PHENOTYPIC AND GENOTYPIC ANTIMICROBIAL RESISTANCE PROFILES OF ARCHIVED *KLEBSIELLA* ISOLATES FROM CHILDREN UNDER FIVE YEARS OF AGE IN KISII AND HOMABAY COUNTY HOSPITALS

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Phenotypic and Genotypic Antimicrobial Resistance Profiles of Archived Klebsiella Isolates from Children Under Five Years of Age in Kisii and Homabay County Hospitals

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this work to God Almighty who has been my source of strength throughout my study period. This study is dedicated to my family for their great financial support and source of inspiration and encouragement during the study period.

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ABBREVIATIONS AND ACRONYMS

AMC	Amoxicillin Clavulanic
AMR	Antimicrobial Resistance
API	Analytical Profile Index
AZM	Azithromycin
BSI	Blood Stream Infections
C	Chloramphenicol
CA	Community Acquired
CAUTIS	Catheter Associated Urinary Tract Infections
CAZ / CLAV	Ceftazidime / Clavulanate
CAZ	Ceftazidime
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CN	Gentamicin
CPS	Capsular polysaccharide
CRE	Carbapenem Resistance Bactericeae
CRF	Case Report Form
CRO	Ceftriaxone
CTX / CLAV	Cefotaxime / Clavulanate

CTX	Cefotaxime
ECDC	Economic Cooperation among Developing Countries
ESBL	Extended beta Lactamases
FOX	Cefoxitin
HA	Hospital Acquired
HCTRH	Homabay County Teaching and Referral Hospital
ICU	Intensive Care Unit
IMI I	Imipenem Hydrolyzing Beta-Lactamase
IMP	Imipenem
KEMRI	Kenya Medical Research Institute.
KPC	*Klebsiella Pneumoniae* Carbapenemase
KTRH	Kisii Teaching Referral Hospital.
MBL	Metallo Beta-Lactamase
MDR	Multiple Drug Resistance
MH	Mueller Hinton
NDM	New Delhi Metallo beta-lactamase
OXA	Oxacillinase
PCR	Polymerase Chain reaction
PMQR	Plasmid-Mediated Quinolone Resistance

QC	Quality Control
SERU	Scientific Ethics and Review Unit
SHV 1	Sulfhydryl Variable 1
SOP	Standard Operation Procedure
SPM	Sao Paulo Metallo beta-lactamase
SSA	Sub Saharan Africa
TEM	Temoneira
UK	United Kingdom
UNICEF	United Nations Children's Fund
USA	United States of America
UTI	Urinary tract infections
UV	Ultraviolet
VAP	Ventilator associated Pneumonia
VIM	Verona Integron Encoded Metallo Beta-Lactamase
VP	Voges Proskauer
WHO	World Health Organization

ABSTRACT

The genus *Klebsiella* belongs to the *Enterobacteriaceae* family and comprises Gram-negative, opportunistic, non-motile pathogen with a mucoid appearance. The gastrointestinal tract serves as a reservoir for transfer of resistance genes and is often the potential source for infections. Multidrug resistance strains of *Klebsiella* cause severe nosocomial and community-acquired infections that are difficult to eradicate using available antibiotics**.** The increase in the incidence of extended spectrum β lactamases (ESBL) producing *Klebsiella spp*, has become a serious problem worldwide, because of their antibiotic resistance. The emergence and spread of β-lactamase-producing *Klebsiella spp* has been associated with a substantial healthcare burden resulting from therapeutic failures. Multidrug resistant *Klebsiella* strains are resistant to extended-spectrum beta-lactam antibiotics, aminoglycosides, and fluoroquinolones. The aim of this study was to determine phenotypic and genotypic antimicrobial resistance profiles of *Klebsiella species* from children under five years of age in Kisii and Homabay County hospitals (Western Kenya). Although AMR bacterial infections pose a disproportionate public health challenge in sub-Saharan Africa, epidemiological data are scarce in Kenya. This was a cross-sectional study involving the use of 245 archived *Klebsiella* isolates. Systematic random sampling was used, and Excel formula randomization was used to generate random numbers to have a representative sample size from the two study sites**.** Antimicrobial resistance profiling was conducted to determine phenotypic resistance patterns and ESBLs, and producing *Klebsiella Pneumoniae and Klebsiella oxytoca* using antimicrobial susceptibility tests methods described by Kirby Bauer, ESBL resistance genes were analyzed using conventional PCR. Descriptive analyses were used to characterize phenotypic AMR and carriage of β-lactamase-producing genes. The modified Poisson regression models were used to assess correlates of phenotypic AMR. The prevalence of β-lactamase carriage among *Klebsiella* spp. isolates at hospital discharge was 62.8% (154/245). The study reported more than 58.2% *Klebsiella* spp. isolates were resistant to a third-generation cephalosporin; more specifically, 154 (62.6%) were resistant to ceftriaxone, 148 (60.0%) were resistant to cefotaxime and 127 (52.0%) were resistant to ceftazidime. Antibiotic use during hospitalization (adjusted prevalence ratio [aPR] =4.51; 95%CI: 1.79-11.4, p<0.001), longer duration of hospitalization (aPR=1.42; 95%CI: 1.14-1.77, p<0.002), and access to treated water (aPR=1.38; 95%CI: 1.12-1.71, p<0.003), were significant predictors of phenotypically determined β-lactamase. All the 154 phenotypically determined β-lactamase-producing *Klebsiella* spp. isolates had at least one genetic marker of β-lactam/third-generation cephalosporin resistance. The most prevalent genes were CTX-M (92.2%; 95%CI: 86.8–95.9) and SHV (92.2%; 95%CI: 86.8–95.9) followed by TEM (57.1%; 95%CI: 48.9–65.1) and OXA (31.2%; 95%CI: 24.0–39.1), respectively. Carriage of β-lactamase producing *Klebsiella* spp in stool is common among children discharged from hospital in western Kenya and is associated with longer duration of hospitalization, antibiotic use, and surprisingly access to treated water. These findings emphasize the need for continued surveillance of antimicrobial susceptibility patterns to inform the development and implementation of appropriate treatment guidelines. In addition, the study recommends measures beyond antimicrobial stewardship and infection control within hospitals, improved

sanitation, and access to safe drinking water to mitigate the spread of β-lactamase-producing *Klebsiella* pathogens.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Klebsiella species are important opportunistic pathogen and a frequent cause of nosocomial and community-acquired infections in immunocompromised individuals (Martin & Bachman, 2018; Wanyiri *et al*., 2013). Gastrointestinal colonization frequently precedes infections with *Klebsiella spp.*, and the gastrointestinal tract is believed to be the most important reservoir for transmission of resistance genes to other *Enterobacteriaceae* strains (Gorrie *et al*., 2017).

Multidrug resistance (MDR) is of significant concern in developing countries due to the widespread use of antimicrobial agents, availability of counterfeit or substandard drugs, and poor infection control measures (WHO, 2015). The scarcity of reliable up to date information, particularly in sub-Saharan Africa, may further limit epidemiological surveillance and effective stewardship efforts (WHO, 2018). *Klebsiella sp*ecies are one of the multidrug-resistant pathogens identified as a threat to human health by the World Health Organization, US Centers for Disease Control and Prevention and the UK Department of Health (Prestinaci *et al.*, 2015). Epidemic and endemic nosocomial infections caused by *Klebsiella* species are the leading cause of morbidity and mortality worldwide.

Resistance to a commonly used class of antibiotics, beta-lactams, as measured by extended spectrum beta-lactamases (ESBLs), is associated with a 50% higher case fatality rates (Li *et al*., 2023). ESBLs are of public health concern because they not only suggest resistance to an entire class of antibiotics but can facilitate selection for resistance determinants in other antimicrobial classes, including aminoglycosides and fluoroquinolones (Ruppé *et al*., 2015). Nosocomial isolates are frequently resistant to numerous antibiotics as a result of the acquisition of multidrug resistance (MDR) plasmids.

Klebsiella is facultative anaerobic Gram-negative rod that are usually encapsulated and non-motile. They are indole and ornithine decarboxylase negative, ferment lactose, and have a positive Voges-Proskauer reaction. *Klebsiella pneumoniae* and other *Klebsiella spp* are the common intestinal commensals with a potential to cause extra intestinal infections in severely ill patients and diarrhea in HIV/AIDS patients (Martin & Bachman, 2018; Wanyiri *et al.*, 2013). *Klebsiella spp* acquire, accumulate, and transfer myriad antimicrobial resistance determinants and represent a significant reservoir for resistance within the gut, and may increase the risk of resistant infections in hospital environments (Taitt *et al*., 2017).

According to Kenyan guidelines (Ministry of Health, 2016), initial treatments for suspected severe bacterial infections involve the use of β - lactam antibiotics (penicillin or ampicillin) along with the aminoglycoside gentamicin. Subsequently, intravenous ceftriaxone, a cephalosporin antibiotic, is recommended.

Nosocomial spread of *Klebsiella spp* are prevalent, especially in crowded hospital environments where children are frequently exposed to antibiotics. These hospital settings serve as a particularly significant reservoir setting for antibiotic resistant *Klebsiella spp.* The hospital environment represents a reservoir of potential pathogens, continuously spread by healthcare personnel, visitors and hospitalized patients. Advocacy for proper sanitation is essential to curb AMR in hospital settings. Children returning home from hospital who develop infection with AMR *Klebsiella* may have limited treatment options and may spread these AMR bacteria within households and the community. Although AMR bacterial infections pose a disproportionate public health challenge in Sub-Saharan Africa (SSA), epidemiological data are scarce. The current AMR study was nested within a clinical trial investigating the impact of azithromycin for prevention of morbidity and mortality in the six months following discharge from hospitals in western Kenya (Pavlinac *et a*l., 2021). This study sought to describe the proportion of phenotypic resistance to commonly used antibiotics, characterize β-lactamase genes among the phenotypically resistant isolates and assess the correlates of ESBL-producing *Klebsiella* isolates among children discharged from hospital in western Kenya in Homabay and Kisii County Teaching and referral hospitals.

1.2 Statement of the Problem

Antimicrobial resistance is now a global problem, and resistance in *Enterobacteriaceae,* specifically *Klebsiella pneumoniae*, is a critical threat to human health (WHO, 2018). Antimicrobial resistance (AMR) is a leading cause of death worldwide, with the highest burden reported in sub-Saharan Africa (SSA) where approximately 1.2 million deaths in 2019 were attributed to bacterial AMR (Kariuki *et al*., 2022; Murray *et al*., 2022). AMR contributes to significant mortality in children under five years of age in SSA accounting for 128,000 deaths annually (Kowalski *et al.*, 2024). Nearly all AMR deaths related to under five years of age occur in low- or middle-income countries (LMICs) (Romandini *et al*., 2021) where AMR is associated with a substantial healthcare burden resulting from empirical antimicrobial treatment failure (Godman *et al.,* 2022). The increase in multidrug resistance has occurred concomitantly with a drastic reduction of discovery and development of new antimicrobial agents, making these infections more difficult to control. *Klebsiella spp.* are one of the most important gram-negative bacteria associated with AMR in SSA and in Kenya (Henson *et al*., 2017; Taitt *et al*., 2017). The burden associated with MDR *Klebsiella* infections includes failure of treatment strategy, extended hospital stay, increased medical costs and increased mortality and morbidity rate (Murray *et al*., 2022). AMR genes can easily be disseminated through mobile genetic elements (MGEs) from commensal to pathogenic bacteria and vice versa. This is due to their ability to easily acquire and share MGEs among members of the Enterobacteriaceae group (Tao *et al*., 2022).

Kisii and Homabay County referral hospitals are located in Western Kenya. Basic services such as insufficient drugs, inadequate health workers and infrastructure for providing adequate sanitation and clean water are insufficient in both health facilities contributing to poor health outcomes leading to significant mortality in children under five years of age. In addition high disease burden in malaria, diarrhea diseases, HIV/AIDS and non-communicable diseases and systemic weakness in health care drives increased mortality among children. There is limited data on antibiotic resistance associated with *Klebsiella spp.* infections as this is not routinely practiced in Kenya, yet such data is important in patient clinical care management.

