *E. coli* clinical isolates

All the *E. coli* clinical isolates were identified using the automated VITEK® compact machine (Biomerieux). This was achieved by using the VITEK® Gram-negative (GN) cards for

identification and VITEK® AST-GN83 and GN86 cards for antimicrobial susceptibility (AST) profiles. Three millilitres of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5-7.0) was aseptically transferred into a clear polystyrene 12×75 mm test tube. A homogenous organism suspension was prepared using sterile cotton swabs by transferring several isolated colonies from the plates to the saline tube. The 0.5 McFarland of the suspension was determined using a Densicheck (Biomerieux). For AST, a Vitek GN 145 microliter pipette was used for pipetting from the 0.5 McFarland suspension into 3 mls of sterile normal saline. The GN ID and AST cards were then placed in the tubes accordingly before loading them into the VITEK® 2 Compact instrument. Each batch of Vitek cards were quality controlled using *E. coli* ATCC 25922 strain.

ii. *E. coli* isolates from Healthy Community (non-clinical isolates)

Identification was based on biochemical reactions. The biochemicals used were Triple Sugar Iron Agar (TSI), Lysine Iron Agar (LIA), Sulphide Indole Motility (SIM), Citrate Utilisation tests, Indole and Eosin methylene blue (EMB). On EMB, *E. coli* gives a distinctive metallic green sheen due to the metachromatic properties of the dyes; the green metallic sheen indicates *E. coli* is able to ferment lactose to produce strong acid end products. Other lactose fermenters, such as some species of *Citrobacter* and *Enterobacter*, also react this way to EMB hence the need to further confirm the identification of *E. coli*. As seen in Table 1, biochemical changes were considered presumptive *E. coli*. All biochemicals were quality controlled using *E. coli* ATCC 25922 strain.

Table 1: Typical *E. coli* biochemical identification characteristics

Characteristics	***E. coli***
TSI	Acid/Acid
LIA	Alkaline/Alkaline
H_2S	Negative
Indole	Positive
SIM (Motility)	Motile
Citrate	Negative
Urease	Negative
Gas	Positive
Eosin methylene blue (EMB)	The distinctive metallic green sheen

Polymerase chain reaction (PCR) was used to confirm *E. coli*. This was achieved by using the *uidA* gene (Table 2), which encodes the beta-glucuronidase enzyme (Godambe et al., 2017). DNA was extracted using the Nuclisense® Biomeriux easyMAG®. Eight hundred microliter of the Nuclisens Lysis buffer was aliquoted into well-labelled sterile Eppendorf micro-centrifuge tubes. Using a sterile colony loop, a loopful of pure isolate colonies from the nutrient agar culture plate (Oxoid, Basingstoke, UK) was picked and added to the lysis buffer in a labelled Eppendorf micro-centrifuge. The cap was then placed on the micro-centrifuge, followed by vortexing. This was left to stand for 10 minutes, after which 400µl of each lysate was aliquoted into easyMAG disposable sample loading vessels and vortexed. Nuclisens Magnetic Silica was then added to each well containing the individual lysed sample. The extraction run was set up on the EasyMAG machine as outlined in the manufacturer's manual with an elution volume of 50 µl. The vessels were then loaded onto the machines, after which the extraction run was as described in the easyMAG manual. After the run, the extracted nucleic acid was transferred into labelled micro-centrifuge tubes and stored at −20 °C for PCR analysis.

PCR amplification was performed as described by (Godambe et al., 2017). The Veriti 96 Well Thermal Cycler-Applied Biosystems, Pittsburg, PA, USA was used. For *E. coli*, PCR amplification was initiated at 94 °C for 5 min, 35 cycles of annealing at 55.2 °C for 10 seconds, extension at 72 °C for 1 minute, denaturation at 94 °C for 10 seconds, and a final extension of 72 °C for 10 minutes. The PCR products (1/10 volume) were analysed by gel electrophoresis (Bio-Rad, Hercules, CA, USA) at 100 volts for 30 minutes using 1.5 % agarose gels (BD Difco™) in 1X TAE buffer (Tris-acetate EDTA). The gels were stained with ethidium bromide (Sigma, St. Louis, MO, USA), and the PCR products were visualised under ultraviolet light.

Table 2: Primers used for confirmation of *E. coli*

Purpose	Target gene	Primers sequence F/R (5'-3')	Amplicon size (bp)	Annealing Temperature (°C)
***E. coli* confirmation**	*uidA*	F-CGGAAGCAACGCGTAAACTC R-TGAGCGTCGCAGAACATTACA	147	55

Confirmed *E. coli* isolates from the healthy community and poultry isolates were then subjected to conventional AST testing using the Kirby-Bauer disc diffusion method (Hudzicki, 2009). Isolated colonies on nutrient agar plates (Oxoid, Basingstoke, UK) were picked and emulsified in normal saline to make a suspension of 0.5 McFarland. Using a sterile swab, this was plated and evenly distributed onto Mueller Hinton agar (Oxoid, Basingstoke, UK), after which discs impregnated with antibiotics, as listed in Table 3 were placed 24mm apart. These plates were incubated aerobically at 35-37°C for 18-24 hours. The susceptibility results were measured and interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI (Clinical and Laboratory Standards Institute), 2020; Standards and Testing, 2018).

iii. Stored *E. coli* isolates from poultry.

The stored *E. coli* isolates from poultry were presumptively identified using conventional bio-chemicals and confirmed using the Analytical Profile Index (API 20E) (Biomerieux) and 16S rDNA sequencing. AST was determined using the Kirby-Bauer disc diffusion method (Muligisa-Muonga et al., 2021; Phiri et al., 2020). Table 3 shows the antibiotics used.

Table 3: Antibiotics tested on *E. coli* using Kirby-Bauer disc diffusion method

Antibiotic class	Antibiotic
Penicillins	Ampicillin (10µg)
Second generation cephalosporins	Cefoxitin (30µg)
Third generation cephalosporins	Cefotaxime (30µg)
	Ceftazidime (30µg)
Fourth generation cephalosporins	Cefepime (30µg)
Carbapenems	Imipenem (10µg) OR
	Meropenem (10µg)
Sulphonamides and trimethoprim	Co-trimoxazole (1.25/23.75µg)
Fluoroquinolones	Ciprofloxacin (5µg)
Aminoglycosides	Gentamicin (10µg)
	Amikacin (30µg)
Tetracyclines	Tetracycline (30µg)
Nitrofurans	Nitrofurantoin (300µg)
Monobactum	Aztreonam (30µg)

Isolates that were resistant to third-generation cephalosporins (3GC) were screened for ESBLs using MacConkey agar (Oxoid, Basingstoke, UK) supplemented with cefotaxime sodium (Hoechst Marion Roussel, Bridgewater, NJ, USA) 1.0 mg/L. MacConkey agar was prepared according to the manufacturer's instructions, after which cefotaxime sodium solution was filter-sterilised and added aseptically to the sterilised MacConkey agar.

3.3.2 Identification and AST of *Enterococcus* species

The greyish-looking colonies on blood-agar, Gram-positive cocci and catalase-negative were plated on Bile Esculin Azide (BEA) agar (Oxoid, Basingstoke, UK). BEA agar was used to identify *Enterococcus* species presumptively. The detection of *Enterococcus* species is based on the hydrolysis of esculin in the media into glucose and esculetin (Chuard and Reller, 1998). The esculetin reacts with a ferric iron salt to produce a phenolic iron complex, which turns the medium dark brown or black. BEA agar contains ingredients such as bile salts and sodium azide to inhibit the growth of other Gram-positive and Gram-negative organisms, respectively (Pillay et al., 2018). Single colonies that turned the media black (bile esculin-positive colonies) were subjected to PCR for confirmation. The Kirby-Bauer disc diffusion method was used for AST determination as earlier described in section 3.3.1. All the media used for the identification of Enterococcus species were quality controlled using the *E. faecalis* ATCC 29212.

All the clinical isolates were identified using the automated VITEK® compact machine (Biomerieux). This was achieved by using the VITEK® Gram-positive (GP) cards for identification and VITEK® AST-GP67 cards for antimicrobial susceptibility (AST) profiles. All the Vitek cards were quality controlled using the *E. faecalis* ATCC 29212.

The presumptive *Enterococcus* species from the healthy community and poultry were subjected to PCR as described in section 3.3.1. The *Tuf* gene was used for confirmation of the *Enterococcus* genus, while the *SodA* gene was used to speciate *E. faecium* and *E. faecalis* respectively (Li et al., 2012a; Pillay et al., 2018). The *tuf* gene which encodes the elongation factor EF-Tu, is involved in peptide chain formation and is located in the short tandem repeat region on the bacterial chromosome (Li et al., 2012b). It has been found to be more discriminative than the 16S rRNA gene for identifying strains belonging to the genera *Enterococcus* (Li et al., 2012b). PCR was performed in a DNA thermal cycler with 30 cycles of 94°C for 30 seconds, 50–55°C for 1 minute, 72°C for 1 minute 30 seconds, followed by a final extension step of 72°C for 10 minutes (Li et al., 2012a). Table 4 shows the primers used that were used.

Table 4: Primers used for confirmation of *Enterococcus* genus and speciation of *E. faecalis* and *E. faecium*

Purpose	Target gene	Primers sequence F/R (5'-3')	Amplicon size (bp)	Annealing Temperature (^{o}C)
***Enterococcus* genes**	*Tuf*	F-TACTGACAAACCATTCATGATG R-AACTTCGTCACCAACGCGAAC	112	53
E. faecalis	*SodA*	F-ACTTATGTGACTAACTTAACC R-TAATGGTGAATCTTGGTTTGG	360	55
E. faecium	*SodA*	F-GAAAAAACAATAGAAGAATTAT R-TGCTTTTTTGAATTCTTCTTTA	215	48

Confirmed *Enterococcus* species from the healthy community and poultry isolates were then subjected to conventional AST testing using the Kirby-Bauer disc diffusion method. The antibiotics that were used are listed in Table 5. All the antibiotics were quality controlled using *E. faecalis* ATCC 29212 strain.

Table 5: Antibiotics tested on *Enterococcus* species using Kirby-Bauer disc diffusion method

Antibiotic class	Antibiotic
Aminoglycosides	High level Gentamicin (120µg)
Phenicols	Chloramphenicol (30µg)
Glycopeptides	Vancomycin (30µg)
Macrolides	Erythromycin (15µg)
Nitrofurans	Nitrofurantoin (300µg)
Penicillins	Ampicillin (10µg)
	Penicillin (10 units)
Quinolones	Ciprofloxacin (5µg)
Streptogramins	Quinupristin-dalfopristin (15µg)
Tetracyclines	Tetracycline (30µg)
Oxazolidones	Linezolid (30µg)

3.3.3 Identification and AST of *Salmonella* species

i. *Salmonella* Clinical isolates

The blood culture samples were processed as described in section 3.2.1.3. The non-lactose fermenting colonies on MacConkey were subjected to Gram stain. The identification and confirmation of suspected *Salmonella* was determined using the automated VITEK® compact machine (Biomerieux) and utilising the VITEK® Gram-negative (GN) cards. AST was determined using VITEK® AST-GN83 and GN86 ID cards. All Vitek cards were quality controlled used *E. coli* ATCC 29522.

3.3.4 Characterisation of resistant gene determinants using PCR and WGS

3.3.4.1 Determination of resistant genes using PCR

The selection criteria for molecular characterisation of isolates was determined by the diversity of phenotypic resistance profiles. Seventy *E. coli* isolates from the healthy community resistant to 3rd generation cephalosporins, ciprofloxacin and/or carbapenems were screened for ESBL, ampC, PMQR and Carbapenemase resistance gene determinants using PCR. The primer selection and PCR protocol were based on (Farkas et al., 2019) (Table 6). Similarly, *Enterococcus* species that were MDR from the healthy community were subjected to PCR to determine resistant genes. The primer selection and PCR protocol were based on (Zou et al., 2011) (Table 7).

Table 6: Primers used to determine resistance genes in *E. coli* isolated from healthy communities

Antibiotic class	Target gene	Primers sequence F/R (5'- 3')	Amplicon size (bp)	Annealing temperature (°C)
Penicillins	*bla*$_{TEM}$	F-TCCGCTCATGAGACAATAACC	1080	50
		R-TTGGTCTGACAGTTACCAATGC		
	bla$_{SHV}$	F-GCGTTATATTCGCCTGTGTATTAT	385	55
		R-GCCTGTTATCGCTCATGGTAATG		
Cephems	*bla* $_{CTX-M}$	F-TCTTCCAGAATAAGGAATCCC	400	57
		R-CCGTTTCCGCTATTACAAAC		
	ampC	F-AGAAGGACCAGGCACAGATC	671	60
		R-CTCGGCATTGGGATAGTTGC		
Carbapenems	*bla*-$_{VIM}$	F-GATGGTGTTTGGTCGCATA	449	59
		R- CGAATGCGCAGCACCAG		
	bla-$_{NDM}$	F-GGTTTGGCGATCTGGTTTTC	621	57
		R- CGGAATGGCTCATCACGATC		
	bla-$_{OXA-48}$	F-GCTTGATCGCCCTCGATT	705	53
		R-GATTTGCTCCGTGGCCGAAA		
Fluoroquinolones	*qnr*A	F-ATTTCTCACGCCAGGATTTG	541	55
		R-GATCGGCAAAGGTTAGGTCA		
	*qnr*B	F-GATCGTGAAAGCCAGAAAGG	368	55
		R-ACGATGCCTGGTAGTTGTCC		
	*qnr*S	F-ACGACATT CGTCAACTGCAA	496	55
		R-TAAATTGGCACCCTGTAGGC		

Table 7: Primers used to determine resistance genes in *Enterococcus* species from healthy community

Antibiotic class	Target Gene	Primers sequence F/R (5'- 3')	Amplicon size (bp)	Annealing temperature (°C)
Macrolides	*erm*A	F-AAGCGGTAAAACCCCTCTGAG	645	50
		R-TCAAAGCCTGTCGGAATTGG		
	*erm*B	F-CATTTAACGACGAAACTGGC	639	52
		R-GGAACATCTGTGGTATGGCG		
	*erm*C	F-CAAACCCGTATTCCACGATT	642	58
		R-ATCTTTGAAATCGGCTCAGG		
Glycopeptides	*VanA*	F-GCGCGGTCCACTTGTAGATA	314	65
		R-TGAGCAACCCCCAAACAGTA		
	VanB	F-AGACATTCCGGTCGAGGAAC	220	65
		R-GCTGTCAATTAGTGCGGGAA		

3.3.4.2 Whole genome sequencing of *E. coli*, *Enterococcus* and *Salmonella* species

i. *E. coli* and *Enterococcus* species

One hundred and forty *E. coli* isolates resistant to third-generation cephalosporins (3GCs) and those that were multi-drug resistant (MDR) were selected for WGS. The selection of *E. coli* isolates was as follows; 90 human clinical isolates from blood and urine, 25 from the healthy communities and 25 from poultry. Nine clinical MDR *Enterococcus* isolates from blood were also selected for WGS. The strains selected for WGS were streaked on Luria Broth (LB) agar plates (Thermo Fisher Scientific, UK) and incubated aerobically for 18-24 hours, after which the plates were examined to confirm the absence of contamination. A single colony of each strain was then

inoculated in liquid LB broth and incubated in an aerobic shaker incubator. In order to assess the microbial growth, the Optical Density at 600 nm (OD600nm) was measured to verify that cells were in the exponential growth phase, which is usually between 0.5 and 1.0. OD measurements were only considered valid when below 1.0, and cells were harvested during the exponential growth phase. The harvested cells were required to be an equivalent of 8-12 OD600nm (8-12mL culture in 1.0 OD600nm). The cells were then concentrated by centrifugation in a 1.5 mL to 2 mL microfuge tube, washed with 1 mL phosphate-buffered saline (PBS), and then pelleted again. The final pellet was then re-suspended in the 0.5 mL of 1x DNA/RNA Shield buffer (Zymo Research, USA) in a 2 mL screw cap tube. The samples were then shipped to MicrobesNG (Birmingham, UK) for sequencing.

Five to forty microliters of the suspension were lysed with 120 µL of Tris-EDTA buffer (TE) containing lysozyme (final concentration 0.1 mg/mL) and RNase A (ITW Reagents, Barcelona, Spain) (final concentration 0.1 mg/mL), then incubated for 25 min at 37°C. Proteinase K (VWR Chemicals, Ohio, USA) (final concentration 0.1mg/mL) and SDS (Sigma-Aldrich, Missouri, USA) (final concentration 0.5% v/v) were added and incubated for 5 min at 65°C. Genomic DNA was purified using an equal volume of solid-phase reversible immobilisation (SPRI) beads and re-suspended in elution buffer (EB) (Qiagen, Germany). DNA was quantified with the Quant-iT dsDNA HS kit (Thermo Fisher Scientific) assay in an Eppendorf AF2200 plate reader (Eppendorf UK Ltd, UK).

Genomic DNA libraries were prepared using the Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's protocol with the following modifications: input DNA was increased 2-fold, and PCR elongation time was increased to 45s. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR automated liquid handling system (Hamilton Bonaduz AG, Switzerland). Pooled libraries were quantified using the Kapa Biosystems Library Quantification Kit for Illumina. Libraries were sequenced using Illumina sequencers (HiSeq/NovaSeq) using a 250bp paired-end protocol. Reads were adapter trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 (Bolger et al., 2014). De novo assembly was performed on samples using SPAdes version 3.7 (Bankevich et al., 2012), and contigs were annotated using Prokka 1.11 (Seemann, 2014).

ii. *Salmonella* species

One hundred and seventeen *Salmonella* species isolates were selected for WGS. The isolates were shipped to the National Institute for Communicable Diseases (NICD), South Africa, for whole-genome sequencing (WGS). Genomic DNA was isolated from bacteria using an Invitrogen PureLink Microbiome DNA Purification Kit (Invitrogen, Waltham, Massachusetts, USA). WGS was performed using Illumina NextSeq (Illumina, San Diego, California, USA) next-generation sequencing technology. DNA libraries were prepared using a Nextera DNA Flex Library Preparation Kit (Illumina) followed by 2 × 150 bp paired-end sequencing runs with ~80 times coverage.

3.3.5 Determination of genotypic relatedness of *E. coli* isolated from humans- (Clinical and non-clinical) and poultry

Genotypic related of *E. coli* strains from humans and poultry was determined using the SNP (close relatedness) as described in SnapperDB (Dallman et al., 2018), and phylogeny was determined using EnteroBase and visualized using Microreact.

3.3.6 Data management and analysis

Social demographic, clinical and antibiotic use data

The socio-demographic, clinical, empiric treatment and antibiotics commonly prescribed in primary healthcare facilities data, was entered into CSPro. The antibiotics given as empiric treatment, as well as those commonly prescribed in primary healthcare facilities were categorized as Access, Watch and Reserve as per the WHO Access, Watch, Reserve (AwaRe) classification (WHO, 2019).

Antimicrobial sensitivity data

AST data was entered into WHOnet 2020, after which it was exported into Excel spreadsheets and analysed in STATA version 28.0. The inhibition zones were analysed for AMR interpretation using the CLSI 2020 guidelines and WHONET 2021. The antimicrobial susceptibility results were interpreted as Resistant (R), Intermediate (I), and Susceptible (S) isolates according to the CLSI 2020 M100 guidelines. The prevalence of resistant *E. coli*, *Enterococcus* and *Salmonella* species was calculated by dividing the number of resistant isolate test results by the total number of isolates tested. Results were presented using proportions, frequency distribution and percentages.

WGS analysis for E. coli, Salmonella and Enterococcus species

For *E.coli,* the raw sequencing data (30X FastQ files for paired-end reads) were uploaded and investigated at the EnteroBase web-based platform (https://enterobase.warwick.ac.uk/species/index/ecoli) and SnapperDB, the United Kingdom Health Security Agency (UKHSA) (Dallman et al., 2018). *Salmonella*, raw genomic (30X FastQ files for paired-end reads) sequencing data were assembled using SPAdes software (version 3.15). The assembled data for all the three pathogens were analysed using online bioinformatics pipelines at the Centre for Genomic Epidemiology (CGE) of the Technical University of Denmark (http://www.genomicepidemiology.org/services/). These pipelines included KmerFinder 3.2 for species identification, MLST 2.0 for determining MLST profile, and ResFinder 4.1 for identifying antimicrobial resistance determinants. *Salmonella* serovar determination was performed using the online bioinformatics pipeline SeqSero2 version 1.1.0 (http://denglab.info/SeqSero2). Further raw sequencing data were uploaded and investigated at the EnteroBase web-based platform (http://enterobase.warwick.ac.uk/species/index/senterica). EnteroBase analysis included serotyping, phylotyping and MLST confirmation using various genomic tools and genomic comparison of isolates based on core-genome multilocus sequence typing (cgMLST) data using the cgMLST V2 + HierCC V1 scheme. The *E. coli* phylogenetic cluster and geographic distribution of group diversity analysis of cgMLST data were illustrated using Microreact (https://microreact.org/). The cgMLST scheme analysis for *Salmonella* incorporated 3002 genes, and phylogenetic cluster analysis of cgMLST data was depicted using a GrapeTree-generated minimum spanning tree using the MSTree V2 algorithm (https://bitbucket.org/enterobase/enterobase-web/wiki/GrapeTree).

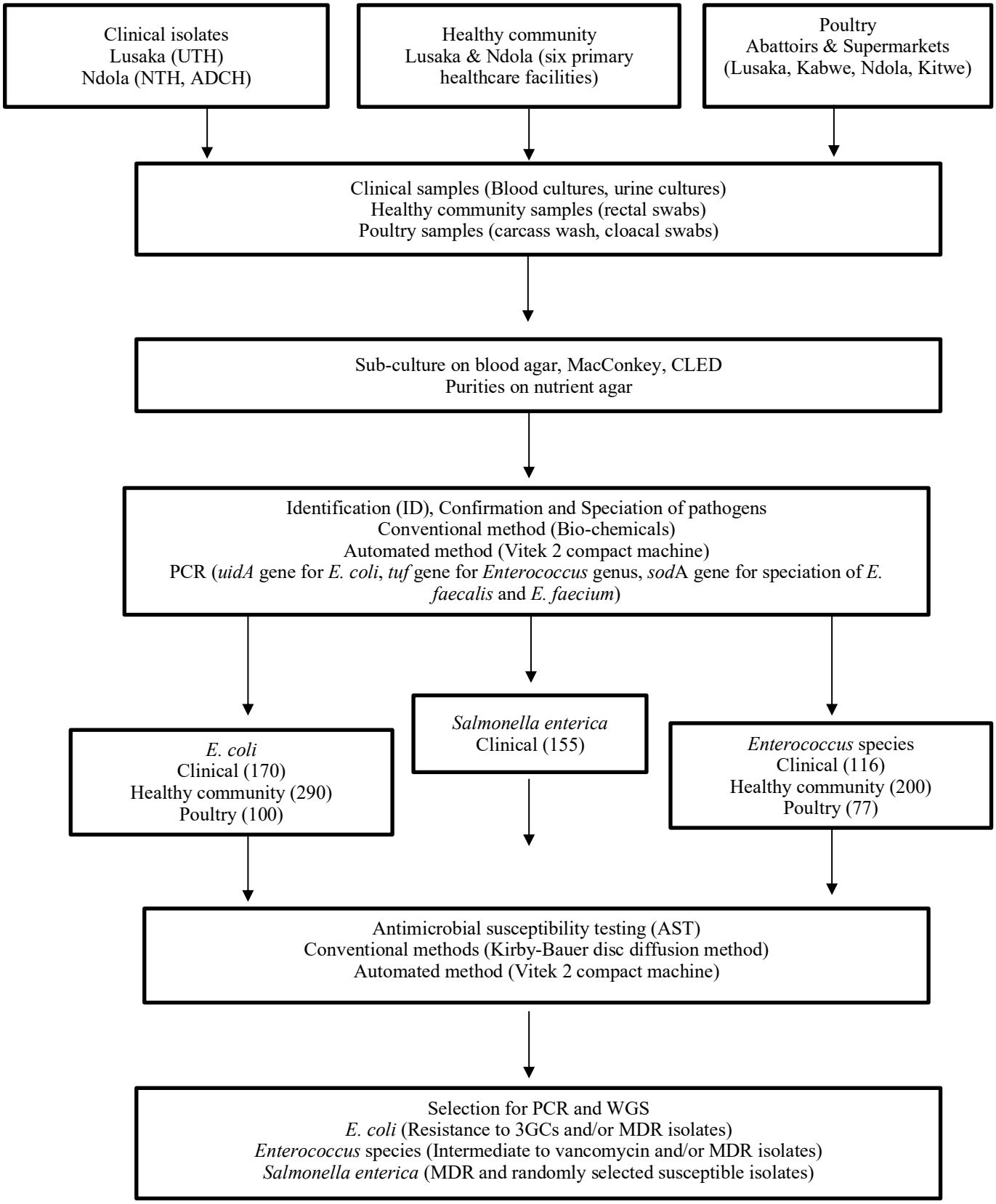

Figure 2: Flowchart for sample collection, identification and Antimicrobial Susceptibility testing
Abbreviations: MDR – Multi-drug resistance, 3GCs – Third generation cephalosporins, PCR – polymerase chain reaction

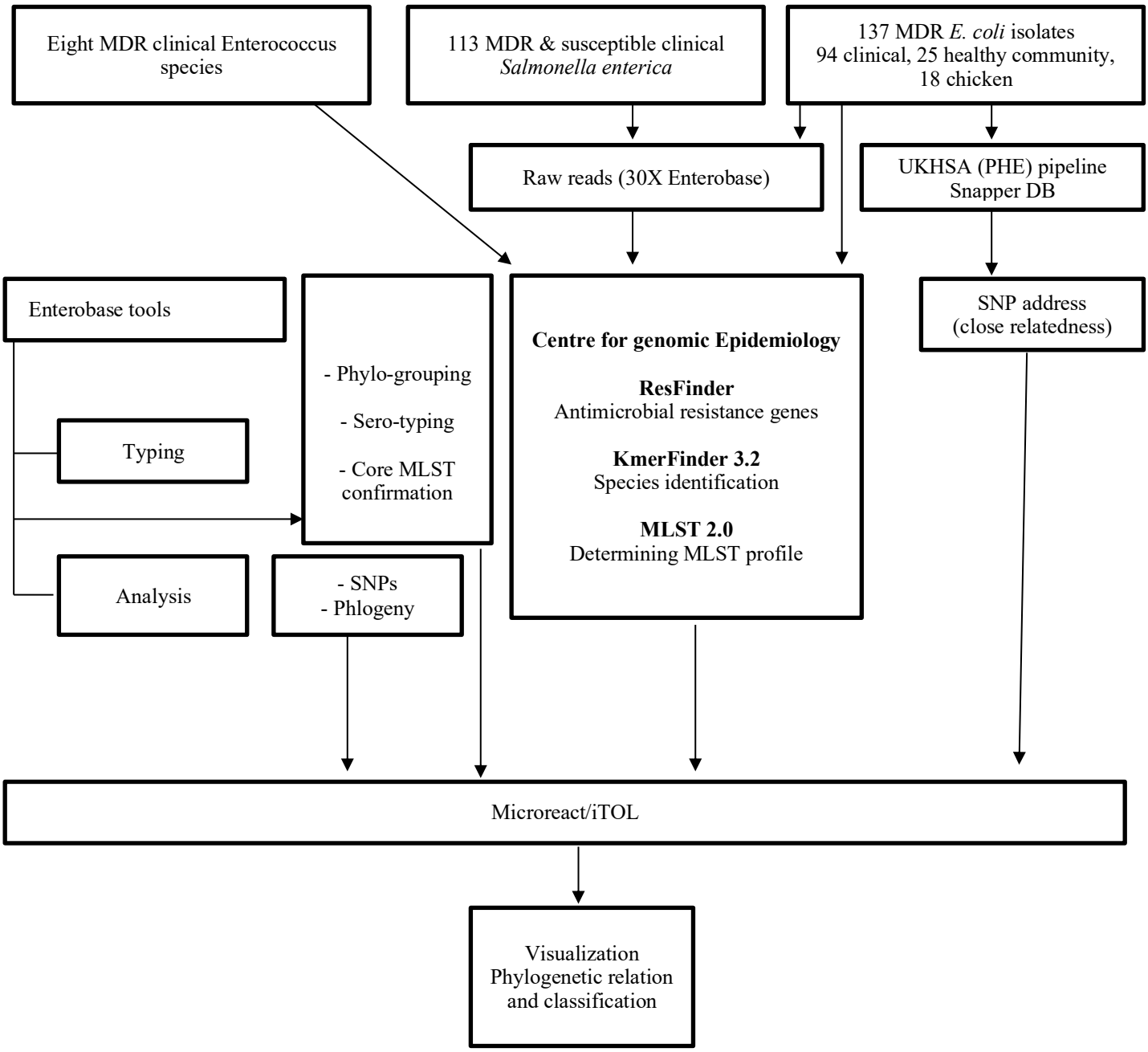

Figure 3: Flowchart for WGS analysis for all the three pathogens *Enterococcus* species, *Salmonella enterica* and *E. coli*

Abbreviations: MDR – multidrug resistance, UKHSA – United Kingdom Health Security Agency, PHE – Public Health England, MLST – Multi-locus sequence typing, SNP – Single nucleotide polymorphisms, iTOL – Interactive Tree of life

3.3.7 Ethics consideration

3.3.7.1 Ethical Clearance

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee at Eres Converge institutional review board (Ref. No. 2019-Aug-017). The regulatory approval and the transfer of samples to a third party were sought from National Health Research Authority (NHRA). This was in line with the legal agreement in the Material Transfer Agreement (MTA) approved by NHRA of Zambia. Permission to conduct the study at the different hospitals/institutions was obtained from the Senior Medical Superintendents of all the hospitals included in the study.

3.3.7.2 Consent

Before administering the oral questionnaires and collecting samples, written informed consent was sought and obtained from the participants and for paediatric patients, their guardians gave consent. Only those that gave consent were included in the study.

3.3.7.3 Confidentiality

The study participants were assured confidentiality by not identifying them by name but by codes, age and sex. The data was secured, and the results were used for research purposes only. In order to allow for further academic investigation, as required by the research community, the isolates were stored at -80°C.

CHAPTER FOUR

4.0 RESULTS

4.1 Hospitalised patients with BSIs and UTIs

4.1.1 UTH in Lusaka District of Lusaka Province

Escherichia coli, *Enterococcus* species, and *Salmonella enterica* isolates from UTH were collected in two phases (2018-2019) and (2020-2021). The *E. coli*, *Enterococcus* species, and *Salmonella enterica* isolates from 2018-2019 were achieved isolates from BSIs and UTIs. In the 2018-2019 collection phase, 83 *E. coli* isolates were included in this study, of which 53% (44/83) were from BSIs, and 47% (39/44) were from UTIs. Eighty-five *Enterococcus* species isolates were included, of which 72% (61/85) were from BSIs, and 28% (24/85) were from UTIs. All the 76 *Salmonella enterica i*solates from 2018-2019 included in this study were isolated from BSIs at UTH.

In the 2020-2021 collection phase, samples were collected prospectively from BSIs only. Between October 2020 and March 2021, 1,781 blood culture specimens were processed, out of which 206 patients were enrolled in our study. Among the 206 enrolled patients, adult patients were relatively more represented 54% (112/206) than paediatric patients 46% (94/206), while females (61%) were relatively more than males (39%). A total of 158 pathogens were isolated, and the patients with confirmed BSI caused by Enterobacterales were 43% (88/206). *E. coli* was the most prevalent at 23% (37/158), followed by *Salmonella enterica* at 20% (20/158), *and Enterococcus* BSI at 15% (24/158) (Table 8). The prevalence levels of other pathogens not included in this study are also highlighted in Table 8.

4.1.2 NTH and ADCH in Ndola district of Copperbelt Province

Between October 2020 and March 2021, 169 pathogens were isolated. Of these, 110 GNB were isolated from blood and urine cultures. Thirteen GNB isolated from BSIs were from ADCH, and eight were from NTH. Eighty-one isolates were from urine from NTH, and nine were from ADCH. *E. coli* was the most prevalent in both urine and blood culture specimens. Twelve *Enterococcus* species were isolated from both hospitals, seven from NTH and five from ADCH, with only five *Staphylococcus aureus* BSIs from ADCH. All 42 *Salmonella enterica* isolates from BSIs were from ADCH (Table 8).

Table 8: Distribution of pathogens isolated from blood and urine (2020 to 2021)

Pathogens isolated	UTH (blood cultures)		NTH and ADCH (Blood and urine cultures)	
	Frequency (n)	Proportion (%)	Frequency (n)	Proportion (%)
Escherichia coli	37	23	50	30
Klebsiella pneumoniae	26	16	28	17
Salmonella enterica	32	20	42	25
Acinetobacter baumannii	13	8	6	4
***Enterococcus* species**	24	15	12	7
Pseudomonas aeruginosa	7	4	4	2
Staphylococcus aureus	14	9	5	3
Klebsiella aerogenes	3	2	-	-
Citrobacter freundii	1	1	-	-
Proteus mirabilis	1	1	10	6
Enterobacter cloacae	-	-	8	5
Morganella morgannii	-	-	4	2
Total	158	-	169	-

4.2 Healthy Community Survey

One hundred and sixty-eight rectal swabs were collected from primary health care facilities in Lusaka from pregnant and under-five children (Table 9). Similarly, 122 rectal swabs were collected from primary health care facilities in Ndola from pregnant and under-five participants (Table 9).

Table 9: Distribution of rectal swabs collected from primary healthcare facilities in Lusaka and Ndola District

Study area	Health facility	Total number of participants	Pregnant women Frequency (n)	Under-five Frequency (n)
Lusaka District	Kanyama first level hospital	65	40	25
	Kalingalinga health centre	55	20	35
	Chilenje first-level hospital	48	21	27
Ndola District	Mapalo health centres	51	26	25
	Lubuto health centre	40	10	30
	New Masala health centre	31	24	7
	Total	**290**	**141**	**149**

4.3 Prescribing patterns at UTH, NTH, ADCH

Empiric treatment data was only collected for 153 enrolled patients that presented with fever and symptoms of BSIs and UTIs in the 2020-2021 data collection phase. The breakdown of antibiotics used is highlighted in Table 10. Of note, 29% (44/153) of the patients received more than one antibiotic with different combinations, such as Tuberculosis (TB) fixed-dose with cefotaxime (6%), metronidazole with cefotaxime (9%), co-trimoxazole with cefotaxime (2%) and cefotaxime with penicillin (2%). Fourth-generation cephalosporins were mainly prescribed in the Neonatal Intensive Care Unit (NICU) and other paediatric wards. Ceftriaxone, a third-generation cephalosporin (3GC), was the most prescribed antibiotic as empiric treatment in all three hospitals. Cefepime, a fourth-generation cephalosporin (4GC) and penicillins followed, with imipenem, being the least prescribed.

Table 10: Antibiotics given as part of empiric treatment at UTH, NTH and ADCH (2020-2021)

Name of antibiotic	Frequency (n)	Per cent (%)	AWaRe classification
Ceftriaxone	97	63%	Watch
Cefepime	50	33%	Watch
Penicillin	32	21%	Access
Metronidazole	22	14%	Access
Gentamicin	16	10%	Access
Ciprofloxacin	7	5%	Watch
Cephalexin	6	4%	Access
Doxycycline	5	3%	Access
Co-trimoxazole	4	3%	Access
Erythromycin	3	2%	Watch
Amoxicillin	3	2%	Access
Chloramphenicol	1	1%	Access
Imipenem	1	1%	Watch

4.4 Prescribing patterns in Primary healthcare facilities

Most antibiotics prescribed in the primary healthcare facilities in both Lusaka and Ndola belonged to the Access group of antibiotics (Table 11, Table 12 and Figure 4). Only Chilenje first-level hospital had the highest use of ceftriaxone 98% (1759/1792) and the least use 2% (34/1792) was at Kalingalinga clinic. Lusaka had amoxicillin the most prescribed where as cephalexin was the most prescribed in Ndola. The other antibiotics that fell in the top five of the most prescribed were metronidazole, ciprofloxacin and co-trimoxazole. Ciprofloxacin was the most prescribed antibiotic from the WHO Watch group of antibiotics in all the primary healthcare facilities (Tables 11 and 12).

Table 11: Prescribing patterns at Chilenje and Kanyama first level hospital and Kalingalinga clinic (2020-2021)

Name of antibiotics	Indication	Frequency (n)	Per cent (%)	AWaRe classification
Amoxicillin	RTI, Otitis Media	7,272	23%	Access
Metronidazole	UTI, Diarrhoea	5,871	18%	Access
Ciprofloxacin	UTI, PID, STI	4,955	15%	Watch
Cephalexin	RTI, UTI, Pyelonephritis	4,873	15%	Access
Co-trimoxazole	Diarrhoea, Prophylaxis in HIV patients	2,242	7%	Access
Cefotaxime/Ceftriaxone	RTI, UTI, Sepsis	1,792	6%	Watch
Azithromycin	RTI, Covid related symptoms, Tonsillitis	1,469	5%	Watch
Doxycycline	UTI, STI	1,284	4%	Access
Benzathine penicillin	Tonsillitis, Syphilis,	882	3%	Access
Gentamicin	STI	751	2%	Access
Cloxacillin	RTI	378	1%	Access
Penicillin V	URTI, Tonsillitis	289	1%	Access
Nitrofurantoin	UTI	134	0%	Access
Chloramphenicol	Otitis media	25	0%	Access
Ampicillin	Sepsis	8	0%	Access
Total	**-**	**32, 205**	**100%**	**-**

Abbreviations: RTI – Respiratory tract infection, HIV – human immunodeficiency virus, URTI – Upper respiratory tract infection, STI – Sexually transmitted infection, PID – Pelvic inflammatory Disease

Table 12: Prescibing patterns at Lubuto, Mapalo and New Masala health centers in Ndola District of Copperbelt province (2020-2021)

Name of antibiotic	Indication	Frequency (n)	Per cent (%)	AWaRe classification
Cephalexin	RTI, UTI	7,038	20%	Access
Amoxicillin	RTI	5,834	17%	Access
Metronidazole	Diarrhoea	5,558	16%	Access
Co-trimoxazole	URTI, RTI,	5,403	16%	Access
Ciprofloxacin	UTI	3,182	9%	Watch
Cloxacillin	RTI, Wounds, Burns,	1,830	5%	Access
Penicillin	Febrile illness, Sepsis	1,634	5%	Access
Doxycycline	UTI	1,416	4%	Access
Axithromycin/Erythromycin	RTI	1,338	4%	Watch
Gentamicin	UTI	411	1%	Access
Chloramphenicol	Conjunctivitis	89	0%	Access
Total	-	**34,594**	**100%**	-

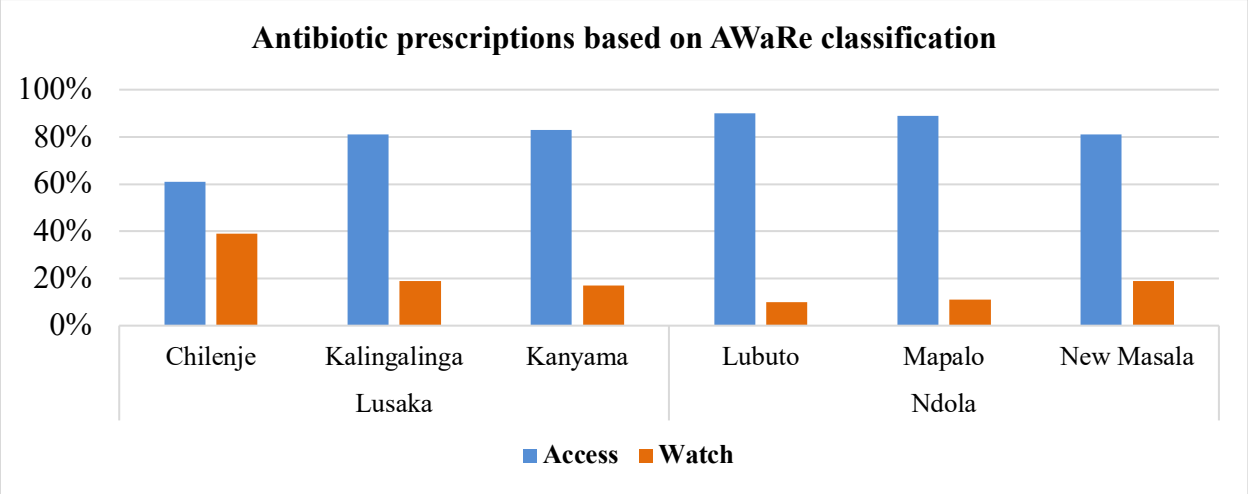

Figure 4: Antibiotic prescriptions based on AWaRe classification of the primary healthcare facilities in Lusaka and Ndola districts.

4.5 *Escherichia coli*

4.5.1 BSI and UTIs (Clinical *E. coli*)

The UTH had the highest *number of E. coli* isolates because of the longer data collection duration (2018-2021), and most of the isolates were from BSIs. *E. coli* from NTH and ADCH were mostly from UTIs (Table 13).

Table 13: Distribution of clinical *E. coli* from blood and urine cultures from UTH, NTH and ADCH

		Institution			
		ADCH	**NTH**	**UTH**	**Total**
		Frequency (n)			
Specimen type	**Blood**	2	4	80	86
	Urine	4	40	40	84
	Total	**6**	**44**	**120**	**170**

Ampicillin and co-trimoxazole resistance were over 80% in all three hospitals, with the highest recorded at ADCH (100%). Ciprofloxacin resistance was over 60%, with the highest also recorded at ADCH (80%). On the other hand, ceftriaxone, amoxicillin-clavulanate, ceftazidime and cefuroxime resistance were highest at UTH. Similarly, Gentamicin and piperacillin-tazobactam resistance was only recorded at UTH. At the same time, imipenem resistance was only recorded at NTH. Although resistance was high in both BSIs and UTIs, resistance was generally higher in BSIs than in UTIs. Only imipenem, co-trimoxazole and piperacillin-tazobactam recorded higher resistance in UTIs compared to BSIs.

4.5.2 Healthy community (Non-clinical *E. coli*)

AMR in the non-clinical *E. coli* from the healthy community was highest to co-trimoxazole (61%), ampicillin (73%) and tetracycline (78%) and lowest to imipenem, piperacillin-tazobactam and ampicillin-sulbactam, all at 1%. Resistance in non-clinical *E. coli* was generally higher in Ndola compared to Lusaka. Though low, resistance to antibiotics such as ceftriaxone, cefepime, and amikacin used to treat invasive infections in hospitalised patients was noted in the healthy community. In some clinics, the most prescribed antibiotics relatively corresponded with the resistance rates observed.

Resistance to ampicillin was noted to be high in all six different healthcare facilities. Similarly, co-trimoxazole and tetracycline resistance was high in all the facilities, with the least resistance in Chilenje. Imipenem resistance was only seen in Chilenje, while piperacillin-tazobactam and ampicillin-sulbactam resistance was recorded in Mapalo and Kalingalinga health facilities. Resistance to other antibiotics such as chloramphenicol, ciprofloxacin, ceftriaxone, gentamicin, amikacin and aztreonam was recorded in all the primary healthcare facilities but at diverse percentages. There was a minimal difference in the occurrence of AMR in adults (pregnant women) in comparison to children ≤ 5 years. Similarly, there were minimal differences in the occurrence of AMR in the different age groups in children ≤ 5.

4.5.3 Poultry (*E. coli*)

AMR was recorded in both abattoirs (cloacal swabs) and ready-to-eat chickens (carcass wash) from retail supermarkets. AMR was higher at the abattoir compared to retail supermarkets and notably was the high resistance in critically important antimicrobials for human medicine.

Figure 5 summarises and compares the resistance profile in clinical, poultry and healthy community *E. coli* isolates to 12 key antibiotics. Although resistance was highest in clinical isolates, more than half of the antibiotics tested showed resistance in isolates from all three sources, highest in ampicillin and co-trimoxazole. Poultry isolates had the highest resistance to tetracycline 78%. In clinical isolates, ciprofloxacin and cefotaxime resistance was at 68% and 62%, respectively (Figure 5).

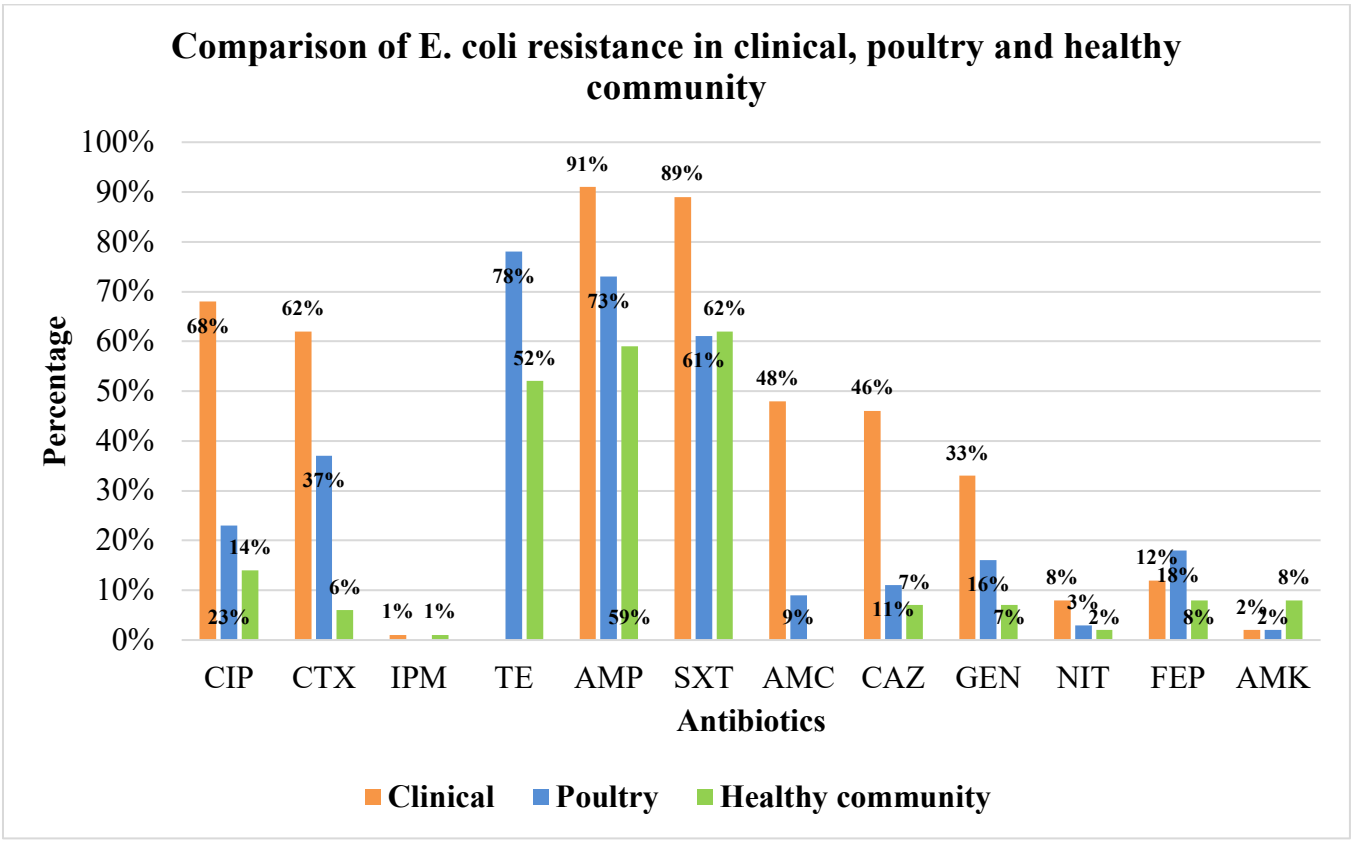

Figure 5: Comparison of *E. coli* resistance profiles in clinical, poultry and healthy community isolates to 12 key antibiotics

(**Abbreviations**: CIP – ciprofloxacin, CTX – cefotaxime, IPM – imipenem, TE – tetracycline, AMP – ampicillin, SXT – co-trimoxazole, AMC – amoxicillin-clavulanate, CAZ – ceftazidime, GEN – gentamicin, NIT – nitrofurantoin, FEP – cefepime, AMK – amikacin)

4.5.4 Phylogenetic analysis of *E. coli* from human and poultry

A total of eight phylogroups were identified among the *E. coli* isolated from blood, urine, healthy community (rectal swabs) and poultry (carcass wash and rectal swabs). Phylogroups B2 and D are known to cause extra-intestinal infections, of which B2 is the most prevalent. The remaining phylogroups belong to intestinal and commensal strains. Similarly, B2 was the most prevalent phylogroup causing BSIs and UTIs, and to a lesser extent, phylogroup D. Notable was the presence of phylogroups B2 and D in the non-clinical isolates from the healthy community and the presence of phylogroups considered to be commensal strains in clinical isolates from blood and urine (Figure 6 and Appendix A). Remarkably, only blood and urine recorded the presence of phylogroup C, a phylogroup closely related to the commensal B1 (Figure 6).

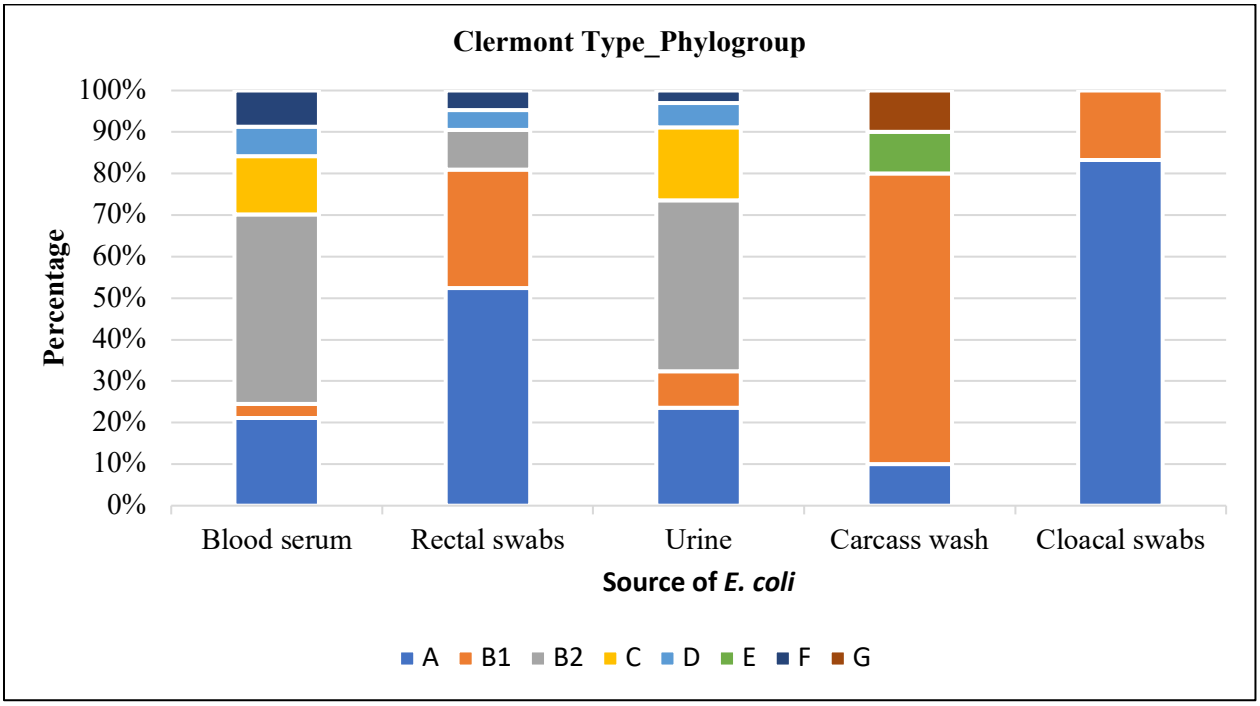

Figure 6: Distribution of *E. coli* phylogroups based on Clermont classification

*Rectal swabs – healthy communities, carcass wash – chickens from retail supermarkets, cloacal swabs – chickens from abattoirs.

Based on MLST analysis, eight main phylogroups were further subdivided into clonal complexes (CC), sequence types (STs) and ST subclones encoding a variant of the type 1 fimbrial (*fim*H). In this study, a total of 12 CCs, 47 STs and 54 serotypes and 29 *fim*H subclones were identified, with some of the STs not fitting into any of the CCs (Figure 7/Figure 8/Appendix A). Of the 47 STs, ST131 was the most prevalent (n = 37), followed by ST410 (n = 13) (Figure 7). Sequence type 131 was predominately in the clinical isolates, with only one in rectal swabs from the healthy community. The prevalent ST131 serotype was O25/H4, with only two O16/H5 serotypes from blood and a rectal swab, respectively. All the ST131 belonged to phylogroup B2. Sequence type 410 was exclusively in clinical isolates, with some strains belonging to O8/H9 (Figure 7 and Appendix A). The distribution of the serotypes in the different sources (urine, blood, rectal swabs, carcass wash and cloacal swabs) was diverse, with some serotypes being found in more than one source, whereas others belonged to only one source type (Figure 8/Appendix A). Strikingly, *E. coli* ST10 and ST48 intersected across the three interfaces (clinical, healthy community and poultry), further characterization by serotyping was diverse with only two strains (one from poultry and one from bloodstream infections) having the same serotype O25/H4 (Appendix A). Despite

this diversity and unrelated, all the strains carried similar resistance gene determinants all the antibiotic classes tested, as well as the same plasmids.