1.3 Justification of the Study

Determining and understanding antimicrobial resistance profiles in Kenya will improve global health in an era when antibiotic choices are increasingly limited because of high prevalence resistance in Gram negative bacteria. Genetically characterizing the ESBL resistance genes and assessing the correlates of ESBL producing *Klebsiella* will enable an improved understanding of the mechanisms of resistance, can give clues to emerging phenotypic resistance and risk factors associated with ESBL carriage.

Few studies in Kenya have been done with main focus on *Klebsiella* infection and antibiotic resistance in all patient ages (Henson *et al*., 2017; Taitt *et al*., 2017; Wairimu *et al*., 2021). However, no study has focused on phenotypic, genotypic resistance and risk factors associated with carriage in *Klebsiella spp* in children under five years being discharged from the hospital.

Data generated from this study will provide knowledge to researchers on the transmission dynamics of *Klebsiella spp.* Understanding on antibiotic resistance patterns and establishment of adequate infectious control programs are essential to guide therapy to reduce longer hospital stays and increased cost of treatment on resistant ESBL Klebsiella infections. The identification of ESBL phenotypes and genotypes necessitates the importance to investigate the circulating ESBL genes as they play a major role in reduced antibiotic susceptibility which could lead to treatment failure and significant mortality to children under five years of age.

1.4 Research Questions

1. What is the prevalence of phenotypic resistance in *Klebsiella spp* from children below five years of age discharged from Kisii and Homabay county hospitals?
2. What phenotypes and genotypes of ESBLs are present in Klebsiella *spp* from children below five years of age discharged from Kisii and Homabay county hospitals?
3. What are the resistance genes associated with ESBL resistance?
4. What are the risk factors associated with ESBL Carriage?

1.5 Objectives

1.5.1 General Objective

To determine phenotypic and genotypic antimicrobial resistance (AMR) profiles of *Klebsiella* species from children under five years of age discharged from hospital in Kisii and Homabay County Hospitals.

1.5.2 Specific Objectives

1. To determine the prevalence of antimicrobial resistance phenotypes (Antibiotic AST profiling) in *Klebsiella spp* in children under five years of age discharged from Kisii and Homabay county hospitals.
2. To determine antimicrobial resistance phenotypes of ESBLs producing *Klebsiella spp* in children under five years of age discharged from Kisii and Homabay county hospitals.
3. To characterize resistance genes associated with ESBL resistance in *Klebsiella Spp* using conventional PCR molecular technique in children under five years of age discharged from Kisii and Homabay county hospitals
4. To determine risk factors associated with ESBL carriage in children under five years of age discharged from Kisii and Homabay county hospitals

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of *Klebsiella*

Klebsiella is a genus of Gram negative rod shaped bacteria in the family *Enterobacteriaceae*. *Klebsiella* is named after German-Swiss microbiologist Edwin Klebs, a 19th century German microbiologist. *Klebsiella* organisms occur in soil and water and on plants, and some strains are considered a part of the normal flora of the human gastrointestinal tract. *Klebsiella* is a genus of Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide capsule (Abbas *et al*., 2024). This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms (Lenchenko *et al*., 2020). Members of the *Klebsiella* genus typically express 2 types of antigens on their cell surface. The first is a lipopolysaccharide (O antigen); the other is a capsular polysaccharide (K antigen) (Choi *et al*., 2020). Both of these antigens contribute to pathogenicity. The structural variability of these antigens forms the basis for classification into various serotypes. The virulence of all serotypes appears to be similar. Three species in the genus *Klebsiella* are associated with illness in humans: *Klebsiella pneumoniae, Klebsiella oxytoca*, and *Klebsiella granulomatis*. In recent years, *Klebsiella spp* has become important pathogens in nosocomial infections (Nordmann *et al*., 2009).

2.2 Epidemiology of *Klebsiella*

Klebsiella spp are found in a wide range of diverse environments including mucosal membranes of humans where they colonize the gastrointestinal tract, pharynx, and the skin among other sites. *Klebsiella spp.* are a major cause of nosocomial infections and a common pathogen in community-acquired infections, bacteremia, and pneumonia. *K. pneumoniae* commonly colonizes humans, with prevalence ranging from 1% to 19% in the nasopharynx and 5% to 38% in the gastrointestinal tract (Martin & Bachman, 2018). The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel.

Klebsiella spp. primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic obstructive pulmonary disease. It is estimated that *Klebsiella spp*. cause 8% of all nosocomial bacterial infections in the United States and in Europe (Ahmad & Abulhamd, 2015). Data is essential to measure the extent of *Klebsiella* infection problems; unfortunately, they remain scarce in SSA, mainly due to limited access to microbiology laboratories and the lack of an AMR surveillance network. *Klebsiella* is responsible for a significant proportion of hospital-acquired infections including septicemia, urinary tract infections, pneumonia, and soft tissue infections especially in the immunocompromised hosts such as neonates. In Western countries, it is estimated that approximately 3% to 5% of all community-acquired pneumonia is related to an infection caused by *K. pneumoniae*, but in developing countries such as Africa, it can account for approximately 15% of all cases of pneumonia (Ashurst & Dawson, 2024). Overall, *K. pneumoniae* accounts for approximately 11.8% of all hospital-acquired pneumonia in the world. In those who develop pneumonia while on a ventilator 8% to 12% are caused by *K. pneumoniae*, while only 7% occur in those patients who are not ventilated. Mortality ranges from 50% to 100% in patients with alcoholism and septicemia (Mim *et al.*, 2025), Host factors that predispose to colonization and infection are as follows: Admission to an intensive care ward, prolonged use of invasive devices, poor infection control strategies, immunocompromised individuals, especially those with alcoholism or diabetes and extensive use of broad-spectrum antibiotics. Bacteria enter the host either by direct inoculation or by following oropharyngeal aspiration.

2.3 Diseases Caused by *Klebsiella*

Nosocomial *Klebsiella* infections are caused mainly by *Klebsiella pneumoniae*, the most medically important species of the genus. To a much lesser degree, *K. oxytoca* has been isolated from human clinical specimens. *Klebsiella* accounts for 6 to 17% of all nosocomial urinary tract infections (UTI) and shows an even higher incidence in specific groups of patients at risk, e.g., patients with neuropathic bladders or diabetes mellitus (Vuotto *et al*., 2014).

K. pneumoniae is the most frequent cause of pneumonia acquired in healthcare settings, accounting for 10% of all bacterial infections acquired in hospitals (Guo *et al*., 2016). Pneumonia is the leading cause of hospitalizations and deaths among children under the age of 5 years worldwide and is associated with significant mortality (Ferdous *et al*., 2018; Marangu & Zar, 2019; von Mollendorf *et al*., 2022). The bacterium typically colonizes human mucosal surfaces of the oropharynx and gastrointestinal (GI) tract. Once the bacterium enters the body, it can display high degrees of virulence and antibiotic resistance. The polysaccharide capsule of the organism is the most important virulence factor and allows the bacteria to evade opsonophagocytosis and serum killing by the host organism.

K. pneumoniae is one of the bacteria that cause healthcare-associated urinary tract infections. It is common in patients who have catheters and people living with kidney disease. *K. pneumoniae* colonizes the urinary tract leading to symptoms such as urgency, dysuria, hesitancy, low back pain, and suprapubic discomfort (Paczosa & Mecsas, 2016). Systemic symptoms such as fever and chills are usually indicative of a concomitant pyelonephritis or prostatitis. The treatment of *K. pneumoniae* UTIs has become increasingly difficult due to the bacterium's resistance to many first-line antibiotics, including fluoroquinolones and β-lactams (Kherroubi *et al*., 2024). The emergence of extended-spectrum β-lactamase (ESBL)-producing strains, which confer resistance to third-generation cephalosporin's, (cefotaxime 73.7%, ceftazidime 65.8%) has further restricted available therapeutic options in treatment of UTIs (Jalil & Al Atbee, 2022).

K. pneumoniae strains have been reported to cause skin and soft tissue infections including diabetic foot ulcers (Du *et al*., 2022) cellulitis (Hu *et al.*, 2020) and necrotizing fasciitis (Murali *et al.*, 2019). Cellulitis caused by *Klebsiella species* is considered potentially severe often occurring in individuals with underlying conditions like diabetes mellitus or compromised immunity. In countries with limited resources, Surgical site infection is the foremost infection in the overall patient population, affecting up to 66% of operated patients and nine times more than in industrialized countries (Billoro *et al.*, 2019).

K. Pneumoniae can cause life - threatening bloodstream infections (BSIs) with significant prevalence and high mortality worldwide (Yang *et al*., 2024). *K. pneumoniae* is major etiological agent of neonatal sepsis globally (Dangor *et al*., 2024). *K. pneumoniae* septicemia in young children (under 5) is a serious, often fatal, bloodstream infection, particularly in developing regions, with children under 2 years of life (Verani *et al*., 2024) being most vulnerable. Key symptoms include lethargy, poor feeding, breathing difficulties, and shock, often stemming from respiratory infections like pneumonia, with high rates of multi-drug resistant (MDR) strains making treatment challenging.

K. pneumoniae is a major cause of bacterial meningitis worldwide (Piednoir *et al.*, 2020). In 2019, the Global Burden of Disease report highlighted meningitis as one of the common disease among children under 5, causing 112,000 deaths and 1.28 million incident cases (Wunrow *et al*., 2023). *K. pneumoniae* can cause bacterial meningitis, or inflammation of the membranes that cover brain and spinal cord necessitating lengthy hospital stays and substantial healthcare costs due to severity of infection (Wall *et al*., 2021). Meningitis is a syndrome consistent with the classical triad of fever, headache, and with inflammation in the subarachnoid space.

K. pneumoniae liver abscesses affect people with diabetes or an alcohol use disorder or have been taking antibiotics for a long time. Capsular serotypes (K1 and K2) and the presence of a specific gene (magA and rmpA genes) in *K. pneumoniae* have been associated with invasive syndrome in pyogenic liver abscess (Turton *et al*., 2010). Symptoms include fever, pain in the upper right of abdomen, nausea, vomiting and diarrhea. The diagnosis of Klebsiella Liver Abscess (KLA) is established when *K. pneumonia* is cultured either from blood or the aspirate of the abscess in the absence of underlying hepatobiliary disease.

2.4 Resistance Mechanisms of *Klebsiella* Species to Various Classes of Antibiotics

Resistance mediated by extended-spectrum beta-lactamases (ESBLs) includes all penicillin's, cephalosporin's (including third-generation cephalosporin's) and aztreonam. New classes of enzymes mediating resistance to β-lactam antibiotics have

emerged over the last few decades because of antibiotic selection pressure; the dangerous ones are the extended spectrum β-lactamases produced by enteric pathogens that have spread worldwide since their first description in 1983 (Bradford, 2001). ESBLs are Gram-negative bacteria of the *Enterobacteriaceae* family that carry ESBL genes in their plasmids or chromosomes, produce β-lactam hydrolyzing enzymes, and are rightly considered to be among the most challenging pathogens by the World Health Organization (WHO). Mobile genetic elements (MGEs) are involved in spreading ESBL genes among the bacterial population. ESBLs hydrolyze 3rd generation cephalosporin's and aztreonam but do not hydrolyze carbapenems, and are inhibited by clavulanic acid and tazobactam (Shaikh *et al.*, 2015). ESBL-producing pathogens frequently exhibit plasmid-encoded multidrug resistance. Plasmids that encode the ESBL genes also have been found to carry genes that express resistance for drugs other than Beta-Lactams, such as aminoglycosides (Vuotto *et al.*, 2014). ESBL-producing organisms are often also able to reduce the susceptibility of other non-β-lactamase antimicrobial classes, such as aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, tetracyclines thus reducing range of antimicrobials used in treating infections caused by *Klebsiella*. ESBL producers as a result of the mutations, more than 200 types of ESBLs are currently described in the *Enterobacteriaceae* family and other non-enteric organisms, such as *Pseudomonas aeruginosa* and *Acinetobacter Spp*.

ESBLs resistance enzymes usually confer resistance in most Gram-negative bacterial pathogens as a result of more-selective pressure from the use of β-lactams: oxyimino-cephalosporins (such as cefotaxime, ceftriaxone, ceftazidime, or cefepime) and monobactams (aztreonam) but not carbapenems, which had undergone hydrolysis and further mutations (Blair *et al.*, 2015; Bush, 2013). The ESBL enzymes result from a point mutation in the parent β-lactamases, TEM-1 and SHV -1 by one-four amino acid changes which form the basis of resistance presumably due to selective evolutionary pressure from the use of β-lactams, such as oxyimino-cephalosporins and aztreonam. To date, the number of known β-lactamases has increased, and there are now over 1000 that have been identified. The most recognizable among the mutants of SHV -1, named SHV -2, deactivated the

extended-spectrum cephalosporin drugs and often carried many other resistance genes on its parent plasmid that conferred reduced susceptibility to other unrelated classes of antimicrobials (Altayb *et al.*, 2014).

Gram-negative infections are often resistant to multiple drug classes, including fluoroquinolones, which are commonly used to treat community-onset infections. Antibiotics such as β-lactams, aminoglycosides, and quinolones are used in the treatment of *K. pneumoniae* infection. With both chromosomal and plasmid-encoded ARGs, this pathogen has diverse resistance genes. Quinolones target bacterial topoisomerases blocking bacterial DNA replication. These drugs have been used in clinical practice since the 1960s, but their use increased extensively after the introduction of the first fluoroquinolones in the 1980s, which has led to the development of bacterial quinolone resistance mechanisms (Naeem *et al*., 2016) . Resistance to fluoroquinolones typically arises as a result of alterations in the target enzymes (DNA gyrase and topoisomerase IV) and changes in drug entry and efflux. Although fluoroquinolone resistance is mainly acquired by modification of their target enzymes, it may also involve the acquisition of plasmid-mediated quinolone resistance (PMQR) determinants (Guillard *et al*., 2016). PMQRs determine relatively small increases in quinolone resistance, but these changes are sufficient to mediate the natural selection of mutants that have higher levels of resistance (Vinué *et al*., 2016)

Plasmids encoding beta-lactams often carry other antibiotic resistance genes, for example, enzymes that modify antibiotic targets (e.g., ribosomal RNA methylation conferring high-level aminoglycoside resistance) or acetyltransferases modifying quinolones and aminoglycosides). Drug modifying enzymes have a narrow spectrum of activity, 16 S rRNA methylases confer resistance to practically all aminoglycosides including plazomicin, the most recent aminoglycoside compound developed (Poulikakos & Falagas, 2013). Plasmids can also encode various efflux pumps that extrude not only beta-lactams but also quinolones, aminoglycosides, and tetracyclines. A strain of *K. pneumoniae* isolated in France was found to have an efflux mechanism that increased resistance of chloramphenicol, the macrolide erythromycin, and the Quinolone nalidixic acid, as well as previously unknown

efflux mechanism against beta-lactams. Efflux pumps, have frequently been associated with antibiotic resistance in *K. pneumoniae* due to their ability to export antibiotics from bacterial cells (Filgona *et al*., 2015). Resistance to macrolides is common in *Klebsiella Spp* due to its production of macrolides esterase's (Zieliński *et al*., 2021).

K. Pneumoniae clones with KPC carbapenemase are a significant problem in the USA, Greece, and Israel, and plasmids encoding the VIM Metallo-carbapenemase have disseminated among *K. pneumoniae* in Greece (Livermore, 2008). Their dissemination is associated with multi-drug resistance plasmids of various types, and notably, one particular clone of K. Pneumoniae, ST 258, is crucial for the dissemination observed so far (Pitout *et al.*, 2015).

KPC β lactamases (KPC-1-KPC-7) confer decreased susceptibility or resistance to virtually all β lactams. Carbapenems (imipenem, meropenem, and ertapenem) (Nordmann et al., 2009) may thus become inefficient for treating enterobacterial infections with KPC-producing bacteria, which are also, resistant to many non-β-lactam molecules, leaving few therapeutic options. Detection of KPC-producing bacteria may be difficult on routine antibiotic susceptibility testing. Therefore, it is crucial to implement effective infection control measures to limit the spread of these pathogens. It is necessary to understand the antimicrobial susceptibility pattern of *Klebsiella sp*ecies due to the variation in antibiotic susceptibility in different geographical locations and to implement the measures to control the rapid spread of drug resistance.

2.5 Carriage of Resistance Genes in *Klebsiella* Species

Enterobacteriaceae are part of the normal gut microbiota; individuals can be colonized asymptomatically and unknowingly serve as a reservoir for spread to others, subset develops infection due to these bacteria (Taitt *et al*., 2017). Carriage of resistant commensal *Enterobacteriaceae* strains in the normal gut flora may serve as a reservoir of resistance genes that subsequently may be acquired by strains that cause infection (Tawfick *et al*., 2022). In hospital settings, the intestinal carriage is the main reservoir. The gut colonization of patients is associated with a high risk for

developing self and cross infections due to ESBL-producers, more so in long-term care units. The spread of ESBL-producing clones results from the movement of patients between various units of the hospital and also between hospitals, nationally or internationally. Patients colonized at admission can introduce the pathogen into hospital units.

ESBL determinants have been detected both in clinical isolates and in commensal bacteria from humans and animals and in isolates from products of the food chain and sewage (Olowe *et al*., 2015). This distribution suggests the presence of environmental reservoirs for these resistance determinants (Olowe *et al.*, 2015). The hypervirulent phenotype of *K pneumoniae* is thought to be attributable to the carriage of a virulence plasmid harboring two capsular polysaccharides (CPS) regulator genes (rpmA and rmpA2) and several siderophore gene clusters that contribute to the hypermucoviscous phenotype (Russo & Marr, 2019).

2.6 Risk Factors for Colonization with Klebsiella Species

2.6.1 Longer Hospitalization with Compromised Immunity

Major risk factor for colonization or infection with ESBL producing organisms are long-term antibiotic exposure, prolonged intensive care unit stay, severe illness, residence in an institution with high rates of ceftazidime and other third-generation cephalosporin's use and instrumentation or catheterization (Razazi *et al*., 2012). Exposure to these resistant pathogens can cause serious infections in patients with the following reported risk factors: immune-suppression, advanced age, admission to ICU, mechanical ventilation, previous exposure to antimicrobials, organ or stem-cell transplantation, and prolonged hospital stay (Ture *et al*., 2022). Health-care associated infections caused by CRE, mainly *Klebsiella pneumoniae*, have been encountered most commonly in ventilator-associated pneumonia, bacteremia, urinary tract, and surgical site infections.

2.6.2 Gastrointestinal Colonization with ESBL Organism

The colon serves as a reservoir for extra intestinal pathogens and therefore to predict the risk of ESBL infection it is of paramount importance to screen intestinal carriage (Birgy *et al*., 2012). Intestinal colonization with extended spectrum beta-lactamase producing Enterobacteriaceae makes available a reservoir of bacteria that may cause infections not only to the host but can also be transmitted to others. Such bacteria may transmit resistance genes to other bacteria across species (Weisenberg *et al.*, 2012)

2.6.3 Age

Although ESBL significantly varies by age, data suggest that the highest pediatric age risk group is 1 to 5 years (Medernach & Logan, 2018). Younger infants particularly those under 12 months , have higher carriage due to developing immune systems, exposure in neonates units, and potentially different gut microbiome (Verani *et al*., 2024). Old age is also documented as a risk factor due to the low immunity. Older adults often have more comorbidities due to weakened immunity, frequent antibiotic exposure, increased hospitalization, and reside in long term care facilities all increasing the ESBL carriage (Zhang *et al*., 2022). Children are protected against bacterial infections by weak maternal antibodies present during early stages of life but their immunity heightens as the age of the child grows. Bacterial infections are minimized as the age of the child advances.

2.6.4 Inappropriate Prescriptions

Inappropriate medical prescribing practices, poor patient adherence to antibiotic therapies, and insufficient oversight and regulation are associated with the development and spread of AMR. Lack of clinical training on AMR and provision of treatment guidelines by the public health sector has contributed heavily to the spread of bacteria resistance. Over prescription rate is high especially in LMIC’s countries where provision of antibiotics occurs without a prescription from health provider due to patient’s demands and this results to increased antibiotic resistance within communities through the spread of resistant strains of bacteria.

2.7 Resistance Mechanisms in *Klebsiella Spp*

2.7.1 Enzymatic Antibiotic Inactivation and Modification

Drug alteration is a major mechanism of antibiotic resistance in *K. pneumoniae* (Santajit & Indrawattana, 2016). Antimicrobial resistance genes may be carried on the bacterial chromosome, plasmid, or transposons. Drug inactivation by transfer of a chemical group to the drug most commonly uses transfer of acetyl, phosphoryl, and adenyl groups. Many bacteria produce enzymes that irreversibly modify and inactivate the antibiotics, such as *β*-lactamases, aminoglycoside-modifying enzymes, or chloramphenicol acetyltransferases. One of the well-characterized enzymes is *β*-lactamases. They are highly prevalent and act by hydrolyzing the *β*-lactam ring which is present in all *β*-lactams; thus, all penicillin's, cephalosporin's, monobactams, and carbapenems are essential to their activity (Jacoby, 2009). They can inactivate penicillin's (except temocillin), third-generation oxyimino-cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone). The genes coding for TEM and SHV enzymes have quite high mutation rates, resulting in a high level of diversity in enzyme types and thus increasing the scope of antibiotic resistance.

2.7.2 Modification of Drug Binding Site

K. pneumoniae causes drug resistance by mutating the target gene or methylating some bases so that the corresponding antimicrobial agents cannot bind to the target site. Changes in the structure and/or number of penicillin binding proteins (PBPs) is one mechanism of resistance to –lactam antibiotics. PBPs are Trans peptidases involved in the construction of peptidoglycan in the cell wall. A change in the number (increase in PBPs that have a decrease in drug binding ability, or decrease in PBPs with normal drug binding) of PBPs affects the amount of drug that can bind to that target. A change in structure (e.g. PBP2a in *S. aureus* by acquisition of the *mecA* gene) may decrease the ability of the drug to bind, or totally inhibit drug binding (Beceiro *et al*., 2013).

2.7.3 Porin Loss and Mutation

K. pneumoniae develops resistance by reducing the entry of antimicrobial agents into the bacteria by reducing the outer membrane pore protein. B-lactamase enzymes destroy antibiotics, while fewer porin channels in the bacterial outer membrane physically blocks the drugs entry, especially crucial for carbapenems, making the bacteria extremely hard to kill. *K pneumoniae* contains two main porins, 0mpk35, and 0mpk36, through which hydrophilic solutes gain access to bacteria- cell (Schulz, 2002). Loss of membrane permeability may be to mutation in porin that renders it non-functional or alteration in expression level or due to complete loss of the porin proteins (Doménech-Sánchez *et al*., 2003). This dual mechanism makes bacteria resistant to even broad spectrum antibiotics, threatening treatment of severe infections.

2.7.4 β-lactamases

The βlactam antimicrobial agents are the most extensively used class of antibacterial agents. The basic structure of this pharmacological class is a four-sided lactam ring. The most important mechanism of resistance to the penicillin's and cephalosporin's is antibiotic hydrolysis mediated by the bacterial enzyme β-lactamase. Resistance to β-lactam antibiotic occur by three different mechanisms: (1) prevention of the interaction between the target PBP and the drug, usually by modifying the ability of the drug to bind to the PBP (this is mediated by alterations to existing PBPs or acquisition of other PBPs; (2) active of efflux of β-lactam drugs through efflux pumps; and (3) hydrolysis of the antibiotic by β-lactamase enzymes (Bush & Bradford, 2016). These enzymes may also be commonly known by their enzyme family; for example: the TEM (named after the first patient) family, the SHV (sulfhydryl variable) family, and the CTX (preferentially hydrolyze cefotaxime) family. The genes coding for TEM and SHV enzymes have quite high mutation rates, resulting in a high level of diversity in enzyme types and thus increasing the scope of antibiotic resistance (Bajpai *et al*., 2017).