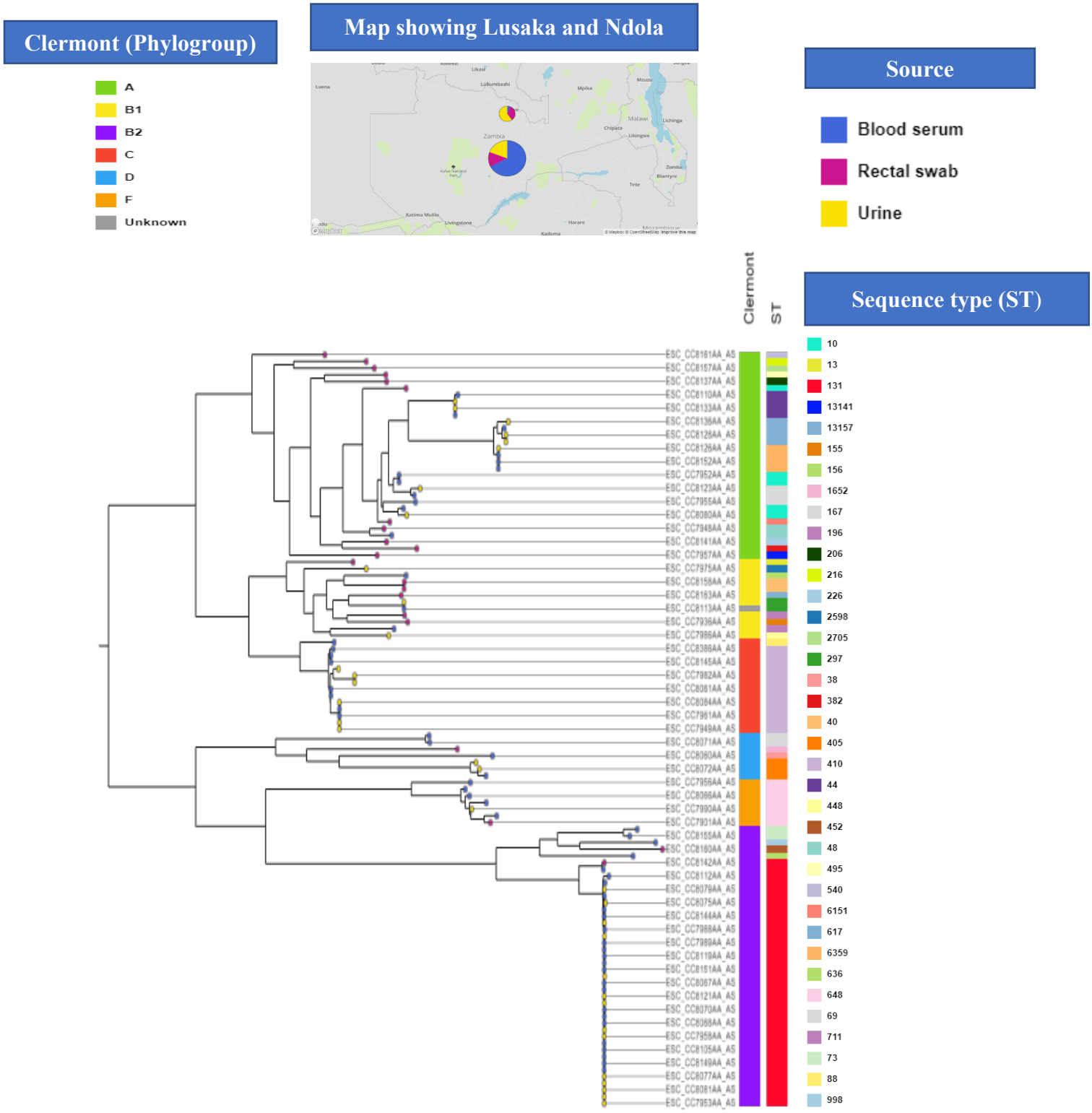

Figure 7: Phylogenetic tree showing the distribution of phylogroups and sequence types from human isolates (clinical and non-clinical)

*Rectal swabs – healthy community

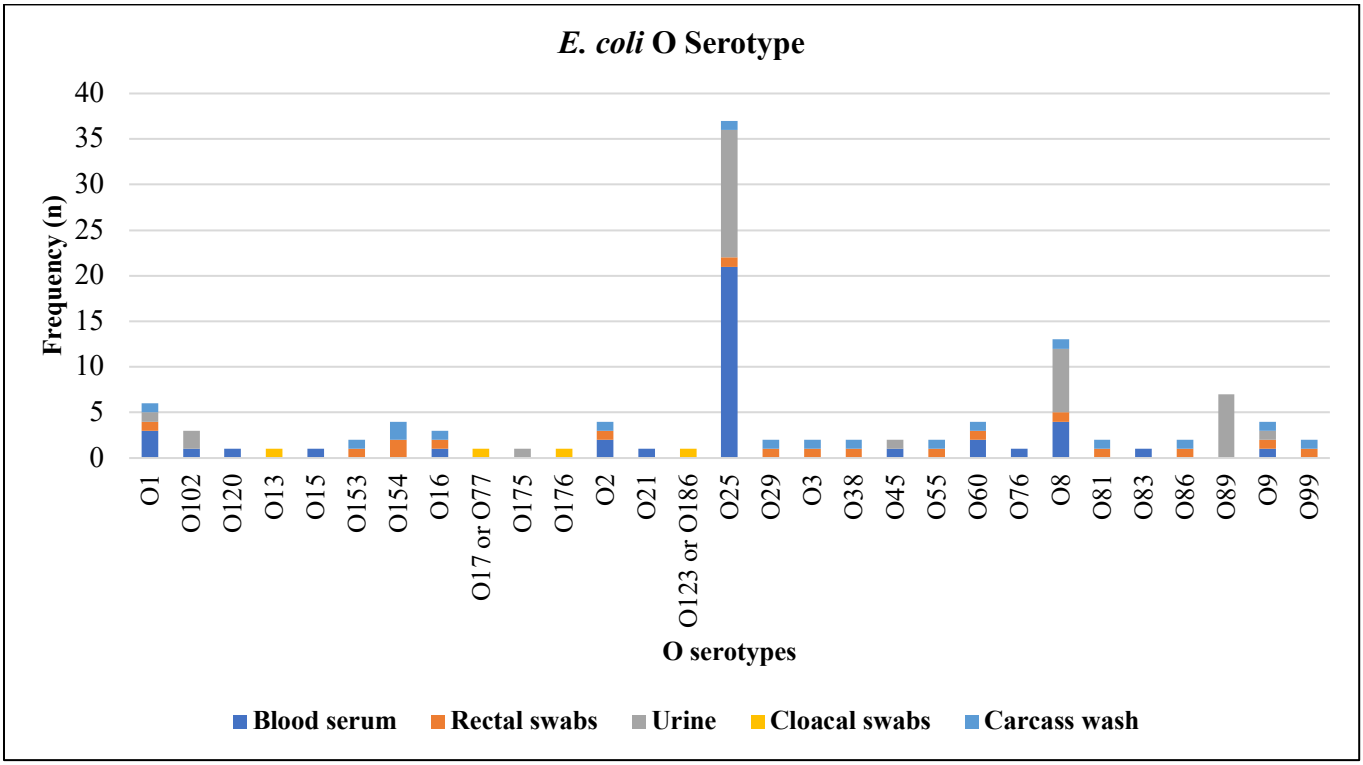

Figure 8: Distribution of *E. coli* O serotypes in the different sample sources

A total of 29 *fim*H ST subclones were identified (Table 14 and Table 15). Notably was the existence of the *fim*H30 subclone (n = 36), the main fluoroquinolone resistance associated subset within the ST131. Thirty-four (94%) of the *fim*H30 subclone were O25/H4-ST131 while the remaining two were ST648 and ST73 respectively. All of the *fim*H30 subclones were from clinical isolates only (urine and blood) and all were resistant to fluoroquinolones and were ESBL producers (mostly $bla_{\text{CTX-M-15}}$). The two O16/H5-ST131 belonged to subclone *fim*H41 while only one O25/H4-ST131 belonged to subclone *fim*H99.

Table 14: *Fim*H (*fim*H10 to *fim*H35) distribution in the different specimen types

	Human			Poultry	
***Fim*H sub-clone**	**Blood**	**Urine**	**Rectal swab**	**Carcass wash**	**Cloacal swab**
***fim*H10**	1	0	0	0	0
***fim*H121**	1	0	0	0	0
***fim*H149**	2	0	0	0	0
***fim*H171**	1	0	0	0	0
***fim*H199**	1	0	0	0	0
***fim*H219**	1	0	0	0	0
***fim*H23**	2	0	0	0	0
***fim*H24**	10	3	0	0	0
***fim*H25**	0	1	0	0	0
***fim*H27**	0	6	0	0	0
***fim*H29**	0	1	0	0	0
***fim*H291**	0	1	0	0	0
***fim*H30**	16	4	0	10	6
***fim*H32**	5	0	0	0	0
***fim*H35**	1	0	0	0	0

Table 15: *Fim*H (*fim*H366 to Unknown) distribution in the different specimen types

	Human			Poultry	
***Fim*H sub-clone**	**Blood**	**Urine**	**Rectal swab**	**Carcass wash**	**Cloacal swab**
***fim*H366**	1	0	0	0	0
***fim*H38**	3	3	0	0	0
***fim*H39**	0	3	0	0	0
***fim*H41**	0	3	0	0	0
***fim*H53**	0	1	0	0	0
***fim*H54**	0	8	7	0	0
***fim*H559**	0	0	0	0	0
***fim*H58**	0	0	1	0	0
***fim*H65**	1	0	0	0	0
***fim*H69**	1	0	0	0	0
***fim*H86**	1	0	0	0	0
***fim*H87**	1	0	0	0	0
***fim*H97**	1	0	0	0	0
***fim*H99**	1	0	0	0	0
Unknown	7	0	8	0	0

E. coli from the three different interfaces were phylogenetically diverse and mostly unrelated, with limited relatedness in some STs between different sources [(strains from poultry and healthy community (ST155) and strains from clinical isolates and healthy community (ST648)]. Relatedness and clustering were observed in the clinical isolates from blood and urine in the following STs (ST410, ST617, ST6359, ST44, ST131). Among the 37 ST131, 35 STs that were phylogenetically related were all from blood and urine, and all belonged to the serogroup O25/H4, while the remaining two that were closely related were from clinical (blood) and healthy community and belonged to serogroup O16/H5 (Figure 9).

Figure 9 shows two main clusters with cluster one being widely diverse and cluster two having ST131 and O25 as the predominant ST and serotype respectively. ST131 was representing across the two provinces (Lusaka and Ndola) but there no evidence of geographical clustering. The presence of ST131 in both urine and blood samples confirms that *E. coli* UTIs are the primary source/origin for BSIs. The other STs in cluster two were ST73, ST117, ST452, ST636, ST648, ST998, all of which belonged to phylogroup B2 except ST117 and ST648 that belonged to phylogroup G and F respectively. All of these STs had similar resistance gene determinants and

plasmids as those found in the predominant ST131. Simialrly, the diverse STs in cluster one carried resistance gene determinants on plasmids.

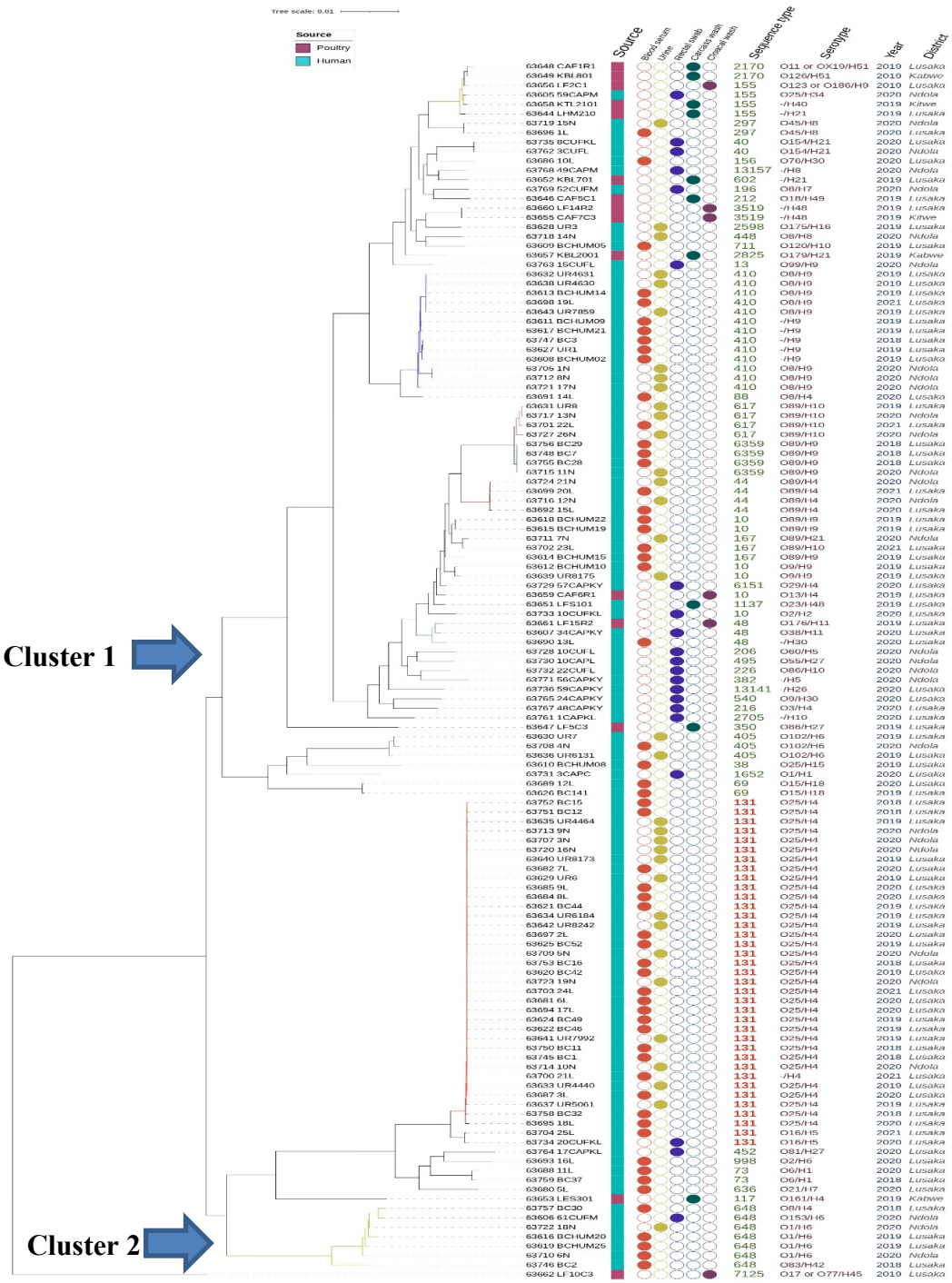

Figure 9: Phylogenetic tree showing relatedness between human (clinical and non-clinical) and poultry *E. coli* isolates with their sequence type and serogroup

In order to compare the close relatedness of the predominant ST131 phylogeny, the ST strains were further analysed with SNPs. Notable was that only two clusters were identified, both of which had urine and blood strains while the others were phylogenetically diverse. A finding that suggests that the ST131 was not only causing healthcare associated infections but community acquired infections as well (Figure 10).

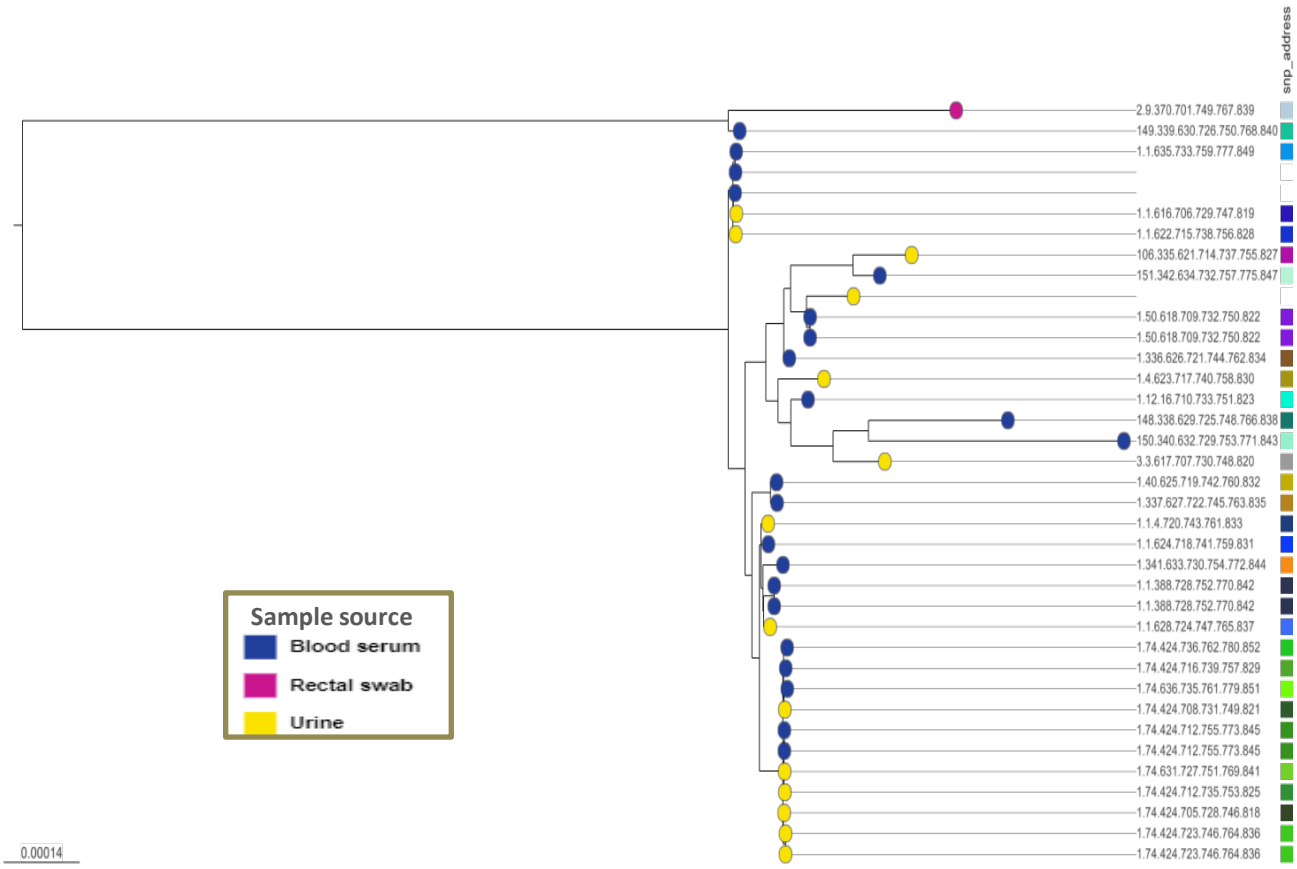

Figure 10: SNP address analysis showing the close related and diversity of ST131

4.5.5 Resistance genes determinants in *E. coli*

While ST10 and ST48 were detected across the three interfaces, further characterisation by OH serotyping revealed that these strains were clonally unrelated but carried similar AMR genes and plasmids. Most of the isolates harboured more than one resistance gene determinant, either from the same antibiotic class or/and from other classes (Appendix B1/B2).

Resistance to chloramphenicol occurs mainly through the production of inactivating enzymes called acetyltransferases (CATs) and chloramphenicol exporters, such as *cml*A. In this study, the CATs were only identified in the human strains, with *cat*A-1being found in clinical and non-clinical isolates. Other CATs that were recorded were *cat*A-2 and *cat*B-3. Notable was the presence of the *flo*R-resistant gene determinant in blood and poultry (carcass wash) and *cml*-1 in poultry isolates. Florfenicol is a derivative of chloramphenicol that is used for the treatment of animal infections. The chloramphenicol exporters *cml*-1 were only found in the poultry isolates (carcass wash and cloacal swabs) (Table 16).

Mutations of DNA gyrase (*gyrA*, *gyrB*) and topoisomerase IV genes (*parC*, *parE*) often mediate fluoroquinolone resistance. In this study, reduced susceptibility and resistance were due to mutation at different codons in *gyrA* and *parC* and, to a lesser extent, through plasmid-mediated quinolone resistance (PMQR). The most common *gyrA* and *parC* mutations recorded were at [83:S-L;87:D-N] and [80:S-I), respectively and these were recorded in all three interfaces. The *qnrS-1* was the most prevalent among the PMQR and was found in all three interfaces. Notably, the presence of another mechanism of PMQR, the efflux pump-encoding genes, *qepA-1*, was recorded in clinical isolates only, whereas *OqxA*/*QqxB* were only recorded in poultry isolates (Table 16/Appendix B2).

Table 16: Resistance gene determinants in phenicol and fluoroquinolones

Resistance gene determinants	HUMAN			POULTRY	
	Blood	Urime	Rectal swabs	Carcass wash	Cloacal swabs
	PHENICOL				
	Frequency (n)				
***catA*-1**	7	4	1	0	0
***flo*R**	1	0	0	2	0
***cml*-1**	0	0	0	2	2
***catB*-3**	0	2	0	0	0
***catA*-2**	1	0	0	0	0
	FLUOROQUINOLONES				
	Frequency (n)				
***gyrA*_EC1[83:S-L;87:D-N]**	26	15	1	0	0
***gyrA*_EC2[83:S-A]**	3	0	0	0	0
***parC*_EC2[80:S-I]**	16	23	1	1	0
***parC*_EC1[80:S-I;84: E-V]**	20	14	0	0	0
***qnrS*-1**	3	1	2	1	1
***parC*_EC1[80:S-I]**	2	0	0	0	0
***qepA*-1**	6	2	0	0	0
***gyrA*_EC2[83:S-L]**	0	0	2	4	0
OqxB	0	0	0	2	4
OqxA	0	0	0	2	4
***gyrA*_EC2[83:S-L;87:D-N]**	18	17	0	0	0
***gyrA*_EC1[83:S-L;87:D-Y]**	1	0	0	0	0
***gyrA*_EC2[83:S-L;87:D-H]**	0	0	1	0	0
***parC*_EC2[56: A-T]**	0	0	1	0	0
***parC*_EC2[80:S-R]**	0	0	1	0	0
***qnrB*-1**	1	0	0	0	0

Tetracycline resistance was through *tet*(A)and *tet*(M) alleles, with *tet*(A)-1 being the most prevalent. Tet (A) was found in all specimen types from all sources while *tet*(M) was only in blood (Table 17/Appendix B2).

Resistance gene determinants in aminoglycosides belong to different resistance mechanisms, such as aminoglycoside modifying enzymes (AME) genes that encode the aminoglycosides acetyl-transferase (ACC), aminoglycoside nucleotides-transferase (ANT) and adenyl-transferase (AAD) enzyme. This study's most prevalent AME genes were *aph (6)-Id-strB*, the streptomycin resistance gene determinant, recorded in all three interfaces. The other AME of note was the fluoroquinolone acetylating aminoglycoside-(6)-N-acetyltransferase (*aac(6')-Ib-cr*), a plasmid-mediated quinolone resistance determinant; this was only recorded in the human isolates from all sources (blood, urine and healthy community). The ANT-resistant gene determinant aadA-5 was found in all three interfaces (Table 17/Appendix B1).

Resistance to co-trimoxazole is determined by resistance gene determinants in sulfamethoxazole (*sul*) and trimethoprim (*dfr*A). Sulfonamide resistance in GNB arises from the acquisition of *sul1* and *sul2* that encode forms of dihydropteroate synthase that are not inhibited by the drug. *The sul1* gene is normally found linked to other resistance genes in class 1 integrons, while *sul2* is usually located on small non-conjugative plasmids and large transmissible MDR plasmids. *Sul3* is a plasmid-borne sulfonamide resistance gene. This study recorded all three *sul* resistance gene determinants with various distributions among the different interfaces.

Table 17: Resistance gene determinants for tetracycline, aminoglycosides and sulfamethoxazole

Resistance gene determinants	HUMAN			POULTRY	
	Blood	Urine	Rectal swabs	Carcass wash	Cloacal swabs
TETRACYCLINE					
	Frequency (n)				
***tet*(A)**	17	15	1	2	0
***tet*(A)-1**	30	11	10	8	3
***tet*(M)**	1	0	0	0	0
AMINOGLYCOSIDE					
	Frequency (n)				
aph (6)-Id, strB	45	22	12	6	4
aac (6')-Ib-cr	33	19	1	0	0
***aadA*-5**	31	9	2	2	1
aac (3)-IId	13	10	1	0	0
aac (3)-IIa	20	6	1	1	0
***aadA*-2**	4	2	0	1	2
***aadA*-22**	0	0	0	1	0
***aadA*-23**	0	0	0	1	0
***aadA*-8b**	4	1	0	0	0
SULFAMETHOXAZOLE					
	Frequency (n)				
***sul*-1**	39	21	2	2	1
***sul*-3**	1	0	1	2	2
***sul*-2**	43	25	15	5	4

The Trimethoprim resistance genes common to all three interfaces were *dfr*A-14 and *dfr*A-17, with various distributions of the other *dfr*A resistance gene (Table 18/Appendix B2). The major beta-lactamase resistance genes in *E. coli* currently comprise the $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{OXA} groups. The bla_{TEM} is known to hydrolyze penicillin and first-generation cephalosporins, while $bla_{\text{CTX-M}}$-encoded enzymes mainly hydrolyze third-generation cephalosporins. Both bla_{TEM} and $bla_{\text{CTX-M}}$ are generally found on plasmids. The *ampC* beta-lactamase resistance genes, such as bla_{CMY}, are also gradually increasing in clinical strains. In this study, a total of [124/140 (89%)] were ESBL-producers and [18/140 (13%)] were *amp*C producers, [16/140 (11%)] of which were in combination with *amp*C and ESBLs.

Bla$_{\text{CTX-M-15}}$ was the most prevalent in the human isolates only (clinical and non-clinical); similarly, but to a lesser extent, *bla*$_{\text{OXA-1}}$ was also only found in the human isolates. In contrast, *bla*$_{\text{CTX-M-27}}$ and *bla*$_{\text{TEM-1}}$ were recorded in all three interfaces. Other *bla*$_{\text{CTX-M}}$ subtypes were *bla*$_{\text{CTX-M-11}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-55}}$ and *bla*$_{\text{CTX-M-65}}$. Although low in frequency, some blood, urine and poultry strains exhibited the *amp*C beta-lactamase *bla*$_{\textit{CMY-2}}$ (Table 18/Appendix B1). Sequence type 131 harbored 81% (30/37) *bla*$_{\text{CTX-M-15}}$ and 16% (6/37) *bla*$_{\text{CTX-M-27}}$ ESBL genes. This was in combination with other beta-lactam resistance gene determinants, predominantly *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{TEM-1}}$ (Appendix B1).

Table 18: Resistance gene determinants for trimethoprim and beta-lactams

Resistance gene determinants	HUMAN			POULTRY	
	Blood	Urine	Rectal swabs	Carcass wash	Cloacal swabs
TRIMETHROPRIM					
	Frequency (n)				
***dfrA*-17**	36	21	3	2	1
***dfrA*-14**	6	3	10	6	2
***dfrA*-1**	4	0	2	1	0
***dfrA*-12**	8	2	0	1	2
***dfrA*-7**	2	0	0	0	0
***dfrA*-8**	1	1	1	0	0
***dfrA*-5**	1	1	0	0	0
***dfrA*-4**	1	1	0	0	0
BETA-LACTAMS					
	Frequency (n)				
***bla*$_{\text{CTX-M-15}}$**	47	26	2	0	0
***bla*$_{\text{OXA-1}}$**	32	23	1	0	0
***bla*$_{\text{CTX-M-55}}$**	1	0	0	3	1
***bla*$_{\text{TEM-1}}$**	34	17	11	4	4
***bla*$_{\text{CMY-2}}$**	4	5	0	3	2
***bla*$_{\text{CTX-M-14}}$**	2	0	0	2	0
***bla*$_{\text{CTX-M-27}}$**	3	4	1	0	0
***bla*$_{\text{CTX-M-106}}$**	1	0	0	0	0
***bla*$_{\text{CTX-M-65}}$**	0	0	0	2	0
***bla*$_{\text{CTX-M-11}}$**	1	0	0	0	0
***bla*$_{\text{TEM-191}}$**	1	0	0	0	0

4.5.6 Plasmid replicons

The plasmid replicons identified in this study, belonged to the IncI (I1), IncF (FII, FIB, FIA, FIC) and IncH (HI2, H4) groups. The IncF group were the most predominant with FII being the most commonly identified. The FII, FIB, FIA and I1 were found in all source types (blood, urine, healthy community and poultry) while the distribution of the other plasmid replicons was varied (Table 19).

Table 19: Distribution of plasmid replicons in the *E. coli* isolates from clinical (blood and urine) and non-clinical (healthy community and poultry)

Source type	Plasmid replicons						
	FII	**FIB**	**FIA**	**I1**	**HI2**	**FIC**	**H4**
Blood	46	47	40	12	1	1	-
Urine	31	29	24	3	-	-	-
Healthy community	15	9	1	2	-	1	1
Poultry	13	9	4	7	4	4	1
Total	**105**	**94**	**69**	**24**	**5**	**6**	**2**

4.6 ENTEROCOCCUS SPECIES

4.6.1 BSIs and UTIs

Most *Enterococcus* isolates were from the UTH from the 2018-2019 data collection phase. *E. faecalis* was the most prevalent of all the species isolated, with the least being *E. durans* (Table 20). Although ADCH had the least *Enterococcus* isolates, resistance was higher than NTH and UTH. The susceptibility results of *Enterococcus* subspecies with intrinsic resistance to certain antibiotic classes/types were not included in the analysis; these include quinupristin-dalfopristin in *E. faecalis* and vancomycin in *E. casseliflavus* and *E. gallinarium*.

Table 20: The distribution of clinical Enterococcus species from UTH, NTH, ADCH

***Enterococcus* species**		**Institution**			**Total**	**Percent (%)**
		ADCH	**NTH**	**UTH**		
		Frequency (n)			**Total**	**Percent (%)**
Organism	*E. faecalis*	0	5	77	82	70%
	E. faecium	3	1	24	28	24%
	E. gallinarium	1	0	2	3	3%
	E. casseliflavus	2	0	0	2	2%
	E. durans	0	0	1	1	1%
Total		**6**	**6**	**104**	**116**	**100%**

4.6.2 *Enterococcus* species from a healthy community

The distribution of *Enterococcus* species in the healthy community revealed *E. faecium* to be the predominant species in comparison to *E. faecalis* and the other species which were in relatively low numbers (Table 21).

Table 21: The distribution of clinical *Enterococcus* species from the healthy community

***Enterococcus* species**		**Frequency (n)**	**Percent (%)**
	E. faecium	93	46%
	E. faecalis	85	43%
	E. gallinarium	10	5%
	E. hirae	8	4%
	E. casseliflavus	2	1%
	E. durans	2	1%
Total		**200**	**100%**

4.6.3 Poultry *Enterococcus* species

Similar to species distribution in human clinical *Enterococcus* species, *E. faecalis* was the most prevalent in poultry isolates, while *E. gallinarium* and *E. durans* were more in poultry isolates than in clinical isolates. *E. hirae* was unique to poultry isolates only (Table 22). Resistance was highest in tetracycline and erythromycin and least in penicillin, linezolid and chloramphenicol.

Table 22: Distribution of *Enterococcus* species from poultry

***Enterococcus* species**		**Frequency (n)**	**Per cent (%)**
	E. faecalis	38	49%
	E. faecium	14	18%
	E. gallinarium	14	18%
	E. durans	8	10%
	E. casseliflavus	2	3%
	E. hirae	1	1%
Total		**77**	**100%**

4.6.4 Resistance profiles of Enterococcus species from clinical, healthy community and poultry

In clinical isolates, resistance was highest to more than seven of the antibiotics tested, with an emergence of resistance to antibiotics such as quinupristin-dalfopristin (1%) and high-level gentamicin (12%) that can be used to treat invasive *Enterococcus* infections such as sepsis, infective endocarditis and osteomyelitis. Although no vancomycin resistance was recorded, six isolates had reduced susceptibility (one from ADCH, one from NTH and four from UTH). Resistance was slightly higher in BSIs than in UTIs. High-level gentamicin was higher in BSIs than UTIs, and the emergence of quinupristin-dalfopristin was observed in BSIs and at ADCH.

In Enterococcus isolates from the healthy community, resistance to tetracycline (39%) was the highest at both Lusaka and Ndola primary healthcare facilities and erythromycin resistance (29%) was the second highest. There was no resistance recorded to linezolid, quinupristin-dalfopristin and vancomycin, though reduced susceptibility (intermediate results) were as follows; linezolid (n = 31) and quinupristin-dalfopristin (n = 16) and vancomycin (n = 14). These three antibiotics are

used to treat invasive infections and are considered last-resort treatment options for MDR *Enterococcus* infections. Erythromycin resistance was highest at Kalingalinga, followed by Chilenje, while tetracycline resistance was highest at New Masala, followed by Chilenje. Kanyama, Kalingalinga and Chilenje recorded the highest frequency of reduced susceptibility to linezolid, quinupristin-dalfopristin and vancomycin, respectively. The reduced susceptibility to the three antibiotics was mostly in adult pregnant women than children ≤ 5 years. Resistance to tetracycline, erythromycin, nitrofurantoin and ciprofloxacin was higher in adult pregnant women than in children ≤ 5 years, while resistance to the remaining antibiotics tested was higher in children ≤ 5 years than in adult pregnant women. Resistance in the different age groups in children ≤ 5 years varied. In children ≤ 5 years, reduced susceptibility to linezolid was (n = 10), quinupristin-dalfopristin (n =9) and vancomycin (n = 5).

Figure 11 summarises and compares resistance profiles of *Enterococcus* species from clinical, poultry and healthy community isolates. The highest resistance in poultry isolates was seen in tetracycline (61%), followed by erythromycin (35%) and ciprofloxacin (26%). Notable was the resistance in poultry *Enterococcus* isolates to antibiotics of clinical importance such as quinuprsitin-dalfopristin (26%), vancomycin (5%) and linezolid (1%) (Figure 11).

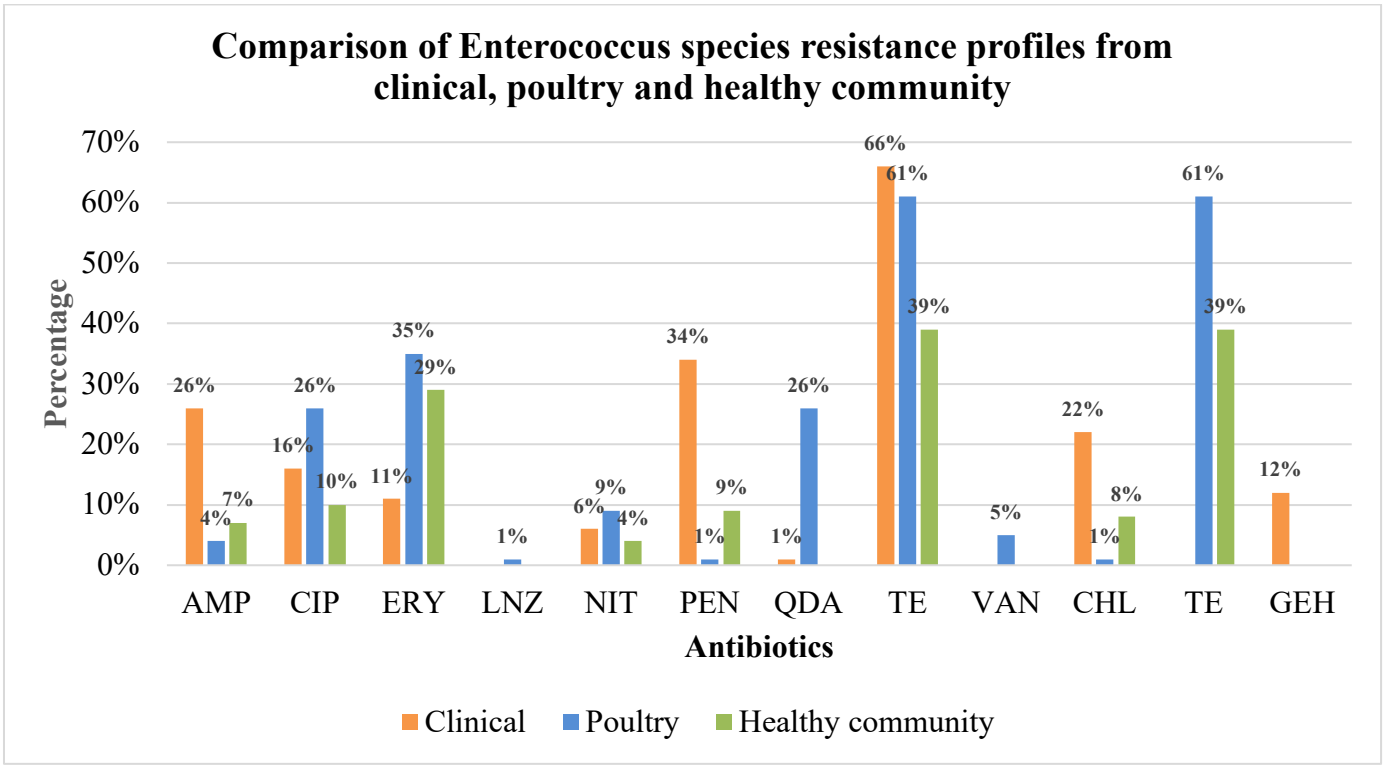

Figure 11: Comparison of *Enterococcus* species resistance profiles in isolates from clinical, poultry and the healthy community.

(**Abbreviations:** AMP – ampicillin, CIP – ciprofloxacin, ERY – erythromycin, LNZ – linezolid, NIT – nitrofurantoin, PEN – penicillin, QDA – quinupristin-dalfopristin, TE – tetracycline, VAN – vancomycin, CHL – chloramphenicol, GEH – high-level gentamicin)

4.6.5 MLST results of clinical E. faecalis and resistance gene determinants of isolates from clinical, healthy community and poultry

The MLST analysis of the eight sequenced *E. faecalis* revealed four different STs; ST58 (n = 6), ST28 (n = 1), ST191 (n = 1), and ST21 (n =1).

The resistance gene determinants found in *Enterococcus* species isolated from BSIs are listed in Table 23, a macrolide resistance gene determinatnt *erm*B was the most prevalent in all the three sources, clinical, poultry and healthy community (Table 23).

Table 23: Antibiotic resistance gene determinants in Enterococcus isolates from clinical, poultry and healthy community

Antibiotic class	Antibiotic	Frequency (n) of Resistance genes
	Clinical isolates	
Beta-lactams	Ampicillin	*bla*$_{\text{TEM-1B}}$ (2)
Macrolides	Erythromycin	*mph*A (2); *erm*B (6)
Tetracycline	Tetracycline	*tet*(A) (1); *tet*(B) (1): *tet*(M) (2)
Quinolones	Ciprofloxacin	aac-(6')-Ib-cr (1);
Phenicol	Chloramphenicol	*cat*B3 (2)
	Healthy commumity isolates	
Macrolides	Erythromycin	*erm*A (4), *erm*B (22), *erm*C (6)
	Poultry isolates	
Macrolides	Erythromycin	*erm*A (10), *erm*B (20), *erm*C (5)

A total of 337 *E. coli* and *Enterococcus* species (clinical and non-clinical from healthy communities) were classified as MDR (52% Lusaka and 48% Ndola), XDR (67% Lusaka and 33% Ndola) and PDR (67% Lusaka and 33% Ndola) respectively. Lubuto primary healthcare facility had the highest MDR isolates, and NTH had the highest XDR isolates (Table 24).

Table 24: Distribution of MDR, XDR and PDR from clinical and non-clinical *E. coli* and *Enterococcus* species from Lusaka and Ndola

Location	Facility	Frequency (n) and Percent (%)					
		MDR (n)	MDR (%)	XDR (n)	XDR (%)	PDR (n)	PDR (%)
Lusaka	Chilenje	36	24%	8	24%	-	-
	Kalingaling a	39	27%	4	12%	-	-
	Kanyama	37	25%	11	32%	1	50%
	UTH	35	24%	11	32%	1	50%
Total (Lusaka)		**147**	**52%**	**34**	**67%**	**2**	**67%**
Ndola	ADH	6	4%	1	6%	-	-
	Lubuto	40	29%	4	24%	-	-
	Mapalo	27	20%	3	18%	-	-
	Masala	25	18%	1	6%	-	-
	NTH	38	28%	8	47%	1	100%
Total (Ndola)		**136**	**48%**	**17**	**33%**	**1**	**33%**
Grand Total		**283**	**-**	**51**	**-**	**3**	**337**

4.7 *Salmonella enterica*

4.7.1 *Salmonella enterica* isolates from BSIs

In both the 2018/2019 and 2020/2021 collection phases, most *Salmonella* infections occurred in the rainy season in comparison to hot-dry and cold-dry seasons. All the *Salmonella enterica* isolates from UTH and ADCH were from blood cultures. The isolates from UTH were from the two collection phases, (n = 76) from 2018/2019 and (n = 37) from 2020/2021, whereas the ones from ADCH (n = 42) were all from 2020/2021. A total of 113 strains were sequenced, and a variety of serovars were identified; *S.* Typhi (86%). *S.* Enteritidis (8%), *S.* Typhimurium (3%), *S.* Heidelberg (2%), *S.* Salamae (2%) S. Paratyphi A (1%), *S.* Weltevreden 1%, and S. Braenderup 1% (Table 25). *S. Typhi* was the most prevalent serovar at both hospitals.

Table 25: Salmonella enterica serovars isolated from BSIs at UTH and ADCH

***Salmonella enterica* serovars**	**ADCH**	**UTH**	**Total**
Salmonella enterica subsp. enterica serovar Typhi	37	97	134
Salmonella enterica subsp. enterica serovar Enteritidis	5	7	12
Salmonella enterica subsp. enterica serovar Typhimurium	-	4	4
Salmonella enterica subsp. enterica serovar Heidelberg	-	3	3
Salmonella Salamae	-	3	3
Salmonella enterica subsp. enterica serovar Paratyphi	-	1	1
Salmonella enterica subsp. enterica serovar Weltevreden	-	1	1
Salmonella enterica subsp. enterica serovar Braenderup	-	1	1
Total	**42**	**113**	**155**

Resistance to ampicillin and co-trimoxazole was highest in the 2018-2019 phase compared to 2020-2021. There was an emergence of resistance to the recommended antibiotics (ciprofloxacin and cefotaxime) for the treatment of *Salmonella* BSIs, all of which were at UTH (Figure 12/Figure 13). Similarly, the emergence of resistance to other priority antibiotics, such as cefepime and imipenem, was recorded at UTH (Figure 13). There was a decrease in *Salmonella* MDR strains from 2010-2019 (46%) to 2020-2021 (5%) (Table 26).

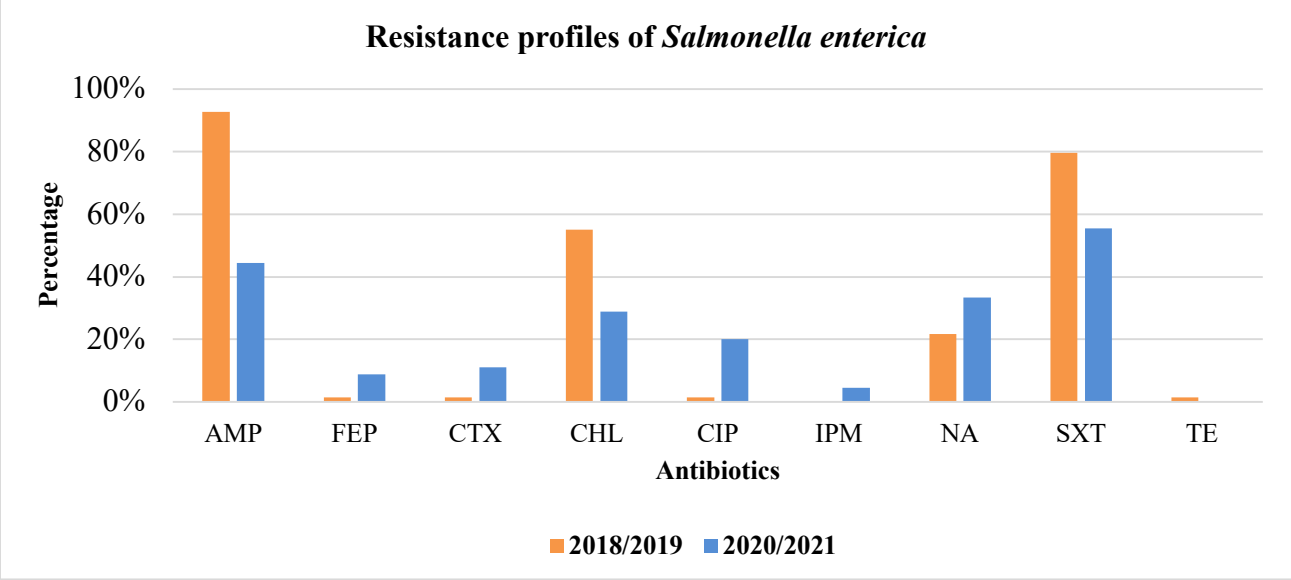

Figure 12: A comparison of resistance profiles of *Salmonella enterica* BSI from 2018-2019 and 2020-2021 phases

(**Abbreviations**: AMP – ampicillin, FEP – cefepime, CTX – cefotaxime, CHL – chloramphenicol, CIP – ciprofloxacin, IPM – imipenem, NA – Nalidixic acid, SXT – co-trimoxazole, TE – tetracycline)

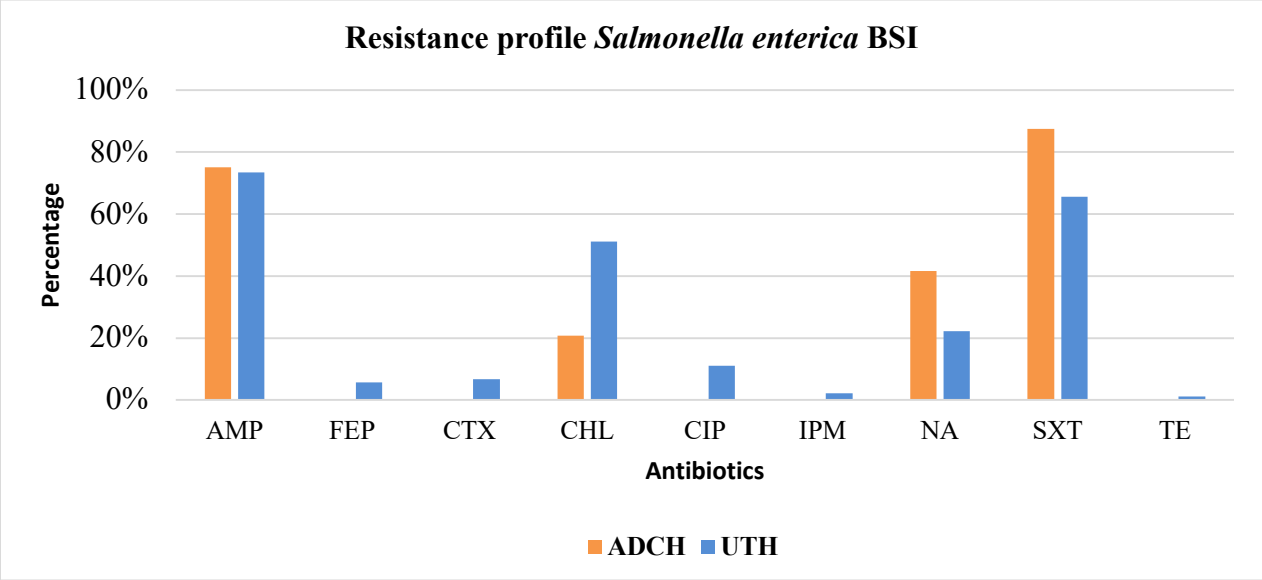

Figure 13: Resistance profiles for *Salmonella enterica* at ADCH and UTH

(**Abbreviations**: AMP – ampicillin, FEP – cefepime, CTX – cefotaxime, CHL – chloramphenicol, CIP – ciprofloxacin, IPM – imipenem, NA – Nalidixic acid, SXT – co-trimoxazole, TE – tetracycline)

Table 26: Distribution of MDR pathogens in 2018-2019 and 2020-2021 phases

Salmonella enterica	**Study Period**			
	2018/2019		**2020/2021**	
	Frequency (n)	**Percent (%)**	**Frequency (n)**	**Percent (%)**
S. Enteritidis	1	20	0	0
S. Heidelberg	1	33	-	-
S. Paratyphi	0	0	-	-
S. Typhi	31	51	3	5
S. Typhimurium	2	100	1	50
S. Weltevreden	0	0	-	-
S. Salamae	0	0%	-	-
Total	**35**	**46**	**4**	**5**

Genes associated with antimicrobial resistance in the different antimicrobial classes were as follows: beta-lactams – $bla_{TEM\text{-}1B}$ (n = 94) and $bla_{CTX\text{-}M\text{-}15}$ (n = 1), phenicols – *cat*A1 (n = 35), folate pathway antagonist (*sul2,* n = 86, *sul1*, n =36, *dfrA1*, n = 6, *dfrA7*, n = 32, *dfrA14*, n = 53). Reduced susceptibility and resistance to quinolones were associated with mutations in the quinolone

resistance-determining region (QRDR) of DNA gyrase and topoisomerase IV, whose subunits are encoded by *gyrA, gyrB, parC*, and *parE* genes. The most prevalent resistance genes were *bla*$_{\text{TEM-1B}}$, *sul2*, and *dfr*A14 (Table 27). Remarkably was the recorded *bla*$_{\text{CTX-M-15}}$, a cefotaximase that presents higher levels of hydrolytic activity against cefotaxime than against ceftazidime (Table 27).

Table 27: *Salmonella enterica* antibiotic resistance gene determinants

Antibiotic class	Resistance genes	Frequency (n)	Total
Beta-lactams	*bla*TEM-1B	94	95
	*bla*CTX-M-15	1	
Sulphonamides	*sul1*	36	122
	sul2	86	
Trimethoprim	*dfr*A1	6	91
	*dfr*A7	32	
	*dfr*A14	53	
Phenicol	*cat*A1	35	35
Fluoroquinolones	*gyr*A D87G	13	35
	*gyr*A D87N	5	
	*gyr*A S83F	3	
	*gyr*A S83Y	4	
	*gyr*B S464F	1	
	*gyr*B S464Y	1	
	*par*C T57S	8	

3.7.2 *Salmonella enterica* sequence types (STs)

A total of eight *Salmonella enterica* STs (1, 2, 11, 15, 85, 313, 365, and 366) were identified using MLST. *S.* Typhi isolates were assigned to two sequence types, ST1 (*85*) and ST2 (2). Similarly, *S.* Enteritidis was assigned to two sequence types, ST11 (*7)* and ST366 (4). The four *S.* Typhimurium isolates were assigned to ST313, a serotype known to cause invasive disease in sub-Saharan African (SSA) countries. Other STs identified were: ST85 (n = 1); *S.* Paratyphi A, ST15 (n = 3); *S.* Heidelberg, ST365 (n = 1); and *S.* Weltevreden (Table 28).

Table 28: *Salmonella enterica* sequence types determined by MLST

***Salmonella enterica* serovars**	**Sequence types**
S. Typhi	ST1 (85), ST2 (2)
S. Enteritidis	ST11 (7). ST366 (4)
S. Typhimurium	ST313 (4)
S. Paratyphi A	ST85 (1)
S. Heidelberg	ST15 (3)
S. Weltevreden	ST365 (1)

Following cgMLST analysis, a cluster of isolates (regarded as highly related isolates) was defined as two or more isolates that differed by no more than five allele differences. A cluster of isolates defines a distinct genotype. Core genome MLST data analysis showed varied genetic diversity (multiple genotypes) among isolates. In total, 31 genotypes were identified among 88 *S*. Typhi isolates. Other *Salmonella* serovars were genetically distant (thousands of allele differences) from *S*. Typhi isolates. *S.* Enteritidis showed eight genotypes, while *S.* Typhimurium showed three genotypes (Figure 14).

3.7.3 Phylogenetic analysis of Salmonella isolates

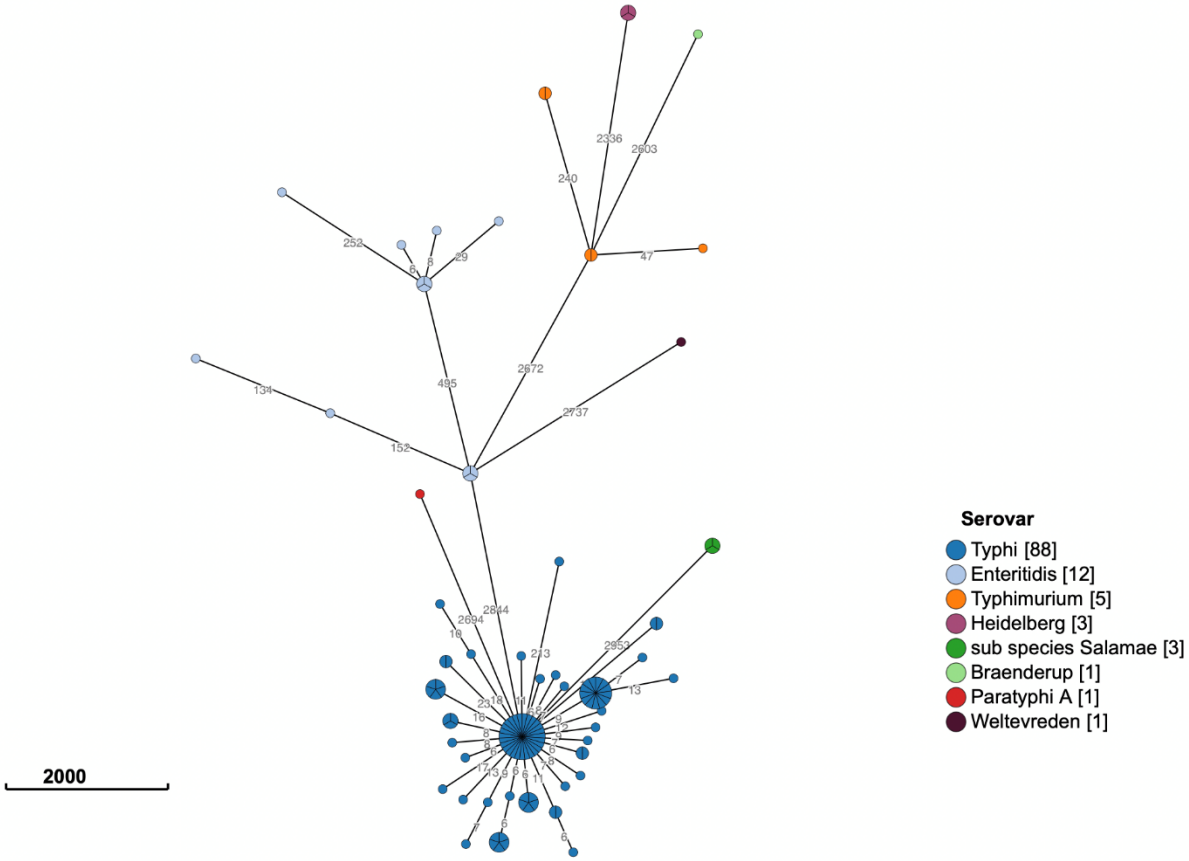

Figure 14: Genetic relatedness of different *Salmonella enterica* serovars

Minimum spanning tree drawn using cgMLST data from *S. enterica* isolated from Lusaka, Zambia, 2018–2021. The circular nodes represent isolate(s) with identical cgMLST profiles; the more significant the node, the more isolates are reflected. The number of values between adjacent nodes indicates the number of allele differences between nodes (isolates). Even with ≤ 5 allele differences, there was a wide range of genetic diversity and varying strains.

In order to determine the phylogenetic relatedness and diversity of our *S.* Typhi strains against others in the existing database, EnteroBase was searched for the nearest strain matches using a search criterion (cut-off) of up to 10 allele differences. Comparative analysis was performed for *S.* Typhi only, and close matches were found from five different countries and a previous outbreak in Zambia. Tanzania had the highest number of *S.* Typhi isolates, with the closest matches to the Zambian isolates. The Tanzanian *S.* Typhi isolates differed from the Zambian isolates by six allele differences, n = 4 from 2008; 7 allele differences, n = 4 from 2008; 9 allele differences, n = 2 from 2009 and 2012; 10 allele differences, n = 1 from 2019. Malawi isolates differed from the Zambian

isolates by ten allele differences, n = 8 from 2010 and 2011; South Africa, nine allele differences, n = 1 from 2018, Zambia 6 allele differences, n = 1 from 2011 and 9 allele differences, n = 3 from 2011. The United States isolates differed from the Zambian isolates by six allele differences, n = 2 from 2017, while the United Kingdom differed by nine allele differences, n = 1 from 2014 (Figure 15).