2.7.5 Increased Efflux Pump Expression of the Antibiotic

Efflux pumps are membrane proteins involved in substance expulsion that reduce intracellular drug concentrations by releasing antimicrobial cells outside the cell, thereby reducing susceptibility to multiple antibiotics (Tang *et al.*, 2020). The efflux pumps function primarily to rid the bacterial cell of toxic substances, and many of these pumps will transport a large variety of compounds (multi-drug [MDR] efflux pumps)The active efflux system AcrAB-TolC can exocytosis many kinds of antibiotics, including β-lactam, macrolides, fluoroquinolones, and tetracycline, which is an important reason for the MDR *K. pneumoniae* (Bharatham *et al.*, 2021).

2.7.6 Biofilm Formation

K. pneumoniae is prone to form biofilms, and structures such as capsular and pili play an important role in the formation of biofilms (Desai *et al*., 2019) . Biofilms have osmotic barrier properties and are resistant to antimicrobial agents, and one study showed that *K. pneumoniae* biofilms reduced sensitivity to gentamicin, ampicillin, and ciprofloxacin (Chung, 2016). Colistin resistance has also been linked to biofilm formation.

2.8 Mechanism of Pathogenesis of *Klebsiella spp*

2.8.1 Capsule

Klebsiella is surrounded by a capsule, which increases its virulence by acting as a physical barrier to evade the host's immune response. This capsule also protects the cell from desiccation. The capsule is considered to be the dominant virulence property and consists of an elaborate layer of surface-associated polysaccharides, the composition of which is very much strain dependent. In *K. pneumoniae*, at least 77 distinct polysaccharides (designated the K antigens) have been reported (Follador *et al*., 2016) Capsule polysaccharides contribute to pathogenesis by mediating resistance to phagocytosis and killing by serum.

2.8.2 Fimbrial Adhesins

The ability of bacteria to form biofilms on medical devices, e.g. catheters, has a major role in development of many nosocomial infections. Most clinical *K. pneumoniae* isolates express two types of fimbrial adhesins, type 1 fimbriae and type 3 fimbriae. Types 1 and 3 fimbriae are produced and assembled on the surface of clinical isolates of *K. pneumoniae* (Gao e*t al.*, 2024; Schroll *et al*., 2010). Type 1 fimbria is the best characterized, highly prevalent in many species of the *Enterobacteriaceae* family, and is mainly composed of several structural subunits called FimA. FimH, an adhesin that confers the ability to recognize mannose, is located at the tip of the fimbria interacting with FimA (Gao *et al*., 2024; Schwartz *et al.*, 2013). Type 3 fimbriae are characterized by the agglutination of erythrocytes treated with tannic acid, mediate bacterial adherence to endothelial and bladder cell lines and are involved in biofilm formation on antibiotic surfaces (Schroll *et al*., 2010).

2.8.3 Siderophores

Siderophores are secondary metabolites produced by different organisms in order to scavenge iron from their surrounding environment making iron available to the cell. They have high affinity for ferric iron. Siderophores are secreted out to form soluble ferric complexes that can be taken up by the organisms. They exhibit complex chemistry that allows them to form the strongest iron-chelating complexes (Albelda-Berenguer *et al*., 2019).

2.8.4 Serum Resistance

After the onset of inflammation, invading *Klebsiella* strains meet the cellular and humoral bactericidal components of the innate immune system. The host's first line of defense against invading microorganisms includes the bactericidal effect of serum, which is mediated primarily by complement proteins. Lipopolysaccharides (LPS) have been implicated as a major factor in the ability of bacteria to resist serum bactericidal activity by the host (Wells *et al*., 2014). Nine different LPS serotypes (O antigens) in *K. pneumoniae* have been described, the O1 serotype being the most

common O antigen found in clinical isolates (Fang *et al*., 2016). *Klebsiella* spp have various serotypes where O1, O2, and O3 have been attributed to 80% of all *Klebsiella* infections (Follador *et al*., 2016).

2.9 Treatment of *Klebsiella* Infections

The choice of treatment is based on various factors including local antimicrobial sensitivity, site of infection, and comorbid conditions. Cephalosporin's have been widely used as monotherapy and in combination with aminoglycosides for Extended-spectrum beta-lactamase (ESBL) producing strains. Carbapenems, particularly meropenem and imipenem are commonly used. For carbapenemase-producing strains, colistin may be used as monotherapy or in combination with tigecycline. Carbapenems antimicrobials remain the gold standard of therapy for serious infections due to ESBL-producers.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The isolates utilized in this study were isolated from stool samples collected from children aged under five years hospitalized at two referral hospitals based in western Kenya: Kisii Teaching and Referral Hospital (KTRH) and Homa Bay County Teaching and Referral Hospital (HCTRH). Kisii County is fast growing agricultural region with an estimated population of 1.1 million people with children below five years accounting for about 220,000 (Kenya National Bureau of Statistics, 2019). KTRH is a level 6 hospital and the largest public hospital in Kisii County. In 2014, Kisii county had one of the highest prevalence of HIV infections (8%) against a national prevalence of 5.6% and alarmingly high new infection estimated at over 5,000 annually (Nyamoita *et al*., 2019). Among the over 60,000 people living with HIV, over 7,000 are children and annually about 1,000 children are infected with HIV. HCTRH is based in Homabay County with a population of about 1,130,000 people covered on an area of 3,152.5 km^2 with 8 sub-counties (Kenya National Bureau of Statistics, 2019). HCTRH is a level four hospital under the Homa Bay County government and was elevated from a district hospital to a level four hospital in 2013 after introduction of county governments. In 2014, Homa Bay county was one of the leading counties in the country with the highest under five mortality rates (91/1000 live births) (Opon, 2016). UNICEF data in 2014 puts Homa Bay county as one of the counties with a poverty rate of 44% and high maternal mortality rate 583 per 100000 live births higher than the national average rate of 488 per 100000 live births (Arora, 2023; Opon, 2016). In 2019, under five mortality rate was 52/1000 live births and 35.5/1000 live births infant mortality higher than the national averages (Kenya National Bureau of Statistics, 2019). In 2018, Homa Bay county had almost five times the national HIV prevalence (26%) with the county accounting for 14% of new HIV infections among children (National AIDS Control Council, 2018). The disparity in health services, HIV prevalence, malnutrition status and significant mortality rates in children under five years between Kisii and Homabay county

referral hospital designates theses site as adequate to conduct this study and make comparison between peri-urban and rural populations.

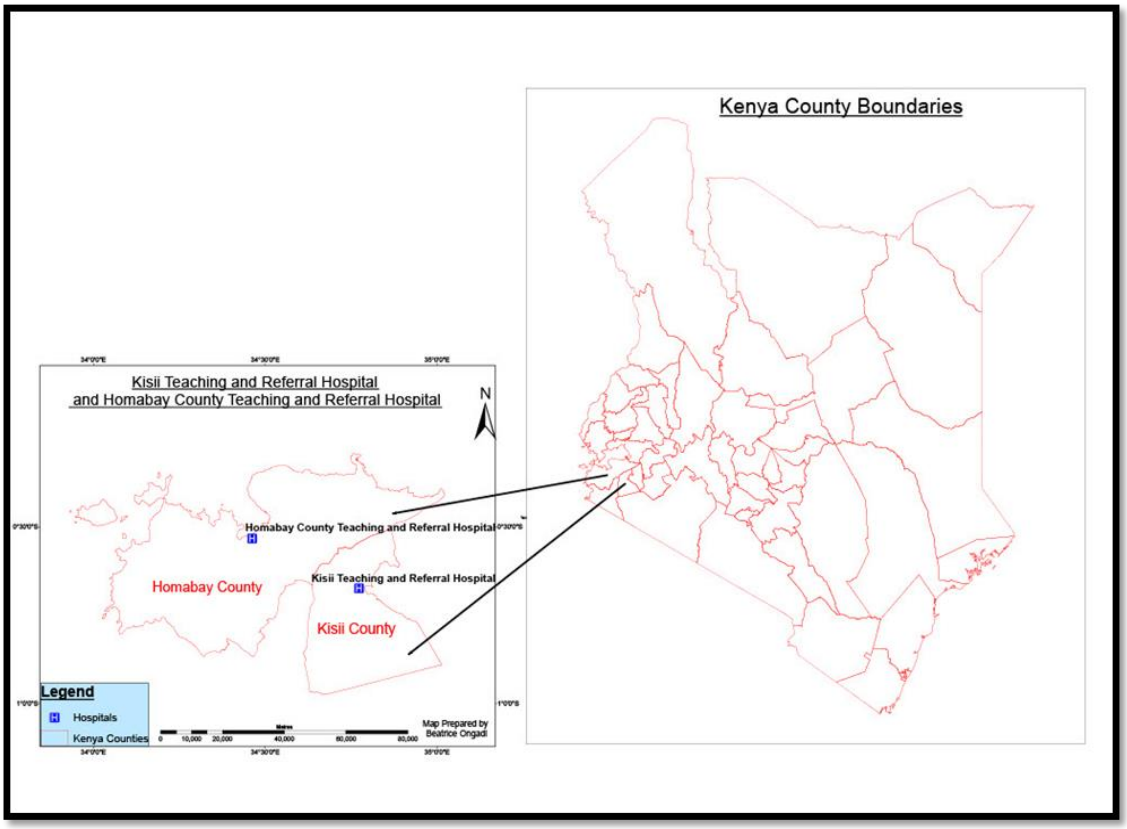

Figure 3.1: Map Showing Location of Kisii and Homabay Counties

3.2 Study Design

The study design is a laboratory-based retrospective cross-sectional study involving the use of archived *Klebsiella* isolates isolated on enrolment visit in reference to Scientific Ethics Review Unit (SERU) Protocol No. 3086 (Azithromycin to prevent post-discharge Morbidity and Mortality in Kenyan Children) (Pavlinac *et al*., 2021). The archived *Klebsiella* isolates used for the study were collected over two year's period from June 2016 to June 2018. *Klebsiella* was isolated and preserved in tryptone soy broth supplemented with 20% glycerol and stored in -80^{o}C freezers.

3.3 Study Population

Children less than five years of age were recruited from KTRH and HCTRH in western Kenya as previously described (Pavlinac *et al*., 2021). Eligible children who were under five years, had been hospitalized and discharged from hospital. Children below five years are vulnerable to infections due to their weak immune system. Clinical and socio-demographic information was collected through interviews after consenting from the child's caregiver while relevant child health history information including clinical data was abstracted from hospital records. A rectal swab or whole stool was collected from the child at discharge before the administration of trial medication and dispatched for analysis (Pavlinac *et al*., 2021).

3.2.1 Inclusion and Exclusion Criteria

Study inclusion criteria involved the use of available archived *Klebsiella spp. (K. pneumoniae and K. oxytoca)* isolates identified in children below five years of age from the parent study.

- *Klebsiella Spp*. identified by phenotypic and biochemical characteristics.
- The caregivers had consented to willing participate in the study.

Isolates were excluded from the study if:

- Bacterial isolates other was identified as any other organism other than *Klebsiella Spp*.
- The caregivers had not consented to participate in the study.

3.4 Sample Size Determination

The sample size for this study was determined using the Fisher exact test formulae (Sprent, 2011) based on archived *Klebsiella spp*. isolates for the last two years. (From June 2016 – June 2018).

Anticipated population proportion: P

Absolute precision (d) = 5%

Confidence interval = 95%

Prevalence 80 % (Henson *et al.,* 2017) Borrowed from a study done in Kilifi on Neonates with *Klebsiella* infection.

$$n = \frac{Z^2_{1-\alpha/2}(1-P)}{d^2} \quad (1)$$ (Kirby *et a*l., 2002)

n= {(1.96*1.96) * 0.8 (1-0.8)}/ (0.05*0.05)

n= {(3.8416) *(0.16)}/ (0.0025)

n= {0.614656}/ (0.0025)

n= 245.8624

n= 245

A minimum of 245 *Klebsiella* spp. isolates were determined as the sample size per organism for statistically significance. A 80% prevalence was considered to determine the number of *Klebsiella* spp. isolates to be tested in the study as reported by a previous study conducted in Kilifi County (Henson *et al.,* 2017). With this consideration, a total of 245 *Klebsiella* spp. available isolates from children being discharged from Kisii and Homa bay county referrals that met all the characteristics in the inclusion criteria were included in this study.