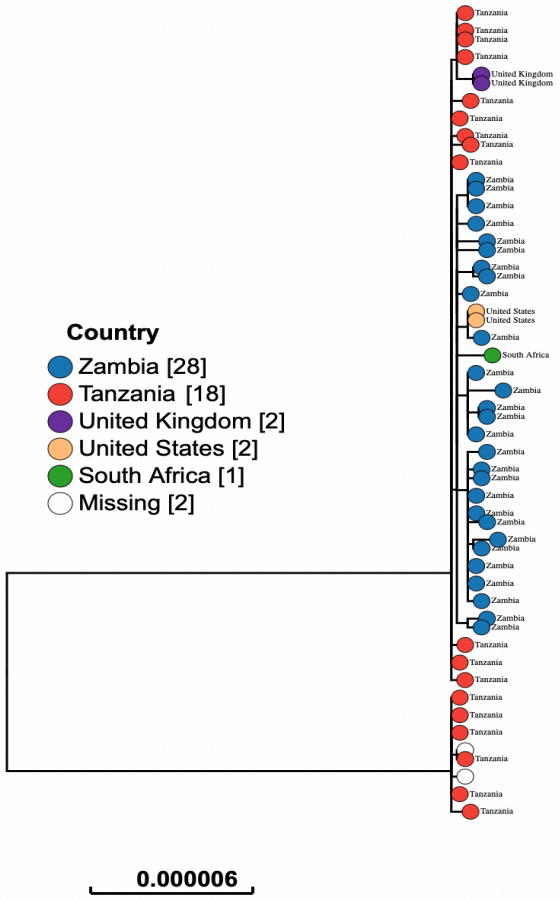

Figure 15: Phylogenetic relatedness of the Zambian *S.* Typhi strains to other countries using a cut-off of 10 allele difference.

CHAPTER FIVE

5.0 DISCUSSION

Antimicrobial resistance is a global challenge concerning human and animal health and the environment. In order to gain insight into the clinical and public health significance of AMR in humans and poultry, *E. coli, Enterococcus and Salmonella* species were investigated as model bacteria. The aim of the study was to investigate the genomic and antimicrobial resistance profiles of these three bacteria isolated from humans and poultry in Lusaka and Copperbelt provinces in Zambia.

5.1 Hospitalised patients with BSIs and UTIs

In this study, *E. coli* and *Klebsiella pneumoniae* were the most prevalent pathogens causing BSIs and UTIs in all three facilities. These findings are comparable to what was reported in a previous Zambian study at the UTH (Roth et al., 2021) and other studies done in Tanzania (Mshana et al., 2009), Ethiopia (Teklu et al., 2019), South Africa and Botswana (Gezmu et al., 2021). Notably, there was a high prevalence of *Klebsiella pneumoniae*-BSI in the Neonatal intensive care unit (NICU), similar to the findings in a previous study at the UTH in Zambia (Kabwe et al., 2016), South Africa and Malawi (Cornick et al., 2021; Dramowski et al., 2022; Essel et al., 2020). This finding can be attributed to extensive physical contact, vertical transmission and poor infection control measures, posing treatment challenges due to AMR and limited permitted antibiotics in this age group (Tam et al., 2019).

Hospital-acquired UTIs and BSIs are caused mainly by Gram-negative pathogens, most of which are MDR and linked to higher mortality, with *E. coli* being the most common cause of BSIs and UTIs with poor treatment outcomes (Diekema et al., 2019; Murray et al., 2022b). *E. coli* was the predominant cause of BSIs and UTIs in this study, most of which were ESBL producers, similar to studies in Africa, the UK and Europe (MacKinnon et al., 2021; Murray et al., 2022b; Silago et al., 2022; Vihta et al., 2018). Considering *E. coli* is the most common cause of BSIs, the increasing burden of third generation cephalosporins resistance negatively impacts patient outcomes (MacKinnon et al., 2021).

5.2 Healthy Community Survey

High resistance levels were observed in the healthy community, especially to antibiotics commonly accessed without prescriptions and used as an empiric treatment in primary health facilities (Kalungia et al., 2016; Mudenda et al., 2022). This agrees with the rising resistance seen in healthy individuals, especially children who have been found to be potential reservoirs of antibiotic-resistant bacteria (Messina et al., 2020). Studies have linked carriage of antibiotic resistance in healthy communities to poor sanitation and infrastructure in high-density areas, environmental contamination, high prevalence of HIV in LMICs and the lack of regulations that allow for access to antibiotics without an indication and prescriptions (Belachew et al., 2021; Kalter et al., 2010; Olaru et al., 2021; Omulo et al., 2021; Sampane-Donkor et al., 2017). In this study, antibiotic resistance was recorded in both high and medium-density areas, which could be attributed to the behaviour towards antibiotic use in the community and the lack of knowledge on the effects of irrational antibiotic use (Cambaco et al., 2020; Tangcharoensathien et al., 2021).

A study in Thailand found that people still used antibiotics to treat flu symptoms, even after massive knowledge and awareness campaigns between 2017 and 2019 (Tangcharoensathien et al., 2021). Similarly, a study in Mozambique recorded common habits that may contribute to resistance, such as purchasing half the course, self-medication, sharing medicines and interruption of treatment (Cambaco et al., 2020). The need to inform and educate the communities on the drivers of AMR and their effects and to put in measures that tackle inappropriate use and behavioural change cannot be overemphasized.

5.3 Prescription patterns

The prevalence of BSIs and UTIs caused by Gram negative Bacteria were more prevalent than Gram-positive bacteria, and most of the GNB were resistant to third-generation cephalosporins (3GCs), the most prescribed antibiotics as an empiric treatment choice for in-patients at all the three hospitals. This finding indicates a high prevalence of inappropriate empirical treatment that is not guided by local antimicrobial susceptibility patterns, with ceftriaxone being the most prescribed antibiotic. These results corroborate the findings from a study carried out at UTH three years ago (Masich et al., 2020) and another one that reviewed antibiotic use in first- and second-level hospitals in Zambia (Kalungia et al., 2022). Comparably, during the Covid-19 pandemic, another study that reviewed the antibiotic prescribing patterns according to the WHO AWaRe classification at the UTH revealed inappropriate prescribing patterns, with ceftriaxone being the most prescribed (Mudenda et al., 2023). Contrary to our findings, a study in Cape Town, South Africa, recorded a lower prevalence of inappropriate empiric treatment (Crichton et al., 2018).

This was also true in consolidated findings of the 303 hospitals from 53 countries that participated in a Global point prevalence survey (PPS) and some hospitals in European countries (Saleem et al., 2020; Versporten et al., 2018).

Inappropriate prescribing observed in this study could be attributed to the lack of updated antibiograms, microbiology diagnostic challenges due to inadequate laboratory capacity and reagent stockouts (Kalungia et al., 2022; Masich et al., 2020; Murray et al., 2022a). Inappropriate empirical antimicrobial therapy has been shown to be associated with increased mortality in young children and neonates, thereby highlighting the importance of appropriate empirical antibiotic recommendations (Cook et al., 2021). However, the lack of AMR structured surveillance and reporting required to support the most appropriate local treatment guidelines and the lack of rapid diagnostic tests in our setting delays the de-escalation or commencement of target-specific antibiotics. This has led to the prolonged use of broader spectrum antibiotics and inappropriate regimens, which creates selective antibiotic pressure, thereby increasing resistance among pathogens (Cook et al., 2021; Wen et al., 2021; Zhang et al., 2015). Currently, only the UTH has an updated antibiogram supported by local antibiotic susceptibility data; the antibiogram was launched in 2022 after the data collection phase of this study (Chanda et al., 2022).

Ceftriaxone, a Watch list antibiotic, the most prescribed for empiric treatment, accounted for 68% of all antibiotics prescribed at the three hospitals. Comparably, though at a lower prevalence, other countries recorded ceftriaxone as the most prescribed antibiotic in their hospital settings; Pakistan, 35%; Tanzania, 31% - 32%, Uganda, 24% (D'Arcy et al., 2021; Saleem et al., 2019; Seni et al., 2020; Tadesse et al., 2022) Relatedly, a Global PPS and systematic review that included high-, middle- and low- income countries also found ceftriaxone to be most prescribed (Saleem et al., 2020; Versporten et al., 2018). This was, however, different to the findings from other studies in EU/EEA countries, Ghana and South Africa, where penicillins with beta-lactamase inhibitors, metronidazole and ampicillin were the most used antibiotics, respectively (D'Arcy et al., 2021; Plachouras et al., 2018; Skosana et al., 2022). The dominance of third-generation cephalosporin use among all regions suggests substantial use of broad-spectrum antimicrobials across countries, thereby promoting the selection of resistant *Enterobacterales* by acquiring ESBLs and mutants that hyperproduce chromosomal AmpC β-lactamases (Livermore, 2005). The Watch group comprises broad-spectrum antibiotics, most of which are among the critically important antimicrobials for human medicine and antibiotic classes with a higher resistance potential (WHO, 2019).

Our study found that the "Access" group of antibiotics were the most prescribed in primary healthcare facilities. Although the prescription of antibiotics was above the WHO recommended threshold of 30%, the choice of antibiotic classes in the primary healthcare facilities was in line with the WHO recommendations, which state that more than 60% of all prescribed antibiotics must be from the Access group (Ofori-Asenso et al., 2016; Sharland et al., 2018). Our findings contradicted findings in Ghana, where almost half of the antibiotics prescribed belonged to WHO's "Watch" group (D'Arcy et al., 2021). The limited availability of culture and sensitivity testing (CST) to guide antibiotic choices in primary healthcare facilities results in consistently high rates of empiric prescribing (Kalungia et al., 2022). Over-prescription of antibiotics in primary healthcare facilities has been found to result in AMR, hence limiting the treatment options and increasing the population carriage of resistant organisms in the community (Costelloe et al., 2010). Encouragingly, none of the facilities prescribed antibiotics from the WHO Reserve group, and the three antibiotics from the Watch group were less than 20% in all facilities except one that also serves as a general hospital. Inappropriate prescribing of 'Watch' and 'Reserve' antibiotics outside specialist hospitals reduce their potential to tackle serious and critical infections when needed (Pauwels et al., 2021).

There were varying resistance rates recorded in the three different sectors, and notable was the high resistance to antibiotics such as ampicillin and co-trimoxazole in all three sectors. This was in agreement with a study in Scotland that explored the relationship between antimicrobial use for UTIs in the community (first and second-level hospitals) and antimicrobial resistance in *E. coli* bloodstream infections and subsequent mortality (McCowan et al., 2022b). Their findings indicated that prior antimicrobial use in the community was associated with a greater likelihood of a resistant *E. coli* bacteraemia and a higher mortality risk (McCowan et al., 2022). In this study, ampicillin/amoxicillin was the most prescribed antibiotic in primary healthcare facilities, and although co-trimoxazole was not the most prescribed, its use for prophylaxis in people living with HIV/AIDS (PLWHA) can validate the high resistance profiles recorded (Powis et al., 2017; Seid et al., 2020). A direct relationship between antimicrobial use and antimicrobial resistance has been found in a range of organisms, and the correlation between antimicrobial use in the community and resistance is well-known (Bell et al., 2014; Cheng et al., 2012; Costelloe et al., 2010; Hillier et al., 2007).

Fluoroquinolones (FQ) are highly bioavailable, broad-spectrum agents with activity against gram-negative pathogens, especially those resistant to other classes of antimicrobial drugs (King et al.,

2000). Commonly used as first-line empiric therapy for urinary tract infections, upper and lower respiratory tract infections, enteric infections, and gonococcal infections, all common clinical presentations at primary healthcare facilities (Dalhoff, 2012). In this study, ciprofloxacin was the most prescribed from the WHO "Watch" group of antimicrobials. Resistance to this class of antimicrobials limits the treatment options for a wide range of infections commonly encountered at primary healthcare facilities (Dalhoff, 2012). A matched case-control study that studied the effect of FQ use in primary care on the development of *E. coli* resistance to FQ, revealed that patients taking one or more courses of FQ were at higher risk of FQ-resistant *E. coli* colonisation or infection (Kurotschka et al., 2022). This risk was highest during the first year after FQ was taken, decreased during the second year and became undetectable afterwards (Kurotschka et al., 2022). Comparably, due to the restricted use of quinolones in human and food-producing animals in Australia, resistance to fluoroquinolones in the community was slow to emerge and remained at low levels in key pathogens, such as *Escherichia coli* (Cheng et al., 2012).

Given the extensive dependence on beta-lactams, especially ceftriaxone, for managing BSI in our setting, 3GC-resistant Enterobacterales are of particular concern. Notable is that despite the high prevalence of 3GC resistance in our setting, as was noted in previous studies at UTH, NTH and Livingstone Central hospital (Chanda et al., 2019; Mwansa et al., 2022; Roth et al., 2021), the UTH and ten other primary and secondary hospitals in Zambia still had 3GCs as the most prescribed drugs for empirical treatment (Kalungia et al., 2022; Masich et al., 2020). The high resistance to 3GCs in all three hospitals indicates the overuse of 3GCs in these settings, as the excessive use of 3GCs is a risk factor for the occurrence and spread of ESBL-producing bacterial strains (Urbánek et al., 2007). Our findings replicated a two-decade study in Malawi that found a marked increase in resistance to first-line beta-lactam antibiotics and a systematic review in sub-Saharan countries that found the prevalence of 3GC resistance in *E. coli*-BSI greater than estimates from high-income countries, with *E. coli* being the leading cause of death in 2019 (Bou-Antoun et al., 2012; Lester et al., 2020; Murray et al., 2022b; Tam et al., 2019). These findings warrant improved rapid laboratory diagnostic capacity with a good turn-around time and yearly revision of antibiograms based on local data. Remarkably was the low resistance to carbapenems, the treatment of choice and currently the last resort treatment option for MDR and ESBL-producing-Enterobacterales BSI (Gutiérrez-Gutiérrez and Rodríguez-Baño, 2019).

5.4 *Escherichia coli* resistance profiles and genomic analysis

5.4.1 *E. coli* resistance pattern

E. coli resistance to other classes of antibiotics, most of which are of clinical importance, was recorded at varying frequencies in all three sectors. Ampicillin/amoxicillin and co-trimoxazole resistance were the highest in all three sectors, both of which are commonly prescribed antibiotics without prescription in community pharmacies in Zambia (Kalungia et al., 2016). Notably in the healthy community and poultry isolates, though at a lower prevalence, was the resistance to critically important antibiotics (3GCs, fourth-generation cephalosporins, aminoglycosides, fluoroquinolones and carbapenems) reserved for the treatment of human infections (Scott et al., 2019b). Classifying antibiotics as critically important, highly important, and currently not used in humans is a "One Health" initiative aimed at limiting and optimising the use of critically important antimicrobials for human medicine in all applications, including animal food production (Scott et al., 2019b).

Antibiotic usage is considered a significant factor in promoting the emergence, selection and dissemination of antibiotic resistance in pathogenic bacteria and endogenous bacterial flora (normal flora) of exposed animals and humans (van den Bogaard et al., 2001). The purchase of antibiotics without prescription in the community, the irrational use of antibiotics in hospital settings and poultry farming, mainly as growth promoters, which is perceived as a reasonable management practice or disease prevention as a mitigation measure against the highly prevalent unhygienic conditions and absence of biosecurity, all these factors contribute to the rise and spread of resistance in the three sectors (Byarugaba, 2004; Duffy et al., 2008; Hart and Kariuki, 1998; Kalungia et al., 2016).

5.4.2 E. coli genomic analysis

The distribution of different *E. coli* phylogroups from the human population (clinical and carriage) and poultry may differ based on socio-economic factors, dietary habits, use of antibiotics, level of hygiene, geographical and climatic conditions, and the genetics of the host (Stoppe et al., 2017; Tenaillon et al., 2010). Similar to findings from other studies, this study demonstrated that phylogroups known to cause extra-intestinal infections (B2 and D) could also be found in the healthy community, while commensal or diarrheagenic phylogroup (A) can cause BSIs and UTIs (Staji et al., 2019, 2016). It has been demonstrated that ExPEC strains usually belong to groups B2 and D. Similarly, most of the ExPEC strains in this study belonged to B2 and, to a lesser extent, D, A, B1, C and F, demonstrating that the route of infection can be intestinal pathogenic *E. coli*

and commensal strains and suggesting the possibility that if any *E. coli* acquires the relevant virulence factors it may give rise to a pathogenic form (Bingen et al., 1998; Ludden et al., 2019b; Nowrouzian et al., 2019; Pupo et al., 1997). It has been hypothesised that pathogenic strains were probably derived from commensal strains following the horizontal acquisition of chromosomal and extrachromosomal genes and operons and gene loss (Stoppe et al., 2017).

The phylogenomic analysis demonstrated high diversity, 12 CCs, 47 STs, and 54 serotypes, with some STs predominantly belonging to a particular source type while others were found in more than one source. These findings agree with One Health genomic surveillance of *E. coli* that compared genomes of *E. coli* causing BSIs and those from livestock and found ST131 to be one of the most common STs causing BSIs and ST10 overlapping among the different reservoirs (Ludden et al., 2019b). Comparable to our finding, another study recorded an overlap of ST10, ST131 and ST648 between the clinical strains and those from the healthy community (carriage) (Matsui et al., 2020). Our findings also confirm previous studies showing the predominance of overlapping strains at the human-animal-environment interface and ST131 and/or ST410 as the most common STs responsible for human infections (Fuga et al., 2022; Mbelle et al., 2019; Seni et al., 2018). In this study, *E. coli* ST69 was only found in the blood. However, one health study in Zambia showed a high degree of genetic relatedness between *E. coli* ST69 from poultry and humans, suggesting clonal transmission of MDR *E. coli* between poultry and humans, with the likely acquisition of niche-specific AMR plasmids (Shawa et al., 2022). Establishing high-risk clones overlapping human and animal sources and the interconnectedness of human, animal and environmental habitats creates opportunities for the emergence, evolution and successful exchange of mobile genetic materials (Fuga et al., 2022; Hernando-Amado et al., 2019).

There was no geographic clustering of the *E. coli* isolates in this study suggesting different sources of acquisition. However the presence of similar resistance gene determinants in different STs from the three sources further suggests possible transmission of resistance genes and other genetic material (Leekitcharoenphon et al., 2021). *E. coli* ST131, the most prevalent ST in our study, is a globally dominant MDR clone associated with the spread of the $bla_{\text{CTX-M-15}}$ ESBL strains, most of which are resistant to fluoroquinolones (Nicolas-Chanoine et al., 2014b). This agrees with our finding that 81% of the ST131 isolates carried the $bla_{\text{CTX-M-15}}$ ESBL gene, and all were resistant to fluoroquinolones. This finding suggests the ST131 in this study belong to clades C1 (also known as *H30*R) and C2 (also known as *H30*Rx) that are characterised by a double mutation at the *gyrA* and *parC* genes conferring high-level resistance to FQ associated with ESBL-$bla_{\text{CTX-M-15}}$

resistance genes (Kallonen et al., 2017; Price et al., 2013a). Similarly, a study in Tanzania found *bla*$_{\text{CTX-M-15}}$ to be the premominant ESBL resistance gene determinant associated with fluoroquinolone resistance in pregnant women with *E. coli* UTIs and chidren with *E. coli* BSIs (Seni et al., 2021). This finding is supported by the 94% prevalence of ST131 *fim*H30 sub-clone observed as this subclone is a major MDR clade which accounts for most fluoroquinolone resistance within ST131 (Boll et al., 2020; Price et al., 2013b). Similar to other studies, the non-*fimH*30 subclone observed in this study was *fimH*41, also carrying the *bla*$_{\text{CTX-M}}$ resistance genes (Matsumura et al., 2015; Stoesser et al., 2016b). The alarming increase and dissemination success of *E. coli* ST131 clone is explained by the carriage of ESBL resistance genes, different virulence factors and predominance in the human gut (Can et al., 2015; Morales Barroso et al., 2018).

The most prevalent ST131 serogroup was O25/H4, while serogroup O16/H5 was minimal. Correspondingly, other studies in Egypt and India reported *E. coli* O25-ST131 as the predominant clone serotype (Abdelrahim et al., 2021; Khadega et al., 2016), while O16-ST131 was reported in a very small percentage in Australia, France and Spain (Nicolas-Chanoine et al., 2014b). There was a phylogenetic difference in the 35 isolates of serogroup O25/H4-ST131 that from blood or urine samples and two isolates of serogroup O16/H5-ST131 that were found in a rectal swab from a healthy individual and a blood sample, respectively. This finding confirms the diversity within the ST131 group, with carriage isolates appearing less virulent than BSI/UTI isolates and further suggesting the acquisition of other pathogenetic elements as an essential trait in ST131 transmission (Johnson et al., 2009; Kudinha and Kong, 2022). Increasing rates of intestinal colonisation with CTX-M-15 ESBL-producing *E. coli* ST131 is particularly worrisome as the intestine is the main reservoir for ExPEC strains causing UTIs (Nielsen et al., 2014).

Following the discovery of *E. coli* O25-ST131 in multiple countries on three continents in 2008 (Coque et al., 2008; Nicolas-Chanoine et al., 2008), the predominance of *E. coli* ST131 has been observed globally in the UK, North America, France, Spain, Canada, Japan, Korea, Middle East and African countries such Nigeria, Mozambique, Egypt, Central African Republic (Abdelrahim et al., 2021; Aibinu et al., 2012; Mandomando et al., 2020; Rogers et al., 2011). However, the phylogenetic comparison of *E coli* ST131 strains from Zambia to other African countries exhibited a wide diversity which could be a result of sub-lineages designated according to their characteristic *fimH* allele (type 1 fimbriae adhesin gene), phylogenetic clade (A, B, C1, and C2), and resistance profile (Mathers et al., 2015; Schembri et al., 2015). While the core genome of ST131 is highly conserved, there is variation in the accessory genome, resulting in differences in virulence gene

repertoire and plasmid content (Johnson et al., 2016; Petty et al., 2014). The ST131's ability to balance clonality and the extent of resistance/virulence genes gives ST131 a fitness advantage and the potential to exchange genetic material over other *E. coli* clones (Forde et al., 2019).

The The distribution of resistant gene determinants was similar to that of the STs, where some were only found in particular specimen types while others were cross-cutting among the different interfaces. This study established a high prevalence of ESBL-producers (89%), and most of the resistance gene determinants found in all three interfaces are known to be found on plasmids (Mathers et al., 2015; van den Bogaard et al., 2001b). Conjugative plasmids can shuttle between bacteria of the same or different species, thus spreading resistance phenotypes and potentially causing large-scale outbreaks and the prevalence of resistant bacteria(Mathers et al., 2015; Rafaï et al., 2015). These findings confirm a global trend where the presence of cross-cutting resistant determinants in *E. coli* strains has been found in a wide range of human and non-human reservoirs in Cameroon, South Africa, Tanzania, Nigeria, Norway and the United Kingdom (Aworh et al., 2022; Founou et al., 2022; Kaspersen et al., 2020; Ludden et al., 2019b; Mbelle et al., 2019; Poirel et al., 2012; Seni et al., 2018). Some studies have found food-producing animals play an important role as reservoirs of MDR pathogens and have also suggested that commercial chicken meat and other sources of protein could be a reservoir of *E. coli* strains to harbour *bla*CTX-M (Aworh et al., 2022, 2021; Founou et al., 2022; Fuga et al., 2022; Subramanya et al., 2020b). Similarly, commensal *E. coli* of healthy humans represent an important reservoir for numerous antibiotic-resistance genes (Jannine K. Bailey et al., 2010; Nji et al., 2021; Rolain, 2013).

The presence of the incF plasmid replicons in blood, urine, healthy community and poultry isolates, as well as the distribuition of IncF plasmid in different STs in this study, confirms the findings that IncF plasmids carrying the $bla_{\text{CTX-M-15}}$ gene are not restricted to ST131. This is consistent with findings that identified the incF in ST131 and other *E. coli* STs such as ST405, ST354, ST28, and ST695 (Coque et al., 2008). The IncF plasmid was also found in a *Shigella sonnei* strain isolate from Czech, *S.* Enteritidis from the United Kingdom, and in a *Klebsiella pneumoniae* strain from Spain (Diestra et al., 2009; Hrabák et al., 2008). This further confirms the ability plasmids have to share resistance genes within the Enterobactaerale family and to other GN pathogens.

In this study, the IncI1 plasmid was also recorded in clinical and non-clinical *E. coli* isolates. The IncI1 plasmids has been associated with the spread of several ESBL genes in poultry and humans

(Carattoli, 2009). Similar to our findings, the IncI1 plasmid was also found in poultry faecal samples from 10 slaughter houses in seven districts in France (Girlich et al., 2007). Another study from different parts of France associated the *bla*$_{\text{CTX-M-1}}$ gene with IncI1 in *E. coli* isolated from human patients further suggesting a potential link of dissemination between animals and humans (Marcadé et al., 2009). Consistent with our findings, another study found the IncI1 plasmid in *Enterobacterale*s isolated from food-producing animals and clinically ill human patients (Smith et al., 2015). A key public health concern with IncI1 plasmids is their ability to carry AMR genes, including those associated with critically important antimicrobials used to treat invasive infections, such as third-generation cephalosporins (Foley et al., 2021).

5.5 Enterococcus species AMR profiles and genomic analysis

5.5.1 Enterococcus species resistance patterns

Enterococcus species, especially *E. faecium* which is part of the ESKAPE pathogens, is an important healthcare-associated pathogen (HAPs) causing infections in critically ill and immunocompromised patients (Mogokotleng et al., 2023). In this study, a higher proportion of *E. faecalis* (71%) than *E. faecium* (24%) was observed, comparable to findings from nine provinces in South Africa, Uganda, Italy and Saudi Arabia that recorded a similar species dispersal (Alotaibi and Bukhari, 2017; Boccella et al., 2021; Kateete et al., 2019; Mogokotleng et al., 2023). These findings were correspondingly in agreement with the SENTRY Antimicrobial Surveillance Programme that studied the distribution of Gram-positive pathogens causing BSIs in the United States and European hospitals; during the 6-year surveillance period, *E. faecalis* and *E. faecium* were found to be the second and the third most causative agents of BSIs after *Staphylococcus aureus* (Mendes et al., 2010). On the contrary, a study in China identified 33% *E. faecalis* and 59% *E. faecium* (Jia et al., 2014). The proportion of the other species as causative agents of BSIs and UTIs was relatively low, as was observed in other studies (Alotaibi and Bukhari, 2017; Mogokotleng et al., 2023). The distribution of Enterococcus species in the healthy community and poultry was similar to the clinical distribution, although *E. faecium* (46%) was slightly more than *E. faecalis* (43%) in the healthy community. This was in agreement with findings from Nigeria and Iran (Adesida et al., 2017; Jannati et al., 2020).

High intrinsic resistance levels and the emergence of MDR *Enterococcus* strains are significant challenges in treating Enterococcal infections (Arias et al., 2010). This is further complicated by the need for combination antibiotic therapy to treat invasive Enterococcal infections. Resistance to the three most useful anti-enterococcal antibiotics such as ampicillin, high-level gentamicin and

vancomycin, was recorded in this study, and these findings have been found to complicate treatment further (Sharifzadeh Peyvasti et al., 2020). Resistance to ampicillin/penicillin and erythromycin limits the affordable and readily available treatment options and increases treatment cost, morbidity and mortality (Puchter et al., 2018a) (Kateete et al., 2019). Resistance to ampicillin, GEH, quinupristin-dalfopristin and the emergence of reduced susceptibility vancomycin is of great concern as these are drugs of choice for invasive Enterococcal infections (Arias et al., 2010). The low prevalence of Enterococcal resistance to vancomycin and high-level gentamicin in our setting could be due to the limited usage of these antibiotic classes as empiric treatment options in our facilities (Kalungia et al., 2022; Masich et al., 2020).