3.5 Sampling Method

This study utilized systematic random sampling. A total of four hundred and sixty one children had *Klebsiella* isolates isolated from their stool samples. Excel formular randomization was used to generate random numbers to get a representative sample size of 245 archived isolates from both study sites. 144 archived Klebsiella isolates

were randomly selected from Kisii and 101 archived Klebsiella isolates were selected from Homabay site.

3.6 Laboratory Protocol

3.6.1 Identification and Confirmation of *Klebsiella* from Archived Isolates through Culture

The archived isolates were removed from -80^{o} C freezer and allowed to thaw at room temperature. A loopful of the isolate were inoculated using streak plate technique on MacConkey agar (Oxoid). Incubation was done at 37 ^{o} C for 24 hours aerobically. Presence of *Klebsiella* was identified by the appearance of lactose fermenting mucoid, large, and doom shaped colonies on the culture plate. Further sub culturing of discreet colonies was done on Mueller Hinton agar (Oxoid) and incubated at 37 ^{o} C for 24 hours to get pure colonies for analytical profile indexing in the identification of *Klebsiella.*

3.6.2 Identification using Analytical Profile Index 20 E

Two hundred and forty-five *Klebsiella* isolates (231 *K. pneumoniae* and 14 *K. oxytoca* isolates) were selected and re-confirmed by biochemical testing using analytical profile index (API) 20E strips (bioMérieux, Inc, Durham, NC, United States). A pure colony suspected to be *Klebsiella spp* was picked from an overnight culture using a disposable straight wire loop. The lactose fermenting or mucoid entire margin gram-negative colonies were suspended in 0.85% normal saline to form a 0.5 MacFarland standard. The suspension was distributed in equal volume in the API 20E Biochemical strips wells with dehydrated media using a Pasteur pipette. The mineral oil was added to arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), hydrogen sulfide (H2S), and urea (URE) wells and incubated at 37°C for 24 hours. API Lab software was used to interpret results strip and coded patterns.

3.7 Antibiotic Susceptibility Testing

The antibiotic susceptibility profiles of the *Klebsiella* isolates were determined by the Kirby-Bauer disk diffusion method as described by Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2021). A loop full of the mucoid, lactose, or non-lactose fermenting colonies was placed into 5ml of 0.85% sterile normal saline and adjusted to a 0.5 MacFarland standard (bioMérieux, Inc, Durham, NC, United States). Bacterial suspensions were applied homogenously on the surfaces of MHA (Oxoid, *Hants, United Kingdom)* plates. An antibiotic discs for ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), cefoxitin (FOX, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (CN,10 µg), amoxicillin-clavulanate (AMC, 20 µg/10 µg), meropenem (MEM, 10 µg), imipenem (IPM, 10 µg), azithromycin (AZM, 15 µg), and aztreonam (AZT, 30 µg) were placed on top of the agar and bacteria using a disc dispenser. The plates were incubated at 35°C in ambient air for 18-24 hours. Each lot of antibiotics disks utilized in the study were run quality control testing before the actual process of subjecting isolates to antimicrobial susceptibility testing. *E. coli* ATCC 25922 was used as a negative control strain, and *K. pneumoniae* ATCC 700603 was used as the positive control strain.

Zone diameters, measured in millimeters, established by CLSI-2020 M-100 were used to determine susceptibility, resistance, or an intermediate designation (Clinical and Laboratory Standards Institute, 2021).Isolates showing resistance to three or more antibiotic classes were defined as multidrug-resistant (MDR). The tests results were used if within the QC ranges set by the Clinical and Laboratory standard institute.

3.8 Phenotypic Detection of ESBL Producers

A double-disk-diffusion-synergy test was used for the determination of ESBL producers in both *Klebsiella* species. Synergy was determined between a disk of Ceftazidime 30mg, Ceftazidime-Clavulanate disc, and cefotaxime 30mg, a cefotaxime-Clavulanate disc placed equidistant 30 mm apart on the lawn culture of a test bacterium on a Mueller Hinton (MH) agar plate and incubated overnight at 37 ^{o}

C incubator for 18 hours. ESBL production is inferred when the inhibition around the ceftazidime-clavulanic or cefotaxime-clavulanic discs is expanded by > 5mm compared to respective ceftazidime or cefotaxime discs alone. The test was considered positive when the difference of inhibition zones between ceftazidime-clavulanate and ceftazidime or cefotaxime -clavulanate and cefotaxime was greater or equal to 5mm.

3.9 Genotypic Detection of ESBL Genes Using Conventional PCR

DNA was extracted from ESBL-producing colonies of *Klebsiella spp* using a boiling method. An inoculating loop was placed into bacteria pooled from an overnight culture in MH mixed with 0.5 ml nuclease free water. The cell suspension was heated for 10 minutes at 100^{o}C then centrifuged at 15,000 revolutions per minute for 5 minutes (maintained at 25^{o}C). The supernatant was used as DNA template for amplification. The extracted DNA was quantified using Nano pore. Extracted DNA was amplified using sets of primers targeting ESBL encoding genes (*bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{CTX-M,}$ *bla* $_{OXA}$) as previously described (Demaneche *et al.,* 2008; Hasman *et al.,* 2005). Briefly, a final reaction volume of 25 µl was used in a master mix containing 0.5 µl forward primer (0.2 µM), 0.5 µl reverse primer (0.2 µM), and 9.5µl nuclease free water. A 12.5 µl mix which included Taq DNA polymerase (2.5 units), 1x PCR Buffer, $MgCl_2$ (0.2 µM), and ultrapure dNTPs (200 µM,) followed by addition of 2 µl template DNA was combined with the PCR master mix. Amplification conditions consisted of 30 cycles of 94^{o}C for 30 seconds, 50^{o}C, 55 ^{o}C and 60 ^{o}C for 30 seconds, initial extension of 68 ^{o}C for 1 minute and with a final extension step of 68^{o}C for 5 minutes (Demaneche *et al.,* 2008). Gel electrophoresis of PCR products as carried out at 200 volts on a 1.5% agarose gel, stained with gel red stain and visualized on a Vilber E-Box gel documentation system. All PCR reactions were run with both negative and positive DNA control templates.

Table 3.1: Primers for Detection of ESBL Genes

Gene	Primer sequence	Expected size (bp)	Annealing Temp (ºC)	References
bla **TEM**	F-5'CATTTCCGTGTCGCCCTTATTCC3' R-5'GGCACCTATCTCAGCGATCTGT3'	828	55	(Demaneche *et al.*, 2008)
bla **SHV**	F-5'TTCGCCTGTGTATTATCTCCCTG 3' R-5'TTAGCGTTGCCAGTGYTCG 3'	854	50	(Hasman *et al.*, 2005)
bla **CTX-M**	F-5'ATGTGCAGYACCAGTAARGTKATGGC 3' R-5'TGGGTRAARTARGTSACCAGAAYCAGCGG 3'	593	50	(Hasman *et al.*, 2005)
bla **OXA-1**	F-5'ATGAAAAACACAATACATATCAACTTCGC 3' R-5' GTGTGTTTAGAATGGTGATCGCATT 3'	820	60	(Hasman *et al.*, 2005)

KEY: F: forward primer, R: Reverse primer, B.p: Boiling point, ºC: Degrees Celsius.

3.10 Ethical Approval

A new study approval was sought from the Scientific and Ethics Review Unit (SERU), KEMRI: P00105 (Appendix 1) in addition to the original parent approval (KEMRI (SERU/SSC No. 3086) and Jomo Kenyatta university and technology REF: JKU/2/11/HSB321-0003/2017 (Appendix 3) Confidentiality was maintained throughout and after the study period. Samples were assigned unique codes only identified by the principal investigator.

3.11 Demographic Data

Detailed descriptions of exposure variables and the derived variables were extracted from the main clinical trial. Data extracted included data on sex, child age, study site, length of hospital stays, and antibiotic use during hospitalization, household toilet type, water source and treatment, and household crowding. A household had access to improved water if the caregiver reported access to reliable piped water in the dwelling or community, or if the household primarily used water from a borehole, a

protected spring, a well with a pump, bottled water, or rainwater from storage tanks for household chores. Household crowding was defined as a household with more than two individuals sharing a room.

3.12 Data Management and Analysis

This study reported the proportion of *Klebsiella* spp. isolates resistant to each tested antibiotic and carrying ESBL genotypes. To evaluate correlates of ESBL-producing *Klebsiella* spp, the study constructed univariate and multivariable Poisson regression models with a robust variance for various child, hospital, and household factors, adjusting for key *a priori* confounders (age, sex, and site). Associations were considered statistically significant at an alpha <0.05. All statistical analyses were performed in Stata (Version 17.0, Stata Corp, and College Station, TX, USA).

3.13 Dissemination of Findings

Results obtained from this study were shared with the health facilities for clinical care Purpose during their monthly Continuous medical education (CME). Abstract was presented in KEMRI Annual Scientific and Health conference 2024, and a manuscript was prepared for publication in a BMC Microbiology journal (Appendix II).

CHAPTER FOUR

RESULTS

4.1 Baseline Characteristics

Two hundred and forty-five *Klebsiella* spp isolated were (231 *K. pneumoniae* and 14 *K. oxytoca*) randomly chosen to be included in this sub-study. The enrolled children had a median age of 15 months (IQR 8–30), 144 were from the Kisii site, and 101 from Homabay. Participants' age was stratified to highlight the impact of infection on age groups and were categorized into ages of five months and below, six months to eleven months, twelve months to twenty-three months and twenty-four months to fifty-nine months. In terms of age, majority of children were between age of 24-59 months from both study sites. Participants enrolled in Kisii accounted for 86 (60%) males and 58 (40%) females. 87 (62%) of children included from Kisii were hospitalized for more than four days. Diagnosis at hospital discharge in Kisii included 39 (27%) pneumonia, malaria 18 (13%) and malnutrition 12 (8%) cases. During hospitalization, 138 (96%) children had taken at least one antibiotic: gentamicin 102 (71%) penicillin 121 (84%), ceftriaxone 38 (26%). Majority of children enrolled in Homabay were males 54 (53%). Most of children enrolled from Homabay had a hospital stay of more than four days and at admission were presenting with malaria 23 (23%), pneumonia 18 (18%), and diarrhea 9 (9%). 80% of children included in the study from Homabay were prescribed an antibiotic during hospital admission. Antibiotics received include penicillin 44 (44%), gentamicin 40 (40%), ceftriaxone 39 (39%), ciprofloxacin 2 (2%) and cefuroxime 1 (1%). Ciprofloxacin was least prescribed antibiotic during hospital admission in both facilities with only Homabay accounting for 2% of prescriptions. Further detailed description of the participants' characteristics is shown by site in Table 4.1