Though low, resistance to antibiotics of choice (ampicillin/penicillin, quinupristin-dalfopristin, high-level gentamicin) for treating invasive infections such as BSIs and infective endocarditis was noted to be higher in BSI isolates than UTI isolates. Additionally, reduced susceptibility to vancomycin was only observed in BSI isolates, with no resistance recorded in BSIs or UTIs. This was consistent with findings from a ten-year Germany surveillance on the continuous increase of vancomycin resistance in enterococci causing nosocomial infections, revealing a higher incremental trend in BSI than in UTIs (Rendschmidt et al., 2018). The low prevalence of vancomycin resistance in this study is consistent with findings from South Africa, Europe and Ethiopia (Ayobami et al., 2020; Ferede et al., 2018; Mogokotleng et al., 2023) but different from Southern India (Sivaradjy et al., 2021). The conflicting geographic prevalence of VRE could be a result of higher or lower usage of vancomycin, fully functional antimicrobial stewardship (AMS) and surveillance programs in HICs or the lack of it in LMICs, thereby leading to the lack of published data (Iskandar et al., 2021a; Kern, 2021; Oberjé et al., 2017; Rolfe et al., 2021). The lack of published data suggests a possibility of higher VRE prevalence in Africa and Asia than currently reported (Li et al., 2023).

Similar resistance profiles were seen in isolates from BSIs/UTIs, healthy communities and poultry, more so in antibiotics such as erythromycin, tetracycline, ampicillin/penicillin that are commonly used in the community and first-level facilities in Zambia (Kalungia et al., 2022; Kalungia et al., 2016; Mudenda et al., 2022b). Notably was the appearance and presence of resistance to the last resort antibiotics (vancomycin, quinupristin-dalfopristin, linezolid) used to treat invasive MDR Enterococcal infections (Arias et al., 2010). Comparable to this study, a study that analysed resistance patterns in *Enterococcus* species from laying hens in Lusaka and Copperbelt recorded high levels of MDR and resistance to the same antibiotics (Mudenda et al., 2022). The presence of similar resistant patterns and resistance genes in healthy communities and poultry as those found

in clinical isolates is of great concern as they could enable horizontal gene transfer between bacterial species and become a potential source for the spread to humans (Selaledi et al., 2020). In the hospital setting, VRE infections resulting from VRE colonisation are common in immunocompromised patients. Some associated factors are underlying health conditions such as liver transplantation, neutropenia, diabetes mellitus or renal dysfunction (Puchter et al., 2018).

Although *E. gallinarum* and *E. casseliflavus* are less pathogenic than *E. faecalis* and *E. faecium* and usually highly susceptible to ampicillin, they are intrinsically resistant to one of the most potent and last resort antibiotic, vancomycin (Britt and Potter, 2016). This complicates the treatment of invasive MDR infections caused by these species as it would require using antibiotics such as linezolid and daptomycin, which are not always readily available in our setting (Britt and Potter, 2016). Additionally, linezolid is usually reserved for treating drug resistant-Tuberculosis infections as the Zambia National Tuberculosis has adopted it and Leprosy Control Programme as recommended by WHO (Monde et al., 2023).

Tetracyclines and macrolides were expressed mainly by *erm*B and *tet*M, respectively, consistent with studies in China and Iran (Ahmadpoor et al., 2021; Tian et al., 2019). The *erm*B confers resistance to macrolide, lincosamides, and streptogramin (MLS) antibiotics and has the capacity to transfer horizontally between genetically linked and genetically unrelated isolates from animals and humans (De Leener et al., 2005). The other macrolide resistance gene determinant (*mph*A) found in this study was also reported in Nigeria (Bamigbola et al., 2023). Antibiotic resistance from animals to humans can be transferred directly and/or indirectly. Direct transmission occurs when resistant zoonotic bacteria infect humans, whereas indirect transmission is when resistant bacteria originating from animals transfer their resistance genes horizontally to the human bacterial population, mostly occurring when animal-derived strains can survive in human sites, primarily the gut (Aarestrup et al., 2000; De Leener et al., 2005).

5.5.2 Enterococcus genomic analysis

The STs identified in this study were ST19, ST21, ST28, ST58, and ST191. Studies have shown various distributions of Enterococcus STs, most dissimilar to STs identified in this study (Apostolakos et al., 2022; Aung et al., 2023; Shobo et al., 2022). Consistent with our findings, STs 19, 21 and 58 were identified in a study that sequenced 146 *E. faecalis* strains isolated from hospital outbreaks, food, animals, and colonisation of healthy human individuals (Neumann et al., 2019). Interestingly, the STs that were identified from bloodstream infections in this study had a

diverse distribution in a study done by Neumann et al where ST58 was only found in animals; ST21, were all human sources but from both hospital-associated infections and the healthy community; ST19 were predominantly from the healthy community than hospital-associated infections, with only one from an animal source (Neumann et al., 2019). In another comparative genomic analysis of *Enterococcus* species, ST28 was found in dairy products, and remarkably, all the strains from dairy products could potentially cause human infections (Apostolakos et al., 2022). ST21 has also been found on frequently touched sites by patients and healthcare workers in different wards in a hospital in South Africa (Shobo et al., 2022). The distribution of similar STs and resistance genes in clinical, commensal, food of animal origin and hospital environment strains supports the possibility of cross-transmission and the ability of previously non-pathogenic strains to acquire traits that allowed them to become pathogenic (Zhou et al., 2020b).

5.6 Salmonella enterica AMR profiles and genomic analysis

Salmonella infection cases mainly occur in the rainy season, followed by hot-dry and lastly, cold-dry seasons (Akil et al., 2014). Though the environmental drivers that influence seasonal dynamics are not fully understood, there is increasing evidence that high temperatures and rainfall can influence the occurrence of enteric fever (Saad et al., 2018). Heavy rainfall is known to cause flooding, a recognised risk factor for enteric fever, as this can cause the mixing of drinking water sources with open sewers that contain faecal matter (Vollaard et al., 2004). This is common in LMIC with poor drainage, waste disposal and sanitation facilities (Corner et al., 2013). The rapid replication of bacteria such as *Salmonella* in warmer conditions, coupled with erratic supplies and access to clean water during hot and dry periods, could lead to water scarcity forcing people to consume contaminated water (Akil et al., 2014; Singh et al., 2001).

5.6.1. Salmonella serovars

S. Typhi (86%) was the most predominant causative agent of Enteric fever over the other serovars, consistent with studies from Tanzania, China and India (Misra et al., 2016; Omari A. Msemo et al., 2019b; Qian et al., 2020b), but different from studies carried out in Malawi, Kenya, Ghana, Burkina Faso and the Gambia where they incidence of iNTS cases was higher than *S.* Typhi cases (Dekker et al., 2018b; Feasey et al., 2015; Guiraud et al., 2017; Kwambana-Adams et al., 2015; Labi et al., 2014; Muthumbi et al., 2015). The serovar differences in these countries could be attributed to the differences in climate and landscape (Akil et al., 2014; Maurer et al., 2015) and the prevalence of malnutrition, malaria, and HIV, the predisposing factors associated with iNTS (Guiraud et al., 2017). Zambia attained the 90:90:90 UNAIDS targets for HIV epidemic control in

2020, this has seen the number of new HIV infections reduce and an increase in people living with HIV (PLWH) on antiretroviral therapy, thereby reducing the proportion of the immunocompromised population (UNAIDS, 2015; UNICEF, 2021).

The emergence of rare *Salmonella* serovars such as *S*. Braenderup, *S.* Heidelberg and *S.* Salamae is an indication of infections that occurred as a result of food vehicle transmission from a wide variety of products such as eggs, chicken/turkey, pork, leafy greens, dairy products, and vegetables (Lamas et al., 2018a). Due to the rare and limited pathogenicity potential of non-*enteric S.* Salamae to cause human and animal infections, it has been proposed as an opportunistic pathogen related to a compromised immune system (Lamas et al., 2018a). *S.* Weltevreden has been found to contribute to the global epidemiology of human Salmonellosis due to seafood. It is, however, low and mostly limited to Asia (Hounmanou et al., 2020a). *S.* Heidelberg has emerged as a serovar of increasing prominence, particularly in some egg-associated outbreaks in the USA and Canada (Kaldhone et al., 2017). The emergence of *Salmonella s*erovars predominantly found in high-income countries and Asia supports the concept of travel associated *with Salmonella* infections and food trade among different countries (Hounmanou et al., 2020b; Johnson et al., 2011; Lamas et al., 2018b). The need for food safety cannot be overemphasised.

5.6.2 Salmonella resistance profiles

Resistance to ciprofloxacin, ceftriaxone and cefepime was reasonably low in our setting, although there was a rise in resistance in the 2020/2021 phase, compared to the 2018/2019 phase. These findings are comparable to the findings in Ghana, Kenya and India (Labi et al., 2014; Menezes et al., 2012; Muthumbi et al., 2015), but different to Italy and Pakistan, which recorded high fluoroquinolones resistance and ceftriaxone (García-Fernández et al., 2015; Klemm et al., 2018). A gradual increase in minimum inhibitory concentration (MIC) values of ciprofloxacin raises the alarm towards the judicial use of these antibiotics. Remarkably, carbapenem resistance emerged in the 2020/2021 phase, coupled with a rise in resistance to cefepime, cefotaxime and ciprofloxacin were only recorded at the UTH, the most important referral and specialised hospital that caters for patients from all over the country. This could result from the long-standing indiscriminate use of antibiotics (Masich et al., 2020). Cephalosporins and fluoroquinolones are the drugs of choice for treating *Salmonella*-BSI, therefore resistance to these antibiotic classes, coupled with resistance to carbapenems, the most reliable last-resort treatment for XDR-*Salmonella*-BSI institutes a public-healthcare problem (Elizabeth J. Klemm et al., 2018; Zakir et al., 2021). The accrual of resistance to most of the therapeutic options necessitates an urgent need to track the emergence and spread

of AMR Typhi to guide empiric therapy, prevent treatment failure and adopt preventative interventions such as an introduction and rolling out of typhoid conjugate vaccines (TCVs) and improving WASH infrastructure (Andrews et al., 2019; Carey et al., 2022).

MDR *Salmonella* infections are classified as resistant to ampicillin, co-trimoxazole, and chloramphenicol, whereas XDR *Salmonella* infections are resistant to the three antibiotics listed in MDR with additional resistance to fluoroquinolones and third-generation cephalosporin (Arulmohi et al., 2017; Ishaque et al., 2022). MDR *Salmonella* infections were lower in the 2020/2021 phase compared to 2018/2019, from 51% in 2018/2019 to 5% in 2020/2021. The prevalence of MDR *Salmonella* infections in our setting was lower than reported in Tanzania and Kenya, 81% and 77%, respectively (Kariuki et al., 2010b; Omari A Msemo et al., 2019). Interestingly, there has been a downward trend in the prevalence of MDR *S.* Typhi from 84% in a previous study in Zambia (Kalonda et al., 2015b) to 51% in 2018/2019 and 5% in 2020/2021 in our study. This observation could be attributed to the changes in prescribing patterns and antimicrobial use, such as reduced use of ampicillin, co-trimoxazole and chloramphenicol as first-line antibiotic treatment for Enteric fever (Browne et al., 2020; Menezes et al., 2012). Contrary to our findings, a systematic review observed an increase in MDR *Salmonella* infections in Kenya (60 to 82% between 1990-1994), Malawi (0 to 88% between 1994 to 2009) and Nigeria (37% to 100% between 1998 to 2014) (Browne et al., 2020).

5.6.3 genomic analysis in Salmonella

Resistance gene determinants to the commonly used class of antibiotics (beta-lactams) in treating *Salmonella* infections were predominantly *blaTEM-1B,* a resistance gene that encodes for the predominant plasmid-mediated β-lactamases of *Enterobacterales* and responsible for resistance in ampicillin (Livermore, 1995). This agreed with findings in India that recorded *bla*TEM-1B as the most prevalent than *bla*TEM116 (Katiyar et al., 2020). There was only one *bla*CTX-M-15, a cefotaximase that hydrolyses cefotaxime/ceftriaxone. In contrast, the other isolates resistant to ceftriaxone had no resistance gene determinants. This finding could result from another mechanism of resistance, such as a change in the outer membrane that allows the penetration of antibiotics (efflux pumps and modification of porins) (Alenazy, 2022; Chowdhury et al., 2022). Resistance determinants for phenicols and folate pathway antagonist*s* found in our study were similar to other studies (Das et al., 2017; Gaind et al., 2006; García-Fernández et al., 2015; Menezes et al., 2012).

Fluoroquinolone resistance determinants identified in this study fell in both the quinolone resistance determining regions (QRDR) and PMQR groups. Fluoroquinolone resistance in *Salmonella* involves a build-up of multiple mutations in a stepwise manner and is an endpoint result of the accumulation of several biochemical mechanisms and/or different mechanisms such as over-activation of multidrug efflux pumps and decreased outer membrane permeability (Giraud et al., 2006; Poole, 2000; Qian et al., 2020b). The most common genetic pattern is a single mutation (usually at *gyr*A codon 83 or 87), which results in a moderate increase in ciprofloxacin MIC to 0.06-0.25 mg/L and is associated with prolonged fever clearance times and increased chance of clinical failure when treating with fluoroquinolones (Carey et al., 2022; Day et al., 2018). An accumulation of three QRDR mutations raises ciprofloxacin MIC to 8-32 mg/L and is associated with a higher occurrence of clinical failure (Carey et al., 2022). This explains the findings in this study, where some *Salmonella* isolates that carried some of the resistance gene determinants displayed reduced susceptibility to ciprofloxacin and not full resistance.

S. Typhi strains isolated from various endemic regions over a century confirm the predominance and co-existence of two sequence types (ST1 and ST2) (Yap et al., 2016). This could be due to international travel and the uniqueness of ST1/ST2 virulence genes that support successful dissemination (Yap et al., 2016). Similar to our findings, *S*. Enteritidis isolates ST11 was also found in Ghana and Gambia (Aldrich et al., 2019; Darboe et al., 2020). *S*. Typhimurium ST313 is the leading cause of invasive *Salmonella* infections in Sub-Saharan Africa (SSA) and is rarely isolated outside SSA (CK et al., 2012; Kariuki and Onsare, 2015; Kingsley et al., 2009b), contrary to the low prevalence (3%) observed in our study and no presence in The Gambia (Darboe et al., 2020). ST313 is more virulent than *S*. Typhimurium ST19, which causes human diarrhoea. The virulence could be attributed to genome degradation and conversion to a more host-restricted existent characteristic of *S*. Typhi infections (Kariuki and Onsare, 2015; Okoro et al., 2012).

Phylogenetic analysis of cgMLST confirms a wide genetic diversity of *S*. Typhi. The genetic diversity of *S*. Typhi identified in our study agrees with a study that utilised data from WGS of nearly 2,000 isolates sourced from over 60 countries that found that the global *S*. Typhi population is highly structured and includes dozens of subclades that display geographical restriction (Wong et al., 2016). The genetic diversity of *S*. Typhi strains supports bacterial evolution, which indicates that pathogenesis would lead to the divergent selection of virulence factors and ultimately result in different clinical outcomes of the infection (Sabbagh et al., 2010). Analysing WGS data from different regions helps generate a robust genotyping system that gives an insight into local *S.* Typhi

populations and helps identify recent introductions into new or previously endemic locations, thereby providing information on their likely geographical source (Wong et al., 2016). Another study that used WGS data originating from 52 countries presented a new phylogeny of *Salmonella enterica* comprising new clades and genetic patterns. This finding confirms the rapid evolution of *S. enterica* and its genotype diversity and highlights the potential for additional refinement within *S. enterica* phylogenies (Worley et al., 2018).

An assessment of phylogenetic relatedness and diversity of our *S.* Typhi isolates to strains from other countries revealed the relatedness of the Zambian *S.* Typhi strains to those from five different countries (Tanzania, Malawi, South Africa, the United States and the United Kingdom). This finding suggests travel-associated spread, with a higher prevalence of geographic association among bordering countries in the region (Johnson et al., 2011). Zambia is a land-locked country that depends heavily on trade with Tanzania through its ports ("Tanzania Exports to Zambia - 2023 Data 2024 Forecast 1997-2021 Historical," 2023; "Zambia Overview: Development news, research, data | World Bank," 2023). This could explain why Tanzania had the closest matches of *S.* Typhi strains to those of Zambia.
There needs to be more information related to phenotypic, molecular and WGS data of pathogens, and the phylogenetic analysis of AMR pathogens isolated from humans and poultry has not been exhaustively studied in Zambia.

This study was able to contribute to the knowledge gaps highlighted by providing information on the phenotypic and genomic characteristics of the three pathogens. For *E. coli* WGS and MLST highlighted the diversity of the *E. coli* strain from the same source and across sources, however the existence of similar resistance gene determinants and plasmids suggests possible transmission among the three interfaces (clinical, healthy community and poultry). *E. coli* clinical infections were mostly driven by ST131, a pandemic clone associated with ESBLs and fluoroquinolone resistance. Although few *E. faecalis* isolates from clinical samples were sequenced, the identification of STs known to be found in healthy community, animals, milk and hospital surfaces further suggests easy transmissibility of *Enterococci,* which could promote the sharing of resistance gene determinants and further spread. WGS results from this study found *S.* Typhi to be the most prevalenet cause of *Salmonella* bloodstream infections in both provinces, with an emergence of ST313 an invasive strain of S. Typhimurium known to cause infections in immunocompromised individuals. A few rare *Salmonella* serovars associated with poultry and

diverse food products were also identified. WGS results from this study, gave an in-depth understanding of the interface of AMR in humans and poultry.

5.7 Study limitations

Incomplete social demographic data for the isolates from the 2018/2019 collection phase and the inability to collect outcome data on the patients that had *E. coli*, *Enterococcus* and *Salmonella* infections caused by MDR pathogens and received inappropriate empiric treatment. Due to financial constraints, two AST methods were used (automated and conventional) and only allowed for a few *Enterococcus* species to be sequenced. The study was also limited to only those patients whose blood and urine samples were submitted to the Microbiological Laboratory for analysis which meant a possible exclusion of patients with blood infections but had no opportunity to have their blood tested. This could bias the study population and therefore reduces the external validity of the findings. The use of short-read sequencing by MiSeq could not allow us to determine the location of AMR genes (plasmid vs chromosome), It is therefore recommended to use hybrid assembly that includes both MiSeq and MinION. Despite these limitations, the study has provided valuable insights into understanding the relatedness/unrelated and antimicrobial resistance profiles/gene determinants of the clinical, healthy community and poultry strains.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study found high levels of resistance to a number of antibiotic classes in all the three studied pathogens, highlighting an AMR problem in BSIs, UTIs, carriage in healthy community and food-producing animals (poultry). Notably was the high level of 3GC resistance and ESBL strains in clinical isolates and the presence of ESBL determining genes in isolates from the healthy community and poultry. Interestingly was the presence of similar resistance gene determinants and incompatibilty (Inc) plasmid replicons FII, FIB, FIA and I1 in both clinical and non-clinical *E. coli* strains. In *Enterococcus* species, resistance to affordable drugs of choice in the treatment of invasive infections was on the rise, though the absence of resistance to vancomycin in clinical isolates is comforting. In *Salmonella*, the level of resistance to the drugs of choice (ciprofloxacin and ceftriaxone) was relatively low though there was an upward trend in the 2020/2021 in comparison to the 2018/2019 phase. Based on the antibiotic resistance patterns in the three pathogens in this study, it is evident that antibiotics were given inappropriately in both the tertiary hospitals and primary healthcare facilities. There was a diverse distribution of STs from the different sources (clinical, healthy community and poultry) with same STs being shared in all three interfaces and some limited to certain sources. The most prevalent *E. coli* ST (ST131) is predominantly attributed to the dissemination of $bla_{\text{CTX-M-15}}$ ESBL gene and fluoroquinolone resistance while *Salmonella* infections were mostly caused by genomic diverse *S*. Typhi with an emergence *S*. Typhimurium ST313. The ST313 is known to be MDR and causative agent for invasive *Salmonella* infections in immunocompromised individuals, more especially HIV. The occurrence of strains known to be commensals as causative agents of BSIs/UTIs, the distribution of similar antimicrobial resistance profiles and resistance gene determinant found on transferable plasmids in all the three interfaces supports the possibility of cross transmission and the ability of previously non-pathogenic strains to acquire traits that allow them become pathogenic and further spread AMR.

6.2 Recommendations

Based on the findings in this study the following recommendations are made:

a) Government should establish multi-displinary AMS programs in all healthcare facilities with a focus on the rationale use of antibiotics and infection prevention;

b) The Ministry of Health and the Zambia Medicines and Supplies Agency (ZAMMSA) should undertake annual review and update of the standard treatment guidelines (STGs) with an adoption of the WHO aWaRe classification at all levels of healthcare provision in Zambia;
c) Annually, all healthcare facilities should update their treatment guidelines based on locally generated antimicrobial susceptibility profiles;
d) Clinicians should be sensitised to base selection of antibiotics in the different WHO aWaRe classification based on local antimicrobial susceptibility data;
e) Governement should build capacity in the provision of microbiology laboratory services to support AMR diagnosis and surveillance;
f) Governement should develop an AMR structured surveillance strategy in human health; and aadopt a one health approach to the fight against AMR
g) Governement should promote adoption of the WHO recommendation on reserving medically important priority antibiotics for human medicine
h) Improvement and enforcement of policies, regulations, interventions and activities that address the prevention of diseases and containment of AMR

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8. APPENDICES

Appendix A: Distribution of phylogroups, sequence types and serotypes from clinical and non-clinical (healthy community and poultry) *E. coli*

				Healthy Community	Hospitalised Patients		Poultry		
Phylogroups	**Clonal Complex**	**Sequence Types**	**Serotypes**	Rectal Swabs	Blood Serum	Urine	Carcass wash	Cloacal Swabs	
A	CC10	10, 44, 48, 167, 617, 1137	?H30, O2:H2, O9:H9, O13:H4,O23:H48, O38:H11, O89:H4 O89:H9, O89:21, O176:H11	2	9	7	1	2	21
	CC165	382	?H5	1	0	0	0	0	1
	CC216	216	O3:H4	1	0	0	0	0	1
	ND	206, 226, 495, 540, 2705, 3519, 6151, 6359, 13141	?H10, ?H26, ?H48, O9:H30, O29:H4, O55:H27, O60:H5, O86:H10, O89:H9	7	3	1	0	2	13
B1	CC448	448	O8:H8	0	0	1	0	0	1
	ND	13, 40, 155, 156, 196, 212, 297, 602, 711, 2170, 2598, 2825, 13157	?H8, ?H21, ?H40, O11/OX19:H51, O120:H10, O126:H51, O123/O186:H9, O154:H21, O175:H16, O179:H21, O18:H49, O25:H34, O45:H8, O76:H30, O8:H7, O99:H9	6	2	2	7	1	18
B2	CC73	73	O6:H1	0	2	0	0	0	2
	CC131	131	?H4, O16:H5, O25:H4, O25:H5, O25:H6, O25:H7	1	22	14	0	0	37
	ND	452, 636, 998	O2:H6, O21:H7, O81:H27	1	2	0	0	0	3
C	CC23	88, 410	?H9, O8:H4, O8:H9	0	8	6	0	0	14
Cladel	ND	7125	O17/077:H45	0	0	0	0	1	1
D	CC38	38	O25:H15	0	1	0	0	0	1
	CC69	69	O15:H18	0	2	0	0	0	2
	ST405	405	O102:H4, O102:H6	0	1	2	0	0	3
	ND	1652	O1:H1	1	0	0	0	0	1
E	ND	350	O86:H27	0	0	0	1	0	1
F	CC648	648	O1:H6, O8:H4, O83:H42, O153:H6	1	5	1	0	0	7
G	CC117	117	O161:H4	0	0	0	1	0	1
Unknown	ND	297	O45:H8	0	1	0	0	0	1

Key: Rectal swabs, Blood serum and Urine were Human source, while Carcass wash and Cloacal swabs were from Animal (Poultry) source.

Appendix B: Distribution of resistance genes in *E. coli* from all sources

Appendix B1: Distribution of resistance genes in clinical and non-clinical *E. coli*

Antibiotics Categories	Health Community	Hospitalised Patients		Poultry	
	Rectal Swabs	Blood Serum	Urine	Carcass wash	Cloacal Swabs
Aminoglycosides	aadA-1b[v] (1, 100%)	aac(3)-IIa;aac(6')-Ib-cr (1, 33%)	aac(3)-IIa;aac(6')-Ib-cr (2, 67%)	aadA-2[v] (1, 100%)	aph(3')-IIa[v];aph(6)-Id,strB;aadA-2[v] (1, 100%)
	aadA-5;aph(6)-Id,strB (1, 100%)	aac(3)-IIa;aadA-8b[v];aadA-2 (1, 100%)	aac(3)-IId;aph(6)-Id,strB (1, 33%)	aadA-22[v];aph(6)-Id,strB[v];aadA-3[v] (1, 100%)	aph(6)-Id,strB[v] (1, 11%)
	aph(6)-Id,strB (8, 50%)	aac(3)-IId;aac(6')-Ib-cr (1, 100%)	aac(6')-Ib-cr (1, 50%)	aadA-23[v];aph(3')-IIa[v];aph(6)-Id,strB (1, 100%)	aph(6)-Id,strB[v];aadA-2[v];aph(3')-IIa[v] (1, 100%)
	aph(6)-Id,strB;aadA-5;aac(6')-Ib-cr;aac(3)-Iia (1, 50%)	aac(3)-IId;aph(6)-Id,strB (2, 67%)	aac(6')-Ib-cr;aadA-5 (3, 60%)	aadA-5 (1, 33%)	aph(6)-Id,strB[v];aadA-5 (1, 17%)
	aph(6)-Id,strB[c] (1, 100%)	aac(6')-Ib-cr (1, 50%)	aac(6')-Ib-cr;aadA-5;aac(3)-Iia (1, 50%)	aph(6)-Id,strB (1, 6.3%)	
	aph(6)-Id,strB[v] (2, 22%)	aac(6')-Ib-cr;aadA-5 (2, 40%)	aac(6')-Ib-cr;aph(6)-Id,strB (1, 100%)	aph(6)-Id,strB;aadA-5;aac(3)-Iia (1, 100%)	
	aph(6)-Id,strB[v];aadA-5;aac(3)-Iid (1, 33%)	aac(6')-Ib-cr;aadA-5;aac(3)-Iia (1, 50%)	aac(6')-Ib-cr;aph(6)-Id,strB;aadA-2;aadA-8b[v];aac(3)-Iia (1, 100%)	aph(6)-Id,strB[v] (2, 22%)	
		aac(6')-Ib-cr;aadA-5;aac(3)-Iid (1, 100%)	aac(6')-Ib-cr;aph(6)-Id,strB[v];aac(3)-IId;aadA-5 (2, 100%)		
		aac(6')-Ib-cr;aadA-5[v];aac(3)-Iia (1, 100%)	aac(6')-Ib-cr;aph(6)-Id,strB[v];aadA-5 (1, 100%)		
		aac(6')-Ib-cr;aph(6)-Id,strB;aac(3)-Iia (1, 100%)	aac(6')-Ib-cr;aph(6)-Id,strB[v];aadA-5;aac(3)-Iid (1, 33%)		
		aac(6')-Ib-cr;aph(6)-Id,strB[v];aadA-5;aac(3)-Iid (2, 67%)	aph(6)-Id,strB (2, 12.5%)		
		aac(6')-Ib-cr[c];aadA-5[c];aac(3)-Iia (1, 100%)	aph(6)-Id,strB;aac(3)-IId;aac(6')-Ib-cr;aadA-5 (1, 100%)		
		aadA-1b[v];aph(6)-Id,strB (2, 100%)	aph(6)-Id,strB;aac(6')-Ib-cr (1, 100%)		
		aadA-5 (1, 100%) (2, 67%)	aph(6)-Id,strB;aac(6')-Ib-cr;aac(3)-Iia (1, 25%)		
		aadA-5[v];aac(6')-Ib-cr (1, 100%)	aph(6)-Id,strB;aac(6')-Ib-cr;aadA-2;aadA-8b[v];aac(3)-Iia (1, 100%)		
		aph(6)-Id,strB (5, 31%)	aph(6)-Id,strB;aac(6')-Ib-cr;aadA-5;aac(3)-Iid (1, 33%)		
		aph(6)-Id,strB;aac(3)-Iid (1, 100%)	aph(6)-Id,strB;aadA-5;aac(3)-IId;aac(6')-Ib-cr (2, 100%)		
		aph(6)-Id,strB;aac(6')-Ib-cr;aac(3)-Iia (3, 75%)	aph(6)-Id,strB;aadA-5;aac(6')-Ib-cr;aac(3)-Iia (1, 50%)		
		aph(6)-Id,strB;aac(6')-Ib-cr;aadA-2[v];aadA-15[v];aac(3)-IIa[v] (1, 100%)	aph(6)-Id,strB[v] (2, 22%)		
		aph(6)-Id,strB;aac(6')-Ib-cr;aadA-5;aac(3)-IIa[c] (1, 100%)	aph(6)-Id,strB[v];aac(6')-Ib-cr;aac(3)-IId;aadA-5 (2, 50%)		
		aph(6)-Id,strB;aac(6')-Ib-cr;aadA-5;aac(3)-Iid (2, 67%)	aph(6)-Id,strB[v];aadA-5 (3, 50%)		
		aph(6)-Id,strB;aadA-2;aadA-8b[v];aac(3)-Iid (1, 100%)	aph(6)-Id,strB[v];aadA-5;aac(3)-Iid (1, 33%)		
		aph(6)-Id,strB;aadA-3[c];aadA-8b[c];aac(3)-IIa[c] (1, 100%)			
		aph(6)-Id,strB;aadA-5 (1, 100%)			
		aph(6)-Id,strB;aadA-5;aac(3)-IIa;aac(6')-Ib-cr (3, 100%)			
		aph(6)-Id,strB;aadA-5;aac(6')-Ib-cr (1, 100%)			
		aph(6)-Id,strB[c];aac(6')-Ib-cr;aadA-5;aac(3)-Iia (1, 100%)			
		aph(6)-Id,strB[v] (2, 22%)			
		aph(6)-Id,strB[v];aac(3)-IId;aadA-5 (1, 100%)			
		aph(6)-Id,strB[v];aac(6')-Ib-cr;aac(3)-IId;aadA-5 (2, 50%)			
		aph(6)-Id,strB[v];aadA-5;aac(3)-Iia (4, 100%)			
		aph(6)-Id,strB[v];aac(6')-Ib-cr;aadA-5;aac(3)-Iid (1, 100%)			
		aph(6)-Id,strB[v];aac(6')-Ib-cr[c];aadA-5 (1, 100%)			
		aph(6)-Id,strB[v];aadA-2;aadA-8b[v] (1, 100%)			
		aph(6)-Id,strB[v];aadA-5 (2, 33%)			
		aph(6)-Id,strB[v];aadA-5;aac(3)-Iid (1, 33%)			
Beta-Lactams	CTX-M-15 (1, 17%)	CMY-2;CTX-M-15;OXA-1 (1, 100%)	CMY-2;OXA-1;TEM-1 (1, 100%)	CMY-2 (1, 100%)	CMY-2[c];TEM-1 (1, 33%)
	CTX-M-15;OXA-1;TEM-1 (1, 5%)	CMY-2[u] (1, 100%)	CTX-M-15 (2, 33%)	CMY-2;TEM-1 (1, 100%)	CTX-M-55;TEM-1[v] 91, 100%)
	CTX-M-27;TEM-1 (1, 33%)	CTX-M-106-p[u] (1, 100%)	CTX-M-15;CMY-2;OXA-1;TEM-1 (3, 60%)	CMY-2[c];TEM-1 (2, 67%)	CTX-M-65 (1, 100%)
		CTX-M-11-p[c];TEM-1 (1, 100%)	CTX-M-15;CMY-2;TEM-1 (1, 50%)	CTX-M-14 (2, 67%)	CTX-M-65;CMY-2;TEM-1 (1, 100%)
		CTX-M-14 (1, 33%)	CTX-M-15;OXA-1 (12, 48%)	CTX-M-55 (2, 67%)	TEM-1 (1, 7%)
		CTX-M-14;TEM-1 (1, 100%)	CTX-M-15;OXA-1;TEM-1 (7, 35%)	CTX-M-55;TEM-1 (1, 100%)	
		CTX-M-15 (3, 50%)	CTX-M-15;TEM-1 (2, 17%)		
		CTX-M-15;CMY-2;OXA-1;TEM-1 (2, 40%)	CTX-M-27 (3, 75%)		
		CTX-M-15;CMY-2;TEM-1 (1, 50%)	CTX-M-27;TEM-1 (1, 33%)		
		CTX-M-15;CMY-2[c];OXA-1;TEM-1a (1, 100%)	TEM-1 (2, 14%)		
		CTX-M-15;OXA-1 (13, 52%)			
		CTX-M-15;OXA-1;TEM-1 (12, 60%)			
		CTX-M-15;OXA-1;TEM-1[c] (1, 100%)			
		CTX-M-15;OXA-1;TEM-191-p* (1, 100%)			
		CTX-M-15;TEM-1 (10, 83%)			
		CTX-M-15[c];OXA-1[c];TEM-1 (1, 100%)			
		CTX-M-27 (1, 25%)			
		CTX-M-27;OXA-1[c] (1, 100%)			
		CTX-M-27;TEM-1 (1, 33%)			
		CTX-M-55 (1, 33%)			
		TEM-1 (2, 14%)			

Appendix B2: Distribution of resistance genes in clinical and non-clinical *E. coli* isolates

Antibiotics Categories	Health Community	Hospitalised Patients		Poultry	
	Rectal Swabs	Blood Serum	Urine	Carcass wash	Cloacal Swabs
Fluoroquinolones	gyrA_EC1[83:S-L;87:D-N];parC_EC2[80:S-I] (1, 20%) gyrA_EC1[83:S-L] 1, 50%) gyrA EC2[83:S-L;87:D-H];parC_EC2[80:S-R] (1, 100%) gyrA_EC2[83:S-L] (1, 20%) gyrA EC2[83:S-L];parC_EC2[56:A-T] (1, 100%) qnrS-1 (2, 50%)	gyrA_EC1[83:S-L;87:D-N];parC_EC1[80:S-I;84:E-V] (19, 56%) gyrA EC1[83:S-L;87:D-N];parC_EC1[80:S-I;84:E-V];qepA-1[c] (1, 100%) gyrA_EC1[83:S-L;87:D-N];parC_EC1[80:S-I] (1, 100%) gyrA EC1[83:S-L;87:D-N];parC_EC1[80:S-I];qnrS-1 (1, 100%) gyrA EC1[83:S-L;87:D-N];parC_EC2[80:S-I] (3, 60%) gyrA EC1[83:S-L;87:D-N];parC EC2[80:S-I];qepA-1[v] (1, 100%) gyrA EC1[83:S-L;87:D-Y];parC EC1[80:S-I;84:E-V] (1, 100%) gyrA_EC1[83:S-L] (1, 50%) gyrA_EC2[83:S-A] (3, 100%) gyrA EC2[83:S-L;87:D-N];parC_EC1[80:S-I;84:E-V];qnrB-1 (1, 100%) gyrA EC2[83:S-L;87:D-N];parC_EC2[80:S-I;84:E-A];qepA-1[v] (1, 100%) gyrA EC2[83:S-L;87:D-N];parC EC2[80:S-I] (13, 48%) gyrA_EC2[83:S-L;87:D-N];parC EC2[80:S-I];qepA-1[v] (3, 75%) gyrA EC2[83:S-L;87:D-N];parC EC2[80:S-I];qnrS-1 (1, 50%) parC_EC2[80:S-X;84:E-X] (1, 100%) qnrS-1 (1, 25%)	gyrA_EC1[83:S-L;87:D-N];parC EC1[80:S-I;84:E-V] (14, 42%) gyrA_EC1[83:S-L;87:D-N];parC_EC2[80:S-I] (1, 20%) gyrA_EC2[83:S-L;87:D-N];parC_EC2[80:S-I] (14, 52%) gyrA EC2[83:S-L;87:D-N];parC_EC2[80:S-I];qepA-1 (1, 100%) gyrA EC2[83:S-L;87:D-N];parC_EC2[80:S-I];qepA-1[v] (1, 25%) gyrA EC2[83:S-L;87:D-N];parC EC2[80:S-I];qnrS-1 (1, 50%)	gyrA_EC2[83:S-L] (4, 80%) OqxA;OqxB[v] (1, 100%) OqxA[v];OqxB[v] (1, 50%) qnrS-1 (1, 25%)	OqxA[v];OqxB[v] OqxA[v];OqxB[v];gyrA_EC2[83:S-L] (1, 100%) OqxA[v];OqxB[v];qnrS-1 (1, 100%) OqxB[v];OqxA[v];gyrA EC2[83:S-L;87:D-N];parC_EC2[80:S-I] (1, 100%)
Phenicols	catA-1 (1, 9.1%)	catA-1 (7, 64%) catA-2[v] (1, 9.1%) floR[v] (1, 9.1%)	catA-1 (3, 27.3%) catA-1;catB-3[v] (1, 100%) catB-3[v] (1, 100%)	cml-1* (2, 50%) floR[v] (2, 67%)	cml-1* (2, 50%)
Sulfamethoxazole	sul-1[c];sul-2[c] (1, 100%) sul-2[c] (1, 100%) sul-2[v] (12, 30%) sul-2[v];sul-1[v] (2, 4.9%) sul-3 (1, 33%)	sul-1[c] (1, 100%) sul-1[v] (6, 55%) sul-1[v];sul-2[v] (5, 56%) sul-1a[c] (1, 100%) sul-2[v] (13, 33%) sul-2[v];sul-1[c] (1, 100%) sul-2[v];sul-1[v] (25, 61%) sul-2[v];sul-3;sul-1[v] (1, 50%)	sul-1[v] (5, 45%) sul-1[v];sul-2[v] (4, 44%) sul-2[v] (9, 23%) sul-2[v];sul-1[v] (12, 29%)	sul-2[v] (4, 10%) sul-2[v];sul-1[v] (2, 5%) sul-3 (2, 67%)	sul-2[v] (2, 5%) sul-2[v];sul-3;sul-1[v] (1, 50%) sul-3;sul-1[v] (1, 100%)
Tetracyclines	tet(A) (1, 3.4%) tet(A)-1[u] (1, 16.7%) tet(A)-1[v] (9, 18.4%)	tet(A) (13, 44.8%) tet(A)-1[c] (1, 100%) tet(A)-1[u] (3, 50%) tet(A)-1[v] (22, 45%) tet(A)-1[v];tet(A) (2, 50%) tet(A)-1[v];tet(A)[c] (2, 100%) tet(M)[v];tet(A)-1[v] (1, 100%)	tet(A) (15, 51.7%) tet(A)-1[v] (11, 22.4%) tet(A)-1[v];tet(A) (1, 25%)	tet(A)-1[u] (2, 33.3%) tet(A)-1[v] (4, 8.2%) tet(A)-1[v];tet(A) (1, 25%) tet(A)-1[v];tet(A)[v] (1, 100%)	tet(A)-1[v] (3, 6%)
Trimethoprims	dfrA-1[v] (2, 67%) dfrA-14[v] (10, 43%) dfrA-17 (3, 5%) dfrA-8 (1, 50%)	dfrA-1 (2, 100%) dfrA-1;dfrA-12 (1, 100%) dfrA-1[v] (1, 33%) dfrA-12 (2, 33%) dfrA-14[v] (4, 17%) dfrA-17 (31, 54%) dfrA-17;dfrA-12 (1, 100%) dfrA-17;dfrA-12[c] (1, 100%) dfrA-17;dfrA-14[v] (1, 100%) dfrA-17[c] (1, 100%) dfrA-17[v] (1, 100%) dfrA-5;dfrA-12[c] (1, 100%) dfrA-7 (1, 100%) dfrA-7;dfrA-12 (1, 100%) dfrA-8;dfrA-12 (1, 100%) dfrB-4;dfrA-14[v] (1, 100%)	dfrA-12 (2, 33%) dfrA-14[c] (1, 100%) dfrA-14[v] (3, 13%) dfrA-17 (21, 37%) dfrA-5 (1, 100%) dfrA-8 (1, 50%) dfrB-4 (1, 100%)	dfrA-12 (1, 17%) dfrA-14[v] (5, 22%) dfrA-17 (1, 1.8%) dfrA-17;dfrA-1[v] (1, 100%)	dfrA-12 (1, 17%) dfrA-12;dfrA-14[v] (1, 100%) dfrA-14[v] (1, 4%) dfrA-17 (1, 1.8%)

Key: Rectal swabs, Blood serum and Urine were Human source, while Carcass wash and Cloacal swabs were from Animal (Poultry) source

Appendix C: Plasmid replicons in blood, urine, healthy community, poultry isolates

Appendix C1: Plasmid replicons in blood, urine, healthy community and poultry

Blood	Urine	Rectal swab	Poultry
	FII-4	-	FIB-24; FII-37
FIB-1; FII-31; FIA-4; I1	FIB-10; FII-29; I1	FII-1; FIB-1; FIA-1	FII-29; I1
FII-33; N[v]	B/O; FIB-1; FII-31; FIA-4	-	HI2; I1
FII-29; FIB-10; I1[	FII-1; FIB-33; FIA-2	B/O; FII-14	FII-21; FIB-1;I1
FIB-1; FIA-1; FII-59; I1; L/M-parB; L/M-rep	FIB-26; FII-1; FIA-1	FII-25; FIB-10	FIB-1; FII-36; FIC-1; HI1; I1
FIB-1; FII-31; FIA-4	FII-2; FIA-1	B/O; FIC-1[v]; FII-10; P	-
FIB-26[v]; FII-1; FIA-1	FIB-20; IA-2	B/O; FIB-10	FII-33; N
FIB-1; FII-31; FIA-4; I1	FII-29; FIB-10	-	FII-18; FIC-1[v]; FIA-5; FIB-1; I1
-	FIB-1; FII-31; FIA-4;I1	FIB-3	FIB-1; FII-18; FIC-1; HI2
FIB-1; FII-1; FIA-1	FII-1; FIB-16; FIA-1	FII-29; FIB-10	FIA-1; FII-16; FIB-1
FII-59; I1; L/M-rep; L/M-parB; L/M-traU	FIB-26; FII-1; FIA-1	FIB-33; FII-2	-
-	FIB-30	-	FII-4; FIB-1; I1
FII-2; FIA-2	FII-29; FIB-10	HI1	FIA-1; FII-34
FII-2; FIA-1	FIB-1; FII-36	FIB-33; FII-2	FII-34; FIB-30; I1
FII-1; FIA-2	FII-1; FIA-2	FII-17; I1	-
FII-2; FIB-1	FIB-26; FII-6; FIA-1	B/O	HI2
FIB-20; FII-22; FIA-1	FII-59	-	FII-33; N
FII-2; FIB-1	FII-29; FIB-10	FII-11; FIB-26	FIB-1; FII-18; FIC-1; FIA-5; HI2
FII-1; FIB-20; FIA-2	FIB-20; FII-1; FIA-2	FII-2	
FIB-3; FIA-6; FII-52; HI2; P	FIB-26; FII-48; FIA-1	FII-20; FIB-12; I1	
FIB-1; FII-31[v]; FIA-4;I1	FII-59	FII-12	
FII-1; FIB-1; FIA-1	FIB-10; FII-29	FIB-15; FII-56	
FII-1; FIB-20; FIA-2	FIB-16; FIA-1		
FIB-10; FII-29; I1	FIA-1; FIB-32		
FII-29 ; FIB-10	FIB-1; FII-31; FIA-4		
FIB-10; FII-29	FII-1; FIB-33; FIA-2		

.

Appendix C2: Plasmid replicons in blood and urine isolates

Blood	Urine
I1	-
FII-22; FIB-20; FIA-1	FII-2; FIB-1
-	FII-29; FIB-10
-	FIB-26; FIA-1
FII-2; FIB-12; I1	FII-1; FIB-1; FIA-1
FII-18; FIC-1; FIB-1; I1	FIB-1; FII-36; FIA-4
FII-31; FIA-4; FIB-1	FIB-1; FIA-4; FII-31
FIB-20; FIA-1	FII-1; FIB-1; FIA-1
FII-1; FIB-16; FIA-1	FII-31; FIA-4; FIB-1
FII-29; FIB-10	I1
FII-2; FIB-1; FIA-1	FII-1; FIB-20; FIA-2
FIB-20; FII-1; FIA-2	FII-31; FIA-4
FIB-26; FII-1; FIA-1	
-	
FII-1; FIB-16; FIA-1	
FIB-1; FII-31; FIA-4	
FIB-26; FIA-1; FII-50	
FIB-20; FIA-2; FII-1	
FII-1; FIB-16; FIA-1	
FIB-1; FII-31; FIA-4	
FII-1; FIB-1; FIA-1	
FII-1; FIB-1; FIA-6	
FIB-16; FIA-1	
FII-4	
FIB-1; FII-31; FIA-4; I1	
FIB-32; FIA-1	
FIB-1; FII-31; FIA-4	
FIB-16; FIA-1	
FII-29; FIB-10	
FII-29; FIB-10	
FII-1; FIB-16; FIA-1	
FIB-32; FIA-1; I1	
FIB-32; FIA-1	
FIB-1; FIA-4; FII-31	
FIA-1	
FII-K7	

Appendix D: Questionnaires, consent/assent forms and participants information sheets

Appendix D1: Hospital: BSIs and UTIs questionnaire for parents/guardians

Interviewee: Parents or guardians to children below 18 years

Questionnaire serial number ☐☐☐

Risk factors associated with antimicrobial resistance in patients with BSIs and UTIs

Parent/Guardian

The purpose of this research study is to gather information on the risk factors associated with Antimicrobial resistance in patients with bloodstream infections.
You have been asked to participate in this research study and please note that by completing This questionnaire you are voluntarily agreeing to participate in this study.
You will remain anonymous and all information given will be treated as confidential.

Please mark the appropriate block with an X or write your answer on the space provided.

1.0 Name of interviewer _____

2.0 Date of interview dd/mm/yy ☐☐☐☐☐☐☐☐
Start time _____

3.0 Participant identification _____

Demographic characteristics

For official use

Section A: Child

4.0 Gender of child
☐1 Male ☐2 Female ☐ 1

5.0 Age _____ ☐ 2

Section B: Parent/Guardian

6.0 Gender
☐1 Male ☐2 Female ☐ 3

7.0 Age _____ ☐ 4

8.0 Highest education of parent/guardian
☐1 None (did not attend school)
☐2 Primary (grade 1-7) ☐ 5
☐3 Junior Secondary (grade 8-9)
☐4 Senior Secondary (grade 10-12)

	5	College					
	6	University					
9.0	Location of household						
	1	Urban	2	Rural		6	
10.0	If urban, is it						
	1	High density	2	Low density		7	
	3	Medium density					
11.0	How many people are living in the household?					8	
	1	18 years and below					
	2	18 years and above					
12.0	Is there livestock at your household?						
	1	Yes	2	No		9	
13.0	If yes to question 12.0, which livestock?						
	1	Chickens				10	
	2	Pigs				11	
	3	Cattle				12	
	4	Other specify					13-14
14.0	Has the child had any contact with livestock in the past month?						
		Yes	2	No		15	
15.0	If yes to question 14.0, how many times?						
	1	Once a week	2	Twice a week		16	
	3	Once a month	4	Twice a month			17-18
	5	Other specify					
16.0	Does the child eat chicken?						
	1	Yes	2	No		19	
17.0	If yes to question 16.0, how many times?						
	1	Once a week	2	Twice a week		20	
	3	Once a month	4	Other specify			21-22
18.0	What is the source of your chickens? *Multiple response*						

1	Own production		23
2	Super market		24
3	Open market		25
4	Other specify		26-27

Section C: Clinical details

19.0 Date of onset of illness?

								28

20.0 Admission date

								29

21.0 Fever

1	Yes	2	No	30

22.0 If yes to question 21.0, when did the fever start?

1	Prior to admission		31
2	2 days post admission		32
3	Other specify _____		33-34

23.0 Was the child given any antibiotics prior to admission?

1	Yes	2	No	35

24.0 If yes to question 23.0, list antibiotics taken?

_____

_____

_____

_____

				36-39

25.0 If yes to question 23.0, how long did the child take the antibiotics? *Multiple response*

1	One day		40
2	Two days		41
3	Three days		42
4	Four days		43
5	Five days		44
6	Other Specify _____		45-46

26.0 What was the source of the antibiotics? *Multiple response*

1	Clinic		47
2	Hospital with a prescription from the clinician		48
3	Off the counter without a prescription		49

4	From family members		50
5	Other specify		51-52

27.0 If you got from a drugstore/chemist, did they ask for a prescription?

1	Yes	2	No	53
3	Sometimes			

28.0 Any hospital admission in the past one month?

1	Yes	2	No	54

29.0 If yes to question 28.0, when was the last hospital admission?

1	One week ago	2	Two weeks ago	55
3	Three weeks ago	4	One month ago	

30.0 Other associated illnesses and treatments?

1	HIV status		56
2	Chemotherapy		57
3	Steroids		58
4	Other specify		59-60

Section D: knowledge around antibiotics

31.0 Do know antibiotics?

1	Yes	2	No	61

32.0 What is an antibiotic?

_____ 62-65

_____

_____

33.0 If yes to question 31.0, list at least two antibiotics and when does one use them? 66-69

_____

_____

34.0 Where do you store your antibiotics?

1	Fridge		70
2	Cupboard		71
3	Open shelf cupboard		72
4	Other specify _____		73
			74-75

35.0 Is it acceptable to use antibiotics given by friends/family members as long they were used to treat the same Problem?

1	Yes	2	No	76
3	Sometimes	4	Not sure	

36.0 Do you think antibiotics will always work when you take them to treat disease?

1	Yes	77
2	No	
3	Sometimes	

37.0 Do you know what antibiotic resistance is?

1	Yes	78
2	No	

38.0 If yes to question 37.0, define antibiotic resistance?

_____ 79-80
_____
_____
_____
_____

39.0 What is the effect of antibiotic resistance on human health?

_____ 81-84
_____
_____

40.0 Where do you get information on antibiotic resistance?
Multiple responses

1	Hospitals		85
2	Practitioners		86
3	TV		87
4	Internet		88
5	Social Media		89
6	Friends/neighbours		90
7	others		91-92

End time: _____

Thank you for your time and contribution

Appendix D2: Hospital: BSIs' patient questionnaire

Interviewee: Patients above 18 years with bloodstream infections

Questionnaire serial number ☐☐☐

Risk factors associated with antimicrobial resistance in patients with Bloodstream infections (BSI)

The purpose of this research study is to gather information on the risk factors associated with antimicrobial resistance in patients with bloodstream infections.
You have been asked to participate in this research study and please note that by completing this questionnaire you are voluntarily agreeing to participate in this study.
You will remain anonymous and all information given will be treated as confidential.

Please mark the appropriate block with an X or write your answer on the space provided.

1.0 Name of interviewer _____

2.0 Date of interview dd/mm/yy ☐☐☐☐☐☐☐☐
Start time _____

3.0 Participant identification _____

Section A: Demographic characteristics

For official use

4.0 Gender
☐1 Male ☐2 Female ☐ 1

5.0 Age _____ ☐ 2

6.0 Marital status
☐1 Married
☐2 Single ☐ 3
☐3 Separated/Divorced
☐4 Widowed

7.0 Highest level of education
☐1 None (did not attend school)
☐2 Primary (grade 1-7) ☐ 4
☐3 Junior Secondary (grade 8-9)
☐4 Senior Secondary (grade 10-12)
☐5 College

	6	University					
8.0	Occupation						
	1	Self-employed					
	2	Unemployed			☐	5	
	3	Government/Salary worker					
	4	Allowance					
	5	Others specify					
9.0	Monthly income Zambian kwacha						
	ZMK _____				☐	6	
10.0	Do you live in your own house or rented house?						
	1	Rented house			☐	7	
	2	Own house					
11.0	Location of household						
	1	Urban	2	Rural	☐	8	
12.0	If urban, is it						
	1	High density	3	Low density	☐	9	
	2	Medium density					
13.0	Is there livestock at your household?						
	1	Yes	2	No	☐	10	
14.0	If yes to question 13.0, which livestock? *Multiple response*						
	1	Chickens			☐	11	
	2	Pigs			☐	12	
	3	Cattle			☐	13	
	4	Other specify _____			☐☐		14-15
15.0	Any contact with livestock in the past one month?						
	☐	Yes	2	No	☐	16	
16.0	If yes to question 15.0, how many times						
	1	Once a week	4	Twice a week	☐	17	
	2	Once a month	5	Twice a month	☐☐		18-19
	3	Other specify _____					
17.0	Do you eat chicken?						
	1	Yes	2	No	☐	20	
18.0	If yes to question 17.0, how many times?						

1	Once a week	3	Twice a week		21
2	Once a month	4	Other specify _____		22-23

19.0 What is the source of your chickens? *Multiple response*

1	Own production		24
2	Super market		25
3	Open market		26
4	Other specify _____		27-28

Section B: Clinical details

20.0 Date of onset of illness?

			29

21.0 Admission date

			30

22.0 Do you have a fever?

1	Yes	2	No		31

23.0 If yes to question 22.0, when did the fever start?

1	Prior to admission		32
2	2 days post admission		33-34
3	Other specify _____		

24.0 Did you take any antibiotics prior to admission?

1	Yes	2	No		35

25.0 If yes to question 24.0, list antibiotics taken

_____

_____

				36-39

26.0 If yes to question 24.0, how long did you take the antibiotics? *Multiple response*

1	One day		40
2	Two days		41
3	Three days		42
4	Four days		43
5	Five days		44
6	Other specify _____		45-46

27.0 What was the source of the antibiotics?

1	Clinic		47
2	Hospital with a prescription from the clinician		48
3	Off the counter without a prescription		49
4	From family members		50
5	Other specify _____		51-52

28.0 If you got from a drugstore/chemist, did they ask for a prescription?

1	Yes	3	No	53
2	Sometimes			

29.0 Any hospital admission in the past one month

1	Yes	2	No	54

30.0 If yes to question 29.0, when was the last hospital admission?

1	One week ago	3	Two weeks ago	55
2	Three weeks ago	4	One month ago	

31.0 Co-morbidity conditions

1	HIV status		56
2	Chemotherapy		57
3	Steroids		58
4	Other specify _____		59-60

Section C: knowledge around antibiotics

32.0 Do know antibiotics?

1	Yes	2	No	61

33.0 What is an antibiotic?

_____
_____
_____

62-65

34.0 If yes to question 32.0, list at least two antibiotics?

_____
_____
_____

66-69

35.0 Where do you store your antibiotics?

1	Fridge		
2	Cupboard		70
3	Open shelf cupboard		71-72
4	Other specify _____		

36.0 Is it acceptable to use antibiotics given by friends/family members as long they were used to treat the same problem?