Table 4.1: Characteristics of Children Enrolled in the Study

	KISII	HOMABAY	Total
	N=144	N=101	N=245
Child Characteristics			
Age (months)			
0-5	31 (22%)	9 (9%)	40 (16%)
6-11	31 (22%)	26 (26%)	57 (23%)
12-23	33 (23%)	32 (32%)	65 (27%)
24-59	49 (34%)	34 (34%)	83 (34%)
Sex			
Male	86 (60%)	54 (53%)	140 (57%)
Female	58 (40%)	47 (47%)	105 (43%)
Hospitalization Information			
Length of hospital stay			
<4 days	54 (38%)	48 (48%)	102 (42%)
≥4 days	87 (62%)	53 (52%)	140 (58%)
Discharge diagnosis			
Diarrhea	10 (7%)	9 (9%)	19 (8%)
URTI	3 (2%)	1 (1%)	4 (2%)
Pneumonia	39 (27%)	18 (18%)	57 (23%)
Malaria	18 (13%)	23 (23%)	41 (17%)
Malnutrition	12 (8%)	7 (7%)	19 (8%)
Other diseases	60 (42%)	43 (43%)	103 (42%)
Any antibiotic used during admission			
NO	6 (4%)	20 (20%)	26 (11%)
YES	138 (96%)	81 (80%)	219 (89%)
Ceftriaxone used during admission			
No	106 (74%)	62 (61%)	168 (69%)
Yes	38 (26%)	39 (39%)	77 (31%)
Ciprofloxacin used during admission			
No	144 (100%)	99 (98%)	243 (99%)
Yes	0 (0%)	2 (2%)	2 (1%)
Cefuroxime used during admission			
No	140 (97%)	100 (99%)	240 (98%)
Yes	4 (3%)	1 (1%)	5 (2%)
Gentamicin used during admission			
No	42 (29%)	61 (60%)	103 (42%)
Yes	102 (71%)	40 (40%)	142 (58%)
Chloramphenicol used during admission			
No	127 (88%)	99 (98%)	226 (92%)
Yes	17 (12%)	2 (2%)	19 (8%)
Penicillin used during admission			
No	23 (16%)	57 (56%)	80 (33%)
Yes	121 (84%)	44 (44%)	165 (67%)
Household Information			
Improved water source			
No	25 (17%)	28 (28%)	53 (22%)
Yes	119 (83%)	73 (72%)	192 (78%)
Treated drinking water			
No	78 (56%)	23 (23%)	101 (42%)
Yes	62 (44%)	76 (77%)	138 (58%)
Toilet			
Private for household only	81 (57%)	35 (35%)	116 (48%)
Shared with ≥1 other household	61 (43%)	53 (52%)	114 (47%)
Open defecation	1 (1%)	13 (13%)	14 (6%)

KEY: URTI – upper respiratory tract infection, N-population of enrolled children

4.2 Phenotypic and Genetic AMR

Overall, 231 (94.3%) of the isolates were *K. pneumoniae* and 14 (5.7%) were *K. oxytoca*. A total of 154 (62.8%) isolates carried ESBL. More than 58.2% *Klebsiella* spp. isolates were resistant to third generation cephalosporin's; 154 (62.6%) were resistant to ceftriaxone, 148 (60.0%) cefotaxime and 127 (52.0%) ceftazidime. Resistance to cefoxitin was low (4.5%) and while 55.0% of isolates were resistant to gentamicin. Among the less commonly prescribed antibiotics in Kenyan hospitals, 38% were resistance to chloramphenicol, 32% to ciprofloxacin and 24% to azithromycin. In contrast, 98% of the *Klebsiella* isolates remained susceptible to Carbapenem, including meropenem and imipenem (Figure 4.1).

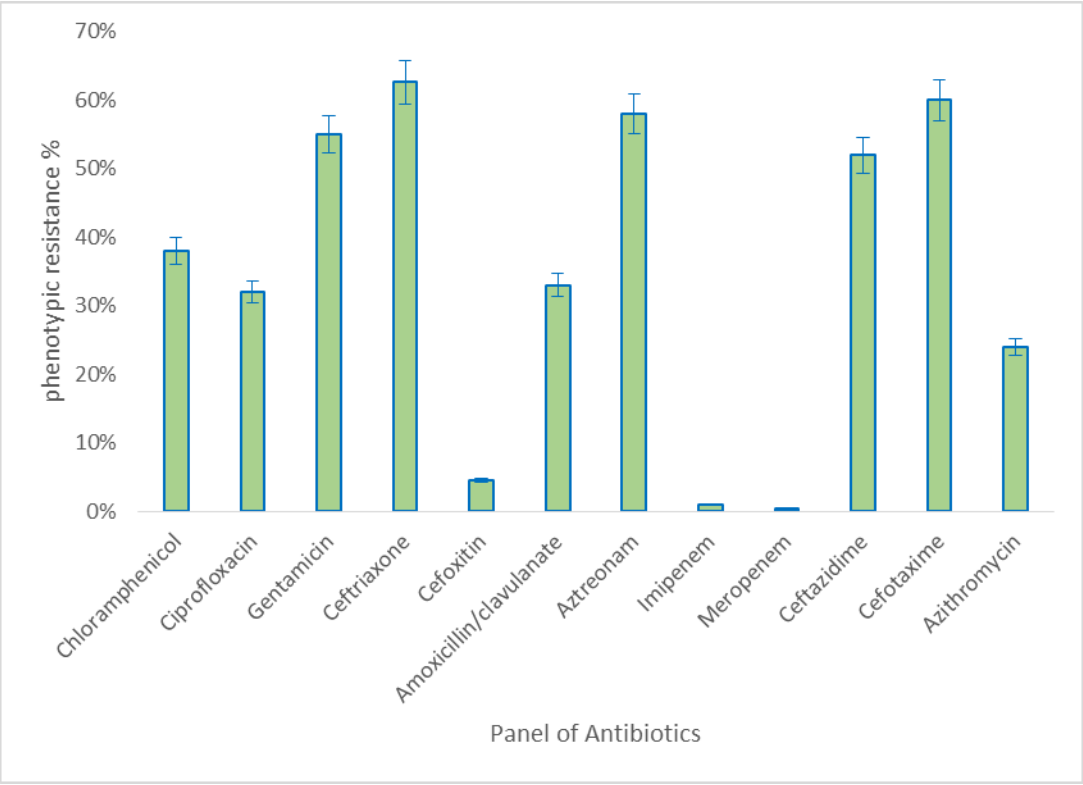

Figure 4.1: Prevalence of Phenotypic Resistance in *Klebsiella* Isolates from Children Discharged From Hospital in Western Kenya

Table 4.2: Phenotypic Resistance of *Klebsiella* species

Antimicrobial Resistance	Kisii	Homabay	
Chloramphenicol			
SUSCEPTIBLE	74 (51%)	68 (67%)	142 (58%)
INTERMEDIATE	5 (4%)	4 (4%)	9 (4%)
RESISTANT	65 (45%)	29 (29%)	94 (38%)
Ciprofloxacin			
SUSCEPTIBLE	94 (65%)	72 (71%)	166 (68%)
INTERMEDIATE	28 (20%)	21 (21%)	49 (20%)
RESISTANT	22 (15%)	8 (8%)	79 (32%)
Gentamicin			
SUSCEPTIBLE	51 (35%)	51 (50%)	102 (42%)
INTERMEDIATE	3 (2%)	5 (4%)	8 (3%)
RESISTANT	90 (63%)	45 (45%)	135 (55%)
Ceftriaxone			
SUSCEPTIBLE	49 (34%)	41 (41%)	90 (37%)
INTERMEDIATE	0 (0%)	1 (0.6%)	1(0.4%)
RESISTANT	95 (66%)	59 (58.4%)	154 (62.6%)
Cefoxitin			
SUSCEPTIBLE	132 (92%)	101 (100%)	233 (95%)
INTERMEDIATE	1 (0.7%)	0 (0.0%)	1 (0.5%)
RESISTANT	11 (7.3%)	0 (0%)	11 (4.5%)
Amoxicillin/clavulanate			
SUSCEPTIBLE	76 (53%)	66 (65%)	142 (58%)
INTERMEDIATE	14 (10%)	8 (8%)	22 (9%)
RESISTANT	54 (37%)	27 (27%)	81 (33%)
Aztreonam			
SUSCEPTIBLE	49 (34%)	43 (43%)	92 (38%)
INTERMEDIATE	10 (7%)	3 (3%)	13 (4%)
RESISTANT	85 (59%)	55 (54%)	143 (58%)
Imipenem			
SUSCEPTIBLE	140 (97%)	101 (100%)	241 (98%)
INTERMEDIATE	2 (1.5%)	0 (0%)	2 (1%)
RESISTANT	2 (1.5%)	0 (0%)	2 (1%)
Meropenem			
SUSCEPTIBLE	142 (99%)	100 (99%)	242 (98.8%)
INTERMEDIATE	2 (1%)	0 (0%)	2 (0.8%)
RESISTANT	0 (0%)	1 (1%)	1 (0.4%)
Ceftazidime			
SUSCEPTIBLE	50 (35%)	49 (49%)	99 (40%)
INTERMEDIATE	8 (5%)	11 (10%)	19 (8%)
RESISTANT	86 (60%)	41 (41%)	127 (52%)
Cefotaxime			
SUSCEPTIBLE	50 (35%)	41 (41%)	91 (37%)
INTERMEDIATE	3 (2%)	1(1%)	4 (3%)
RESISTANT	91 (63%)	59 (58%)	148 (60%)
Azithromycin			
SUSCEPTIBLE	106 (74%)	80 (79%)	186 (76%)
RESISTANT	38 (26%)	21 (21%)	59 (24%)
Species			
K. Pneumoniae	136 (94%)	95 (94%)	231 (94%)
K. oxytoca	8 (6%)	6 (6%)	14 6%)

4.3 Genotypic Detection of ESBLS

At least one ESBL-conferring gene was detected in all the 154 ESBL-producing *Klebsiella* isolates genotyped. CTX-M (92.2%; 95% CI: 86.8–95.9) and SHV (92.2%; 95% CI: 86.8–95.9) were the most prevalent ESBL-conferring gene followed by TEM (57.1%; 95% CI: 48.9–65.1) and OXA (31.2%; 95% CI: 24.0–39.1).

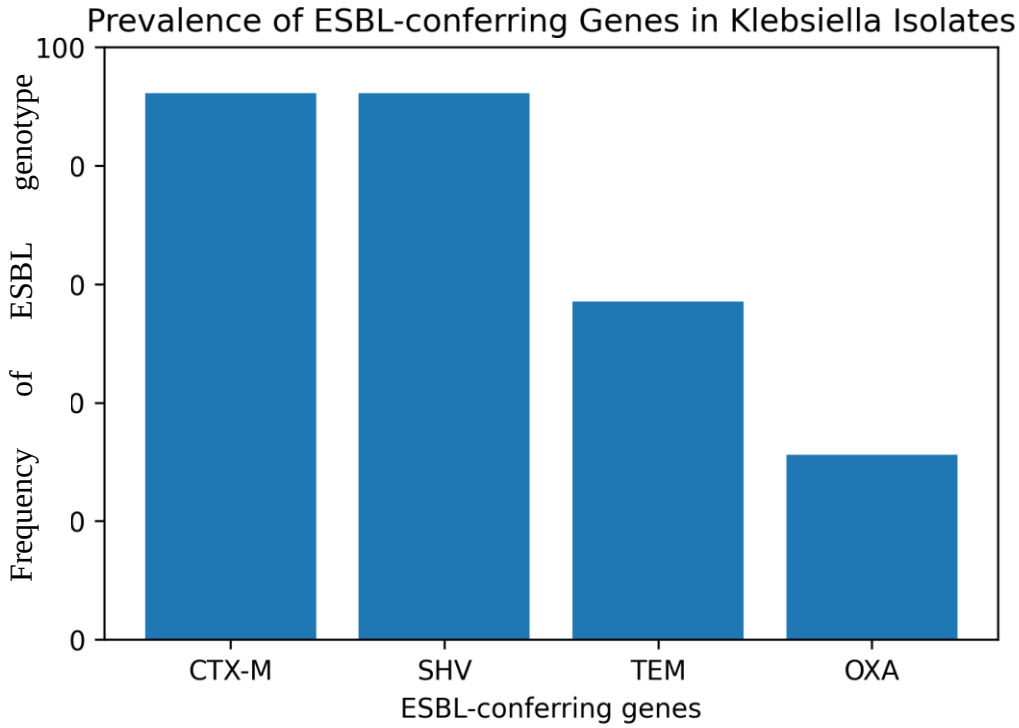

Figure 4.2: Isolated *Klebsiella* ESBL Genotypes

4.4 Co-carriage of *Klebsiella* Genotypes,

A total of 32/154 (20.8%) isolates co-carried all the 4 *bla* genes screened while majority of the isolates 61/154 (39.6%) had co-carriage of 3 β-lactamase genes, followed by Combination of 2 ESBL encoding genes 48/154 (31.1%). Only 13 isolates encoded a single β-lactamase gene, either *bla* $_{\text{CTX-M}}$ or *bla* $_{\text{SHV}}$. A further description of the phenotypic resistance against gene carriage among isolates that were positive for ESBL is shown in Table 4.3.