1	Yes	3	No	73
2	Sometimes	4	Not sure	

37.0 Do you think antibiotics will always work when you take them to treat disease?

1	Yes	74
2	No	
3	Sometimes	

38.0 Do you know what antibiotic resistance is?

1	Yes	75
2	No	

39.0 If yes to question 38.0, define antibiotic resistance

_____ 76-79

_____

_____

_____

_____

40.0 What is the effect of antibiotic resistance on human health?

_____ 80-83

_____

_____

41.0 Where do you get information on antibiotic resistance
Multiple response

1	Hospitals	84
2	Practitioners	85
3	TV	86
4	Internet	87
5	Social Media	88
6	Friends/Neighbours	89
7	Others	90-91

End time _____

<u>Thank you for your time and contribution</u>

Appendix D3: BSIs follow up data collection sheet

Follow up data collection sheet for all participants with confirmed BSIs

Data sheet ☐☐☐

Risk factors associated with antimicrobial resistance in patients with Bloodstream infections (BSI)

1.0 Name of interviewer _____

2.0 Date of interview dd/mm/yy ☐☐☐☐☐☐☐☐

Start time _____

3.0 Participant identification _____

Section A

			For official use	
4.0	Admission diagnosis (Presumptive/Suspected focus or source?			
	1	Suspected Pneumonia (Respiratory)	☐	1
	2	Suspected meningitis (CNS)		
	3	Suspected urinary tract infection (UTI)		
	4	Diarrhoeal disease (gastrointestinal tract)		
	5	Skin and soft tissue infections		
	6	Intra-abdominal surgery		
5.0	Ward admitted	*Multiple response*	☐	2
	1	Medical admission ward	☐	3
	2	Surgical admission ward	☐	4
	3	Main ICU	☐	5
	4	NICU	☐	6
	5	Adult female medical wards	☐	7
	6	Adult female surgical wards	☐	8
	7	Adult male medical wards	☐	9
	8	Adult male surgical wards	☐	10

9	Paediatrics admission ward		11-12
10	Other specify _____		

6.0 Duration of admission

1	24 hours		
2	48 hours		13
3	72 hours		14-15
4	> 72 hours		
5	Other specify _____		

7.0 Co-morbidity conditions

1	HIV status		16
2	Chemotherapy		17
3	Steroid		18
4	Other specify		19-20

8.0 Risk factors

1	Dialysis		
2	Endoscopy		21
3	Intravenous line insertion		
4	Catherization		
5	Urethral dilatation		
6	Dental manipulation		
7	Parenteral feeding		

9.0 Where antibiotics given on admission/Empiric treatment?

1	Yes	2	No		22

10.0 If Yes to question 9.0, list antibiotics given on admission?

_____ | | 23-26

_____

_____

11.0 Where antibiotics given at on-set of fever? | | 27

1	Yes	2	No

12.0 If Yes to question 11.0. list antibiotics given?

_____ | | 28-31

_____

_____

13.0 When was the blood culture collected? *Multiple response*

1	On admission before antibiotics		32
2	On admission after antibiotics		33
3	48 hours after admission		34
4	Other specify _____		35-36

14.0 Organism identified *Multiple response*

_____		37
_____		38
_____		39
_____		40-41

15.0 Was empiric treatment given susceptible to organism isolated?

1	Yes		42
2	No		

16.0 Patient outcome on day 3?

1	Fever still above 38.5◦C		43
2	Fever at 37◦C or below		
3	Fever fluctuating		
4	Death		

END

Appendix D4: Community: Pregnant women's questionnaire

Interviewee: Pregnant women from the community

Questionnaire serial number | | | |

Risk factors associated with antimicrobial resistance in the community - pregnant women

The purpose of this research study is to gather information on the risk factors associated with antimicrobial resistance in healthy pregnant women in the community

You have been asked to participate in this research study and please note that by completing this questionnaire you are voluntarily agreeing to participate in this study.

You will remain anonymous and all information given will be treated as confidential.

Please mark the appropriate block with an X or write your answer on the space provided.

1.0 Name of interviewer _____

2.0 Date of interview dd/mm/yy | | | | | | | | |
Start time _____

3.0 Participant identification _____

Section A: Demographic characteristics

For official use

4.0 Age _____ | | 1

5.0 Marital status

1	Married		
2	Single		2
3	Separated/Divorced		
4	Widowed		

6.0 Highest level of education

1	None (did not attend school)		
2	Primary (grade 1-7)		3
3	Junior Secondary (grade 8-9)		
4	Senior Secondary (grade 10-12)		
5	College		
6	University		

7.0 Occupation

1	Self-employed		
2	Unemployed		4
3	Government/Salary worker		5-6
4	Allowance		
5	Others specify		

8.0 Monthly income Zambian kwacha
ZMK _____ | | 7

9.0 What is the occupation of your husband?

	Self-employed		8
	Unemployed		9-10

			Government/Salary worker				
			Allowance				
			Others specify				
10.0	Monthly income of the husband?						
	ZMK _____						11
11.0	Do you live in your own house or rented house?						
	1		Rented house				12
	2		Own house				
12.0	Location of household						
	1	Urban	2	Rural			13
13.0	If urban, is it						
	1	High density	3	Low density			14
	2	Medium density					
14.0	Is there livestock at your household?						
	1	Yes	2	No			15
15.0	If yes to question 14.0, which livestock?				*Multiple response*		
	1	Chickens					16
	2	Pigs					17
	3	Cattle					18
	4	Other specify					19-20
16.0	Any contact with livestock in the past one month?						
		Yes	2	No			21
17.0	If yes to question 16.0, how many times?						
	1	Once a week	4	Twice a week			22
	2	Once a month	5	Twice a month			23-24
	3	Other specify					
18.0	Do you eat chicken?						
	1	Yes	2	No			25
19.0	If yes to question 18.0, how many times a month?						
	1	Once a week	3	Twice a week			26
	2	Once a month	4	Other specify			27-28

20.0 What is the source of your chickens? *multiple response*

1	Own production	☐	29
2	Super market	☐	30
3	Family and friends	☐	31
4	Open market	☐	32
5	Other specify _____	☐☐	33-34

Section B: Pregnancy and Clinical details

21.0 Pregnancy gestation

1	1st trimester	☐	35
2	2nd trimester		
3	3rd trimester		

22.0 Parity

1	1st Pregnancy	☐	36
2	2nd Pregnancy		
3	3rd Pregnancy		
4	4rd Pregnancy		
5	4rd Pregnancy		
6	>5 Pregnancies		

23.0 Do you consume soil when expecting a baby?

☐	Yes	☐	37
☐	No		

24.0 Any medical problem or discomfort during this pregnancy?

☐	Yes	☐	38
☐	No		

25.0 If yes to question 24.0, what was the problem/symptom? *multiple response*

☐	Nausea		
☐	Abdominal cramp	☐	39
☐	Diarrhoea	☐	40
☐	Fever	☐	41
☐	Headache	☐	42
☐	General body malaise	☐	43
☐	Others specify	☐☐	44-45

26.0 If yes to question 25.0, did you seek any medical help?

	☐ Yes ☐ No	☐	46
27.0	Any antibiotics taken in this pregnancy? 1 Yes 2 No	☐	47
28.0	What was the source of the antibiotics? 1 Clinic 2 Hospital with a prescription from the clinician 3 Off the counter without a prescription 4 From family members 5 Other specify	☐ ☐	48 49-50
29.0	If yes to question 27.0, list antibiotics taken? _____ _____	☐☐☐☐	51-54
30.0	Did you complete the antibiotics course? ☐ Yes ☐ No	☐	55
31.0	If yes to question 30.0, how long did you take the antibiotics? 1 One day 2 Three days 3 Five days 4 Two days 5 Four days 6 Other specify	☐ ☐☐	56 57-58
32.0	If you did not take antibiotics, what did you take instead? _____ _____	☐☐☐☐	59-62

Section C: Knowledge around antibiotics

33.0	Do you know antibiotics? 1 Yes 2 No	☐	63
34.0	What is an antibiotic? _____ _____	☐☐☐☐	64-67

35.0 If yes to question 33.0, name at least two antibiotics and when does one use them? [ ][ ][ ][ ] 68-71

36.0 Where do you store your antibiotics? [ ] 72 [ ][ ] 73-74

1	Fridge
2	Cupboard
3	Open shelf cupboard
4	Other specify

37.0 Is it acceptable to use antibiotics given by friends/family members as long they were used to treat the same problem? [ ] 75

1	Yes	3	No
2	Sometimes	4	Not sure

38.0 Do you think antibiotics will always work when you take them to treat disease? [ ] 76

1	Yes
2	No
3	Sometimes
4	Not sure

39.0 Do you know what antibiotic resistance is? [ ] 77

1	Yes
2	No

40.0 If yes to question 39.0, define antibiotic resistance? [ ][ ][ ][ ] 78-81

41.0 What is the effect of antibiotic resistance on human health? [ ][ ][ ][ ] 82-85

42.0	Where do you get information on antibiotic resistance *Multiple response*		
	1 Hospitals		86
	2 Practitioners		87
	3 TV		88
	4 Internet		89
	5 Social Media		90
	6 Friends/Neighbours		91
	7 Others		92-93
	End time _____		
	Thank you for your time and contribution		

Appendix D5: Participant information sheet for patients with BSIs

Project title: Characterisation of antimicrobial resistant *E. coli*, *Enterococcus* and *Salmonella species* isolated from humans and poultry in Zambia

Invitation
We are doing a research study on medicines used to treat germs that make people sick. The study will help us understand whether the medicines used to treat the germs are still working or not and what could be causing them not to work.
You have been chosen as one of the study participants and I would like to ask you for some of your time to explain the work that we are doing and to request for you to be one of the study participants. As we discuss the information below, please feel free to ask any questions.

What is the Purpose and significance of the Study?
You may be aware that most of the antibiotics used to treat infections in humans are no longer working as they should; this is because of the misuse of antibiotics in humans and poultry. Some of the germs have developed ways to survive even through such treatments. This is what we call the germs are resistant to antibiotics.

For this study we are interested in the resistant germs that cause blood infections. The information gained from this study will be used to know if the antibiotics resistant germs causing blood infections are similar to those found in poultry and healthy individuals and the risk factors that contribute to the development of resistant germs.

Why have I been chosen?
We are inviting you to take part in the study because you have come to the hospital saying are not well and a blood sample was taken from you and sent to the laboratory.

How do I take part in this study?
Participation is voluntary. If you have agreed to take part you will keep this information sheet. You are free to withdraw at any time.

What does the study involve?
If you agree to take part in this study, we will ask a number of questions concerning you and your household. You may choose not to answer any question. We will also look at your hospital and laboratory records to complete the form.

If you do not join the study, you will continue to receive the treatment needed for this infection and you will not lose any health care services. You may choose to leave the study at any time you like.

This exercise will take about 15-20 minutes. The questions that will be asked are general and not personal. There should be no risks to you if you agree to take part in the study.

How the collected information will be managed?
All the information, including your identity will be kept in strict confidence by the research team. The information that will be collected in this study will be used in a general manner and not necessarily linked to particular individuals. In each questionnaire you will be provided a unique code to ensure that your identity is protected.

What will happen to the results of the study?
If you are in the study, the information you give us will help us understand why the medicine is working or not. When we are finished with this study, we will write a report about what was learned. This report will not include your name or that you were in the study
What should I do if I have complaints on how the methods are conducted?
If you encounter any issues or have concern, please contact the Local Principal Investigator (LPI) or the ERES CONVERGE IRB (see below the contact details)
Thank you for reading this information.

Local Principal Investigator Name: Dr. Kaunda Yamba Phone number: +260 977397402 Address: University Teaching Hospital, Pathology and Microbiology, , Nationalist road Email address: kaundayamba@gmail.com	THE ERES CONVERGE IRB Phone number: +260955155633/4 Address: 33 Joseph Mwilwa road, Rhodes Park, Lusaka E-mail: eresconverge@yahoo.co.uk

Appendix D6: Participant information sheet for pregnant women and mothers to children ≤ 5 years (Community)

Project title: Characterisation of antimicrobial resistant *E. coli*, *Enterococcus* and *Salmonella species* isolated from humans and poultry in Zambia

Invitation
We are doing a research study on medicines used to treat germs that make people sick. The study will help us understand whether the medicines used to treat the germs are still working or not and what could be causing them not to work.
You have been chosen as one of the study participants and I would like to ask you for some of your time to explain the work that we are doing and to request for you to be one of the study participants. As we discuss the information below, please feel free to ask any questions.

What is the Purpose and significance of the Study?
You may be aware that most of the antibiotics used to treat infections in humans are no longer working as they should; this is because of the misuse of antibiotics in humans and poultry. Some of the germs have developed ways to survive even through such treatments. This is what we call the germs are resistant to antibiotics.

For this study we are interested in the germs that are normally found in our gastrointestinal tract as normal flora but can also cause disease when our defence against infections is low. The information gained from this study will be used to know if the germs found in our gastrointestinal tract are similar to the antibiotics resistant germs causing blood infections and those found in poultry and healthy individuals and the risk factors that contribute to the development of resistant germs.

Why have I been chosen?
We are inviting you to take part in the study because you are well and healthy and are coming from the community of interest.

How do I take part in this study?
Participation is voluntary. If you have agreed to take part you will keep this information sheet. You are free to withdraw at any time.
What does the study involve?
If you agree to take part in this study, we will ask a number of questions concerning you and your household and collect a rectal swab or ask for a stool sample. There will be minimal discomfort when collecting the rectal swab. You may choose not to answer any question or participate.

If you do not join the study, you will continue to receive the health care services. You may choose to leave the study at any time you like.

This exercise will take about 15-20 minutes. The questions that will be asked are general and not personal.

How the collected information will be managed?
All the information, including your identity will be kept in strict confidence by the research team. The information that will be collected in this study will be used in a general manner and not necessarily linked to particular individuals. In each questionnaire, you will be provided a unique code to ensure that your identity is protected.

What will happen to the results of the study?
If you are in the study, the information you give us will help us understand why the medicine is working or not. When we are finished with this study, we will write a report about what was learned. This report will not include your name or that you were in the study
What should I do if I have complaints on how the methods are conducted?
If you encounter any issues or have concern, please contact the Local Principal Investigator (LPI) or the ERES CONVERGE IRB (see below the contact details)

Thank you for reading this information.

Local Principal Investigator Name: Dr. Kaunda Yamba Phone number: +260 977397402 Address: University Teaching Hospital, Pathology and Microbiology,Nationalist road Email address: kaundayamba@gmail.com	THE ERES CONVERGE IRB Phone number: +260955155633/4 Address: 33 Joseph Mwilwa road, Rhodes Park, Lusaka E-mail: eresconverge@yahoo.co.uk

Appendix D7: Informed Consent form for participant ≥ 18 years with blood stream infection and healthy pregnant women from the community

Project title: Characterisation of antimicrobial resistant *E. coli* and *Enterococcus species* isolated from humans and poultry in Zambia

Have you read the project information (or has it been read to you)?	Yes / No
Has somebody else explained this project to you?	Yes / No
Do you understand what this project is about?	Yes / No
Have you had the opportunity to ask all your questions?	Yes / No
Have your questions been answered in a way you understand?	Yes / No
Do you understand it is OK to stop taking part in this research at any time?	Yes / No
Are you willing to voluntarily take part in the project?	Yes / No

If any answers are 'No' or you do not want to take part, do not sign the form

If you DO want to take part, you can write your name below and sign the form. Keep in mind that:

- Your participation is voluntary.
- Your decision whether or not to participate will not affect your treatment and care at the University Teaching Hospital
- If you have any questions about the study, please feel free to contact [local researcher name: Dr. Kaunda Yamba on +260 977397402 or kaundayamba@gmail.com or University Teaching Hospital, Pathology and Microbiology, Nationalist road. All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously if you wish, ERES CONVERGE IRB office at

33 Joseph Mwilwa road in Rhodes Park, Lusaka. The ERES CONVERGE IRB office contact numbers are +260955155633/4

- Your signature indicates that you have read and understood the information provided above (in the information sheet), that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty and that you will receive a copy of this form.

Your name: _____
Signature: _____
Place and date: _____

Parent/guardian's consent for participants from 15 - 18 years of age: I confirm that I have read and understood the above and freely consent my daughter/niece participating in this study.

Your name: _____
Signature: _____
Place and date: _____

The researcher who explained this project to you:
Name: _____
Signature: _____
Place and date: _____

Appendix D8: Assent form for participants aged 7-17 years with bloodstream infections

Project title: Characterisation of antimicrobial resistant *E. coli* and *Enterococcus species* isolated from humans and poultry in Zambia

Have you read the project information (or has it been read to you)?	Yes / No
Has somebody else explained this project to you?	Yes / No
Do you understand what this project is about?	Yes / No
Have you had the opportunity to ask all your questions?	Yes / No
Have your questions been answered in a way you understand?	Yes / No
Do you understand it is OK to stop taking part in this research at any time?	Yes / No
Are you willing to voluntarily take part in the project?	Yes / No

If any answers are 'No' or you do not want to take part, do not sign the form

If you DO want to take part, you can write your name below and sign the form. Keep in mind that:

- Your participation is voluntary.
- Your decision whether or not to participate will not affect your treatment and care at the University Teaching Hospital
- If you have any questions about the study, please feel free to contact [local principal investigator: Dr. Kaunda Yamba on +260 977397402 or kaundayamba@gmail.com or University Teaching Hospital, Pathology and Microbiology, Nationalist road. All research on human volunteers is reviewed by a committee that works to protect your

rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously if you wish, ERES CONVERGE IRB office at 33 Joseph Mwilwa road in Rhodes Park, Lusaka. The ERES CONVERGE IRB office contact numbers are +260955155633/4

- Your signature indicates that you have read and understood the information provided above (in the information sheet), that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty and that you will receive a copy of this form.

Your name: _____
Signature: _____
Place and date: _____

Parent/guardian's consent for participants from 7 - 17 years of age: I confirm that I have read and understood the above and freely consent my daughter/son/nephew/niece participating in this study.
Your name: _____
Signature: _____
Place and date: _____

The researcher who explained this project to you:
Name: _____
Signature: _____
Place and date: _____

Appendix E: Ethical clearance

ERES CONVERGE

Rhodes Park, Lusaka - Zambia
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 977 493220
Email: eresconvergeltd@gmail.com

I.R.B. No. 00005948
F.W.A. No. 00011697

11th November, 2019

Ref. No. 2019-Aug-017

The Principal Investigator
Dr. Kaunda Yamba
P.O. Box 32379,
LUSAKA.

Dear Dr. Yamba,

RE: CHARACTERISATION OF ANTIMICROBIAL RESISTANT E. COLI, ENTEROCOCCUS AND SALMONELLA SPECIES ISOLATED FROM HUMANS AND POULTRY IN ZAMBIA.

Reference is made to your protocol dated 7th November, 2019. The IRB resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	**Ordinary**	Approval No. **2019-Aug-017**
Approval and Expiry Date	Approval Date: 11th November, 2019	Expiry Date: 10th November, 2020
Protocol Version and Date	Version - Nil.	10th November, 2020
Information Sheet, Consent Forms and Dates	• English, Bemba, Nyanja.	10th November, 2020
Consent form ID and Date	Version - Nil	10th November, 2020
Recruitment Materials	Nil	10th November, 2020
Other Study Documents	Questionnaires.	10th November, 2020
Number of participants approved for study	855	10th November, 2020

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- A reprint of this letter shall be done at a fee.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. Jason Mwanza
Dip. Clin. Med. Sc., BA., M.Soc., PhD
CHAIRPERSON

Appendix F: National Health Research Authority approval letter

NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@gmail.com | www.nhra.org.zm

Ref No:..... **Date: 31st July, 2020**

The Principal Investigator
Dr. Kaunda Yamba
University of Zambia
P.O Box 32379
LUSAKA.

Dear Dr. Yamba,

Re: Request for Authority to Transfer Biological Materials

The National Health Research Ethics Board (NHREB) is in receipt of your request for permission to export biological material under the study entitled **"Characterization of antimicrobial resistant Escherichia coli, Enterococcus and Salmonella species isolated from humans and poultry in Zambia."**

I wish to inform you that following submission of your request to the Board, the review of the same and in view of the ethical clearance, the transfer of Biological Material has been **granted.**

Please note that the Board is mandated to inspect the sites where the samples will be stored and can choose to inspect any site at any given time.

Yours sincerely,

Prof. Patrick Musonda
Chairperson
National Health Research Ethics Board

All correspondences should be addressed to the Director/CEO National Health Research Authority

Yours sincerely,

Prof. Patrick Musonda
Chairperson
National Health Research Ethics Board

All correspondences should be addressed to the Director/CEO National Health Research Authority

Appendix G: Published articles

Appendix G1: Published paper 1

IJID Regions 3 (2022) 248–255

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

Antimicrobial susceptibility and genomic profiling of *Salmonella enterica* from bloodstream infections at a tertiary referral hospital in Lusaka, Zambia, 2018–2019

Kaunda Yamba[1,2,*], Christine Kapesa[1], Evans Mpabalwani[3,4], Lottie Hachaambwa[5,6], Anthony Marius Smith[7,14], Andrea Liezl Young[7], David Gally[8], Geoffrey Mainda[9], Mercy Mukuma[10], Mulemba Tillika Samutela[11,12], Annie Kalonda[2,11], James Mwansa[13], John Bwalya Muma[2]

[1] *University Teaching Hospitals, Pathology and Microbiology Laboratory, Lusaka, Zambia*
[2] *University of Zambia, School of Veterinary Medicine, Department of Disease Control, Lusaka, Zambia*
[3] *University of Zambia, School of Medicine, Department of Paediatrics and Child Health*
[4] *University Teaching Hospitals, Children's Hospital, Lusaka, Zambia*
[5] *University of Maryland, School of Medicine, Centre for International Health, Education, and Biosecurity, Baltimore, MD, USA*
[6] *University Teaching Hospital, Infectious Diseases Unit, Department of Internal Medicine, Lusaka, Zambia*
[7] *National Institute for Communicable Diseases, Centre for Enteric Diseases, Johannesburg, South Africa*
[8] *University of Edinburgh, Roslin Institute, Division of Infection and Immunity, Edinburgh, Scotland*
[9] *Ministry of Fisheries and Livestock Department of Veterinary Services, Public Health Unit, Lusaka, Zambia*
[10] *University of Zambia, Department of Food Science and Nutrition, School of Agricultural Sciences, Lusaka, Zambia*
[11] *Department of Biomedical Sciences, School of Health Sciences, Lusaka, Zambia*
[12] *University of Zambia, School of Veterinary Medicine, Department of Paraclinical Studies, Lusaka, Zambia*
[13] *Lusaka Apex Medical University, Lusaka, Zambia*
[14] *University of Pretoria, School of Medicine, Faculty of Health Sciences, Department of Medical Microbiology, Pretoria, South Africa*

ARTICLE INFO

Keywords:
Salmonella enterica
antimicrobial resistance
genetic diversity
serotype
Zambia

ABSTRACT

Objectives: This study investigated antimicrobial susceptibility and genomic profiling of *S. enterica* isolated from bloodstream infections at a tertiary referral hospital in Lusaka, Zambia, 2018–2019.
Method: This was a prospective hospital-based study involving routine blood culture samples submitted to the microbiology laboratory at the University Teaching Hospital. Identification of *S. enterica* and determination of antimicrobial susceptibility profiles was achieved through conventional and automated methods. Whole-genome sequencing (WGS) was conducted, and the sequence data outputs were processed for species identification, serotype determination, multilocus sequence typing (MLST) profile determination, identification of antimicrobial resistance determinants, and phylogeny.
Results: Seventy-six *Salmonella enterica* were isolated and 64 isolates underwent WGS. *Salmonella* Typhi (72%) was the most prevalent serotype. Notable was the occurrence of invasive non-typhoidal *Salmonella* Typhimurium ST313 (3%), resistance to cephalosporins (4%) and ciprofloxacin (5%), multidrug resistance (46%), and reduced susceptibility to ciprofloxacin (30%) and imipenem (3%). Phylogenetic cluster analysis showed multiple *Salmonella* serovars with a wide range of genetic diversity.
Conclusion: The genetic diversity of *Salmonella* Typhi, high prevalence of multidrug resistance, and the emergence of ciprofloxacin and cephalosporin resistance warrants improved hygiene and water and sanitation provision, continued surveillance to apprise antibiograms and inform policy, and the introduction of the typhoid conjugate vaccine.

Introduction

Salmonella enterica is one of the leading causes of community-acquired bloodstream infections in low- and middle-income countries (LMIC). Invasive *Salmonella* infections (typhoidal and non-typhoidal) cause a considerable burden of illness worldwide, estimated at 3.4 million cases and over 600 000 deaths annually, especially in resource-limited settings (Kariuki et al., 2015). *Salmonella* Typhi (*S.* Typhi) is the most prevalent in crowded, underprivileged populations with poor sanitation and lack of access to safe, clean water (Crump et al., 2015; Parry et al., 2015). It has contributed significantly to the global pub-

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Appendix G2: Published paper 2

PLOS GLOBAL PUBLIC HEALTH

RESEARCH ARTICLE

Phenotypic and genotypic antibiotic susceptibility profiles of Gram-negative bacteria isolated from bloodstream infections at a referral hospital, Lusaka, Zambia

Kaunda Yamba[1,2]*, Chileshe Lukwesa-Musyani[1], Mulemba Tillika Samutela[3,4], Christine Kapesa[1], Mudenda Bernard Hang'ombe[4], Evans Mpabalwani[5], Lottie Hachaambwa[6], Sombo Fwoloshi[6], Raphael Chanda[1], Mirfin Mpundu[7], Glory Kashweka[1], Ruth Nakazwe[1], Steward Mudenda[2,8], John Bwalya Muma[2]

1 Department of Pathology & Microbiology Laboratory, University Teaching Hospitals, Lusaka, Zambia, **2** Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia, **3** Department of Biomedical Sciences, School of Health Sciences, University of Zambia Lusaka, Zambia, **4** Department of Paraclinical Studies, University of Zambia, School of Veterinary Medicine, Lusaka, Zambia, **5** Department of Paediatrics & Child Health, School of Medicine, University of Zambia, Lusaka, Zambia, **6** Department of Internal Medicine, Infectious Diseases Unit, University Teaching Hospital, Lusaka, Zambia, **7** ReAct Africa, Honnington Close, Greystone Park, Harare, Zimbabwe, **8** Department of Pharmacy, School of Health Sciences, University of Zambia, Lusaka, Zambia

* kaundayamba@gmail.com

OPEN ACCESS

Citation: Yamba K, Lukwesa-Musyani C, Samutela MT, Kapesa C, Hang'ombe MB, Mpabalwani E, et al. (2023) Phenotypic and genotypic antibiotic susceptibility profiles of Gram-negative bacteria isolated from bloodstream infections at a referral hospital, Lusaka, Zambia. PLOS Glob Public Health 3(1): e0001414. https://doi.org/10.1371/journal.pgph.0001414

Editor: Rehab Hosny El-Sokkary, Zagazig University, Faculty of Medicine, EGYPT

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Data Availability Statement: All data are in the manuscript and/or supporting information files.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Bloodstream infections (BSI) caused by antimicrobial-resistant (AMR) Gram-negative bacteria (GNB) are a significant cause of morbidity and mortality. Third-generation cephalosporins (3GCs) have been used as empiric treatment for BSI and other invasive infections for years; however, their overuse could promote the emergence of extended-spectrum beta-lactamases (ESBLs). Thus, this study aimed to determine the epidemiological, clinical and microbiological features and the effects of antimicrobial resistance on the outcomes of BSIs at a referral hospital in Lusaka, Zambia. This was a six-month prospective facility-based study undertaken at a referral hospital in Lusaka, Zambia. As part of the routine diagnosis and patient care, blood samples for bacteriological culture were collected from patients presenting with fever and processed for pathogen identification and antimicrobial susceptibility testing using the VITEK 2 Compact instrument. ESBLs and plasmid-mediated quinolone resistance (PMQR) associated genes were determined using the polymerase chain reaction method. Patient information was collected using a structured data collection sheet and entered in CSpro 7.6. Data were analysed in WHOnet and STATA version 14. A total of 88 GNB were isolated, of which 76% were Enterobacterales, 14% *Acinetobacter baumannii* and 8% *Pseudomonas aeruginosa*. Resistance to third and fourth-generation cephalosporins was 75% and 32%, respectively. Noteworthy was the high prevalence (68%) of inappropriate empirical treatment, carbapenem resistance (7%), multi-drug resistance (83%) and ESBL-producers (76%). In comparison to *E. coli* as a causative agent of BSI, the odds of death were significantly higher among patients infected with *Acinetobacter baumannii* (OR = 3.8). The odds of death were also higher in patients that received 3GCs as empiric