Table 4.3: Co-carriage of Resistance Genes among ESBL Positive *Klebsiella* Isolates Segregated by *Klebsiella* Species

Only one ESBL encoding gene	***K. pneumoniae* (N=148)**	***K. oxytoca* (n=6)**	**Total**
bla CTX-M	4	0	4/154 (2.59%)
bla SHV	9	0	9/154 (5.84%)
bla TEM	0	0	
bla OXA	0	0	
Combination of 4 encoding gene			
bla CTX-M +SHV+TEM+OXA	30	2	32/154(20.7%)
Combination of 3 encoding gene			
bla CTX-M+ SHV +TEM	45	0	45/154(29.2%)
bla SHV +TEM+OXA	2	0	2/154(1.29%)
bla CTX-M+TEM+OXA	4	1	5/154(3.24%)
bla CTX-M+SHV +OXA	7	2	9/154(5.84%)
Combination of 2 ESBL encoding Genes			
bla CTX-M+SHV	43	1	44/154(28.5)
bla CTX-M+TEM	3	0	3/154(I.94%)
bla SHV+TEM	1	0	1/154 (0.64%)

Among genes conferring resistance to beta lactam class of antibiotics are demonstrated in the following electrophoresis gels: below figures 4.3 *bla* TEM, figure 4.4 *bla* CTX- M, figure 4.5 *bla* OXA, figure 4.6 *bla* SHV.

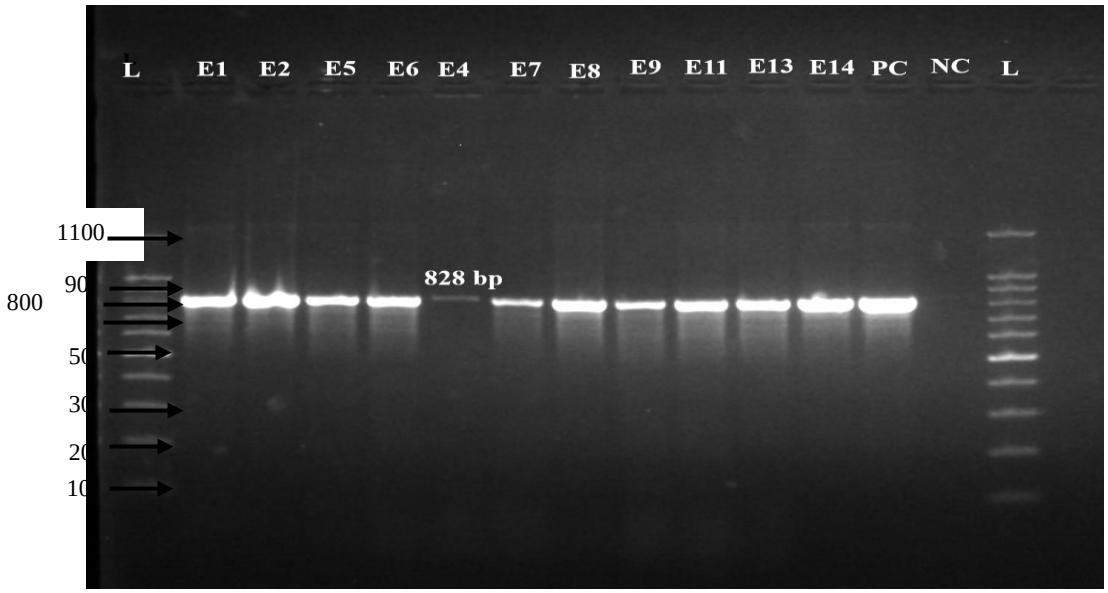

Figure 4.3: Electrophoresis Gel for *bla* TEM (828 bp) L- Molecular Ladder; NC-Negative Control; PC-Positive Control; bp- Base Pairs.

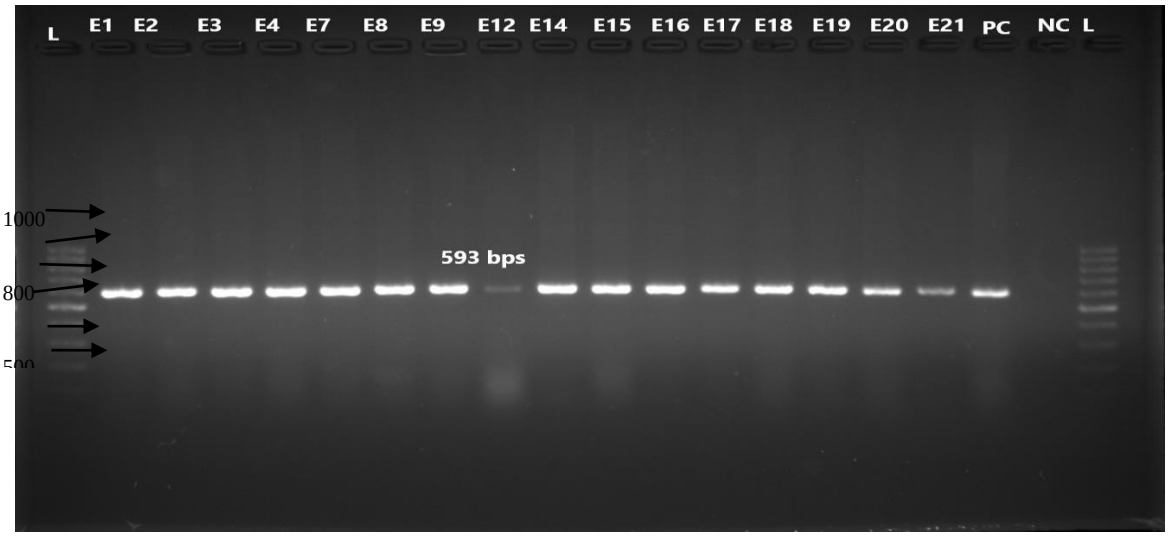

Figure 4.4: Electrophoresis gel for *bla* CTX-M (593 bp) L- Molecular Ladder; NC-Negative Control; PC-Positive Control; bp- Base Pairs

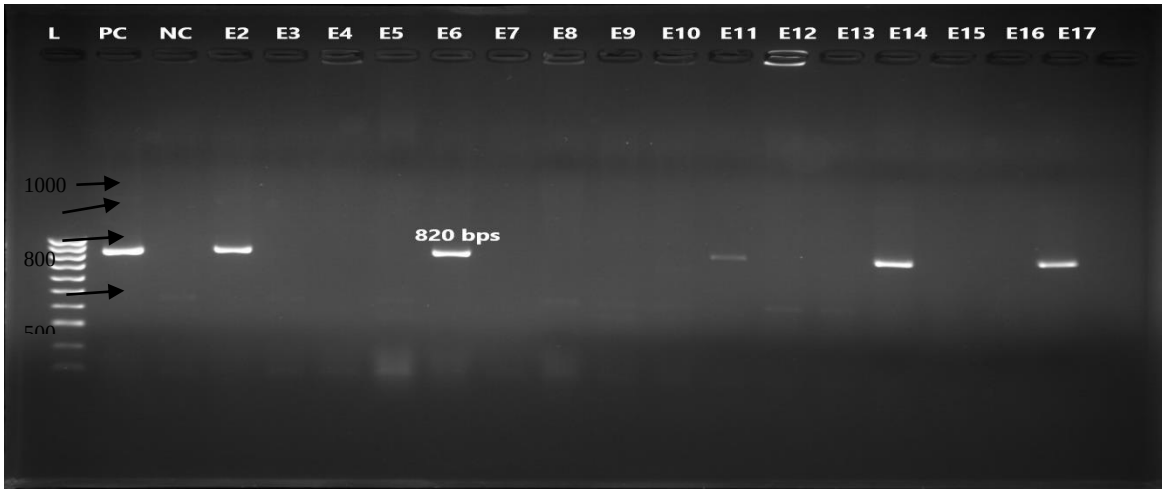

Figure 4.5: Electrophoresis gel for *bla* OXA (820 bp) L- Molecular ladder; NC-Negative Control; PC-Positive Control; bp- base pairs

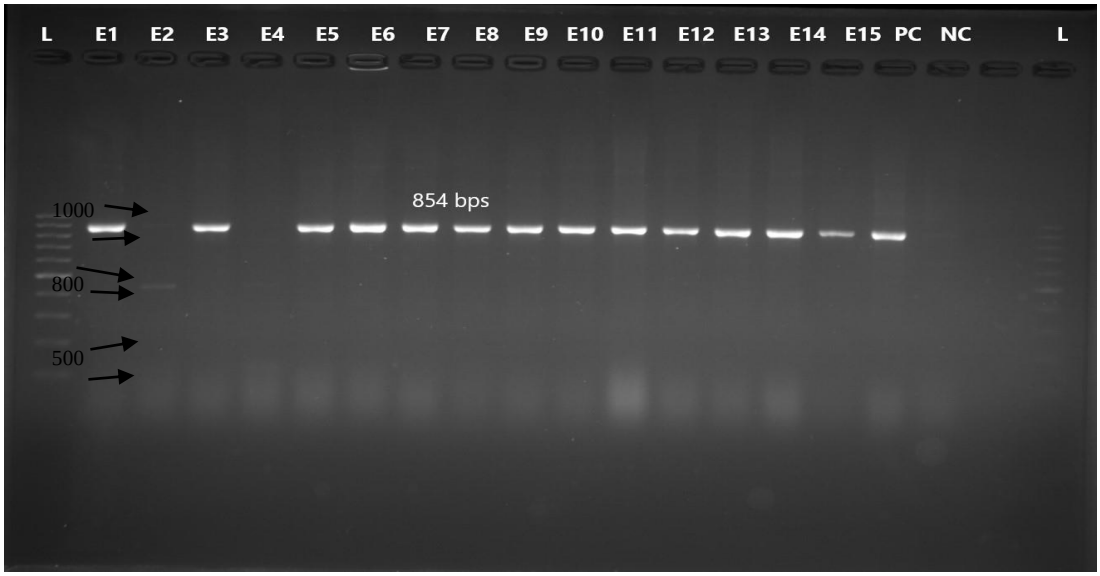

Figure 4.6: Electrophoresis Gel for *bla* SHV (854 bp) L- Molecular Ladder; NC-Negative Control; PC-Positive Control; bp- Base Pairs

4.5 Risk factors of ESBL Carriage among *Klebsiella spp*. Isolates

In the multivariable regression model adjusted for *a priori* confounders (age, site, and sex), any antibiotic administration during hospitalization was associated with a significant increase in the likelihood of *ESB*L-producing *Klebsiella spp* (aPR=4.51;

95%CI: 1.79-11.37, p<0.001). ESBL-producing *Klebsiella spp* isolates were more likely to be found in children who received ceftriaxone (aPR=1.65; 95%CI: 1.37-1.99, p<0.001) and chloramphenicol (aPR=1.32; 95%CI: 1.05-1.67, p=0.019) during hospital admission (Table 2). However, there was no evidence of an association between receiving gentamicin (aPR=0.87; 95%CI: 0.71-1.08, p=0.203) or penicillin (aPR=1.01; 95%CI: 0.80-1.29, p=0.907) on ESBL carriage. Compared to children who stayed in the hospital for 3 days or less, a longer hospital stay (>3 days) was associated with an increased risk of ESBL-producing *Klebsiella* spp. (aPR=1.42; 95% CI: 1.14-1.77, p=0.002). Children residing in households with access to treated water were significantly associated with a higher likelihood of *ESBL* carriage (aPR=1.36; 95%CI: 1.10-1.67, p<0.004). Living in a crowded household and open defecation were not significantly associated with β-lactamase-producing *Klebsiella* spp. Models were adjusted for Age, sex and site only (Table 4.4).

Table 4.4: Predictors of ESBL in *Klebsiella* Isolated from Stool Samples of the Participating Children

	ESBL+ N=154 (%)	ESBL- N=91 (%)	Model 1 PR	95% CI	p-value	Model 2: Adj. PR	95% CI	p-value
Location of the facility								
KISII	96 (62%)	48 (53%)	Ref					
HOMABAY	58 (38%)	43 (47%)	0.86	(0.70, 1.06)	0.152	0.85	(0.68, 1.03)	0.092
Child Characteristics								
Age (months)	14 (9-29)	18 (7-36)	1.00	(0.99, 1.01)	0.696	1.00`	(0.99, 1.01)	0.722
Age (months)								
0-5	23 (15%)	17 (19%)	Ref					
6-11	37 (24%)	20 (22%)	1.13	(0.81, 1.57)	0.469	1.18	(0.84, 1.64)	0.342
12-23	45 (29%)	20 (22%)	1.20	(0.88, 1.65)	0.244	1.26	(0.92, 1.73)	0.152
24-59	49 (32%)	34 (37%)	1.03	(0.74, 1.42)	0.872	1.06	(0.77, 1.46)	0.734
Sex								
Male	88 (57%)	52 (57%)	Ref					
Female	66 (43%)	39 (43%)	1.00	(0.82, 1.22)	1.000	1.02	(0.84, 1.46)	0.874
Hospitalization Information								
Length of hospital stay								
<4 days	51 (34%)	51 (57%)	Ref					
≥4 days	101 (66%)	39 (43%)	1.44	(1.16, 1.80)	0.001	1.42	(1.14, 1.77)	0.002

Any antibiotic used during admission								
NO	4 (3%)	22 (24%)	Ref					
YES	150 (97%)	69 (76%)	4.45	(1.80,11.04)	0.001	4.51	(1.79,11.37)	0.001
Ceftriaxone use during admission								
No	85 (57%)	57 (83%)	Ref					
Yes	65 (43%)	12 (17%)	1.43	(1.21, 1.69)	<0.001	1.42	(1.19, 1.71)	<0.001
Gentamicin use during admission								
No	64 (43%)	13 (19%)	Ref					
Yes	86 (57%)	56 (81%)	0.72	(0.61, 0.85)	<0.001	0.72	(0.60, 0.87)	<0.001
Chloramphenicol use during admission								
No	134 (89%)	66 (96%)	Ref					
Yes	16 (11%)	3 (4%)	1.27	(1.02, 1.58)	0.034	1.28	(1.01, 1.62)	0.042
Penicillin use during admission								
No	44 (29%)	10 (14%)	Ref					
Yes	106 (71%)	59 (86%)	0.78	(0.66, 0.93)	0.005	0.77	(0.62, 0.95)	0.014
Improved water source								
No	32 (21%)	21 (23%)	Ref					
Yes	122 (79%)	70 (77%)	1.05	(0.82, 1.34)	0.681	1.03	(0.81, 1.31)	0.836
Treated drinking water								
No	56 (37%)	45 (52%)	Ref					
Yes	96 (63%)	42 (48%)	1.28	(1.04, 1.58)	0.022	1.38	(1.12, 1.71)	0.003
Toilet								
			Ref					
Shared with ≥1 other household	71 (46%)	43 (47%)	0.98	(0.80, 1.19)	0.813	0.98	(0.80, 1.20)	0.847
Open defecation	8 (5%)	6 (7%)	0.90	(0.56, 1.44)	0.650	1.00	(0.60, 1.65)	0.993

Key: N-Population, %- Percentages, PR- Prevalence ratio, Adj. Prevalence Ratio- adjusted prevalence ratio.

CHAPTER FIVE

DISCUSSION / CONCLUSION AND RECOMMENDATION

5.1 Baseline Characteristics of Children Enrolled in the Study

Antimicrobial resistance (AMR) has become a worldwide public health issue resulting in more than 700,000 deaths per year globally, (Kalungia *et a*l., 2019) with low and middle income countries bearing the highest burden. This study reported that most of children colonized by Klebsiella infection were between the ages of 24-59 months. This contrasts with a study done in South Africa that reported most vulnerable group with *Klebsiella* infection colonization were children between 1-11 months of age (Verani *et al*., 2024). Similar findings were reported in Tanzania where majority of children were below 12 months (Tellevik *et al.*, 2016) and another study in the same region documented that , 205 out of 281 newborns were colonized with *Klebsiella* (Apondi *et al*., 2016).The high acquisition could be due to exposure to antibiotics.

Prevalence of *Klebsiella* infections was higher in males (57%) compared to females (43%) and similar findings were reported in SSA (Worku *et al*., 2024). However this study contradicts a study done in Nairobi, Kenya that documented majority of children colonized were females accounting for (56.67%) (Saisi *et al*., 2019). This contrast can be attributed to differences in immunity factors. Among children discharged from hospital in Western Kenya with *Klebsiella spp* isolated in fecal samples, resistance to commonly used antibiotics was common, and 62.8% were ESBL-producing, this finding is in agreement with previous studies (Gebremeskel *et al*., 2023; Taitt *et al*., 2017; Wairimu *et al.*, 2021). A study conducted in Gambia reported that ampicillin 179/197(90.8.7%), gentamicin 133/197(67.5%.) and ceftriaxone 117/197(59.3%) were the most prescribed antibiotics on children (Chaw *et al*., 2018). Similarly, in the present study, Children received antibiotics at hospital admission were 165/245(67%) and most commonly prescribed antibiotics were gentamicin 142/245(58%) and ceftriaxone 77/245(31%). Ceftriaxone and penicillin are used for initial treatment of severe infections especially in limited resource settings (Aluvaala *et al*., 2025).

5.2 Phenotypic Resistance of Antibiotics

The current study reported phenotypic AMR was associated with longer period of hospitalization and antibiotic use and these findings are consistent with previous findings from Kenya western region (Kariuki *et al.*, 2023; Tornberg-Belanger *et al*., 2022) and findings from studies conducted elsewhere (Chowdhury *et al.*, 2023). A high prevalence of non-susceptibility to 3rd generation cephalosporin's (cefotaxime, ceftriaxone and ceftazidime) was observed across study sites, and has been described elsewhere in sub-Saharan Africa (Doare *et al.*, 2015; Downie *et al.*, 2013; World Health Organisation, 2014). In this study, 62.6% of isolates were resistant to ceftriaxone, aligning with an Eastern Africa systematic review study that documented the level of resistance to ceftriaxone to be 46%-69% (Ampaire *et al*., 2016). In contrast a study done in the similar settings reported higher prevalence rate of over 80 % (Apondi *et al.*, 2016). Resistance to third generation cephalosporin's in *Enterobacteriaceae* is mainly conferred by the acquisition of B. lactamases or by deregulation of genetically encoded B- lactamase enzymes. Extended use of 3rd generation cephalosporin's is directly linked to emergence of resistance bacteria strains, particularly Gram negative bacteria producing ESBLs. Ceftriaxone is widely used in sub-Saharan Africa as first line empiric antibiotic in management of bacterial infections due to its broad spectrum (Meresa *et al.*, 2021). Higher resistance prevalence to 3rd generation cephalosporin's has been reported in previous studies in clinical samples (Chukwu *et al.*, 2022; Lester *et al.*, 2022). Resistance to beta-lactam antibiotics can occur via efflux pumps, altered penicillin binding sites, and beta-lactamases which cleave the beta-lactam ring and inactivate the target antibiotics (Pfeifer *et al.*, 2010). Spread of resistance occurs through clonal dissemination, horizontal genes transfer through plasmids, or translocation of resistance genes between mobile genetic elements. These antibiotics are widely used during hospitalization in the management of bacterial infections (Moges *et al*., 2021). Empiric antibiotic treatment is critical to pediatric hospital care, particularly where access to diagnostic tools such as bacterial culture and antimicrobial susceptibility testing are limited or unavailable. The current study reported carbapenems as the most effective antibiotics with susceptibility prevalence of 98%, findings that are consistent with other studies in our settings (Henson *et al*., 2017; Maveke *et al*.,

2024; Wairimu *et al*., 2021). While antibiotic use is often necessary during a hospital stay, reducing the length of hospital stay may help lower the risk of colonization with ESBL-producing *Klebsiella spp.*, which could complicate patient outcomes after discharge. Beta-lactams are the main antibiotics in the management of bacterial illnesses in sub-Saharan Africa and that ESBLs confer resistance to other antimicrobials that include aminoglycosides, quinolones, and co-amoxiclav (combined amoxicillin and clavulanic acid) (Kibwana *et al.*, 2020; Tornberg-Belanger *et al.*, 2022). In addition, the presence of these beta-lactamases is also associated with resistance to aminoglycosides especially gentamicin which is administered intravenously, for management of bacterial infections. Aminoglycosides are important options for treating life-threatening infections and are generally administered in combination with β-lactam agents (Becker & Cooper, 2013).The most common mechanism of resistance to aminoglycosides involves aminoglycoside-modifying enzymes (AMEs). These enzymes include acetyltransferases (Africa systemic review reported gentamicin resistance in 38.0% of tested *Klebsiella spp* isolates (Tadesse *et al.*, 2017) , another study within the same settings reported 44.0% isolates being resistance to gentamicin (Tornberg-Belanger *et al*., 2022), and these results are consistent with our findings that reported 58% resistance to gentamicin. The percentage of decreased susceptibility to gentamicin (58.0%), a common first-line antibiotics recommended by the WHO in the treatment and management of severe bacterial infections in resource limited settings (Downie *et al.*, 2013), was consistent with previous study conducted in sub-Saharan Africa (Williams *et al*., 2018). This study observed a high degree of susceptibility to cefoxitin likely because cephamycin antibiotics are less likely to be hydrolyzed by ESBLs (Tornberg-Belanger *et al*., 2022; Wairimu *et al*., 2021). Carbapenems' are potent members of the β - lactam antimicrobials that inhibit bacterial cell wall *(*Armstrong *et al.*, 2021) and their capability to possess broad spectrum in vitro activity has demonstrated their effectiveness in treatment of ESBL producing *Klebsiella spp* reported in this study.

5.3 Risk Factors Associated with ESBL Carriage

There was no evidence of an association between ESBL-producing *Klebsiella spp* and social-demographic factors suggesting that ESBL was more likely acquired during hospitalization. Age of children recruited in the study was not significantly associated with ESBL carriage (P-0722) similar findings were reported in Ghana (Akenten *et al*., 2023). However a study in Tanzania reported high ESBL carriage in infants (0-3 months) compared to older group (Tellevik *et al.*, 2016). This could be due to antibiotic usage in early days of life in management of infections. The high rates of AMR acquisition in *Klebsiella spp* during hospitalization is well established (Abban *et al*., 2023; Kariuki *et* al., 2023). For instance, 55% of the neonates admitted without ESBL in Kilifi, Kenya, acquired ESBL during hospitalization whilst only 10% had ESBL at admission to inpatient care (Kagia *et al*., 2019). A study done in Madagascar pediatric ward found prevalence of carriage of ESBL in stool to be 57% at hospital discharge and prevalence of carriage to be only 10% in both adults and children (Andriatahina *et al*., 2010). Children may acquire ESBL-producing pathogens from nosocomial infections during hospital admission or through colonization of ESBL-producing bacteria resulting from antimicrobial selection pressure (Andriatahina *et al*., 2010; Tellevik *et al.*, 2016; Tornberg-Belanger *et al*., 2022). Under antibiotic selective pressure, especially in intensive care units, intestinal colonization by ESBL-PE strains is favored among inpatients, increasing the carriage rates and the risk of nosocomial infections (Trick *et al*., 2001; Andriatahina *et al.,* 2010). In hospital settings, intestinal carriage is the main reservoir of these organisms and the current study reported ESBL carriage in patients who were hospitalized for more than four days. These results are in line with other findings in the region that reported longer hospitalization was associated with ESBL carriage (Kariuki *et al*., 2023). The gut colonization of inpatients is associated with a high risk for developing self and cross infections due to ESBL-producers, especially in long-term care units. The spread of multiresistant strains within the ward, including the spread to other wards of the hospital may be due to transmission from patients to others or from staff to patients.

Phenotypic AMR was associated with antibiotic use and duration of hospitalization, suggesting either selection for antibiotic-resistant bacteria or exposure to ESBL-producing bacteria during hospitalization. The association between ceftriaxone use and ESBL carriage has been previously described in the same settings (Tornberg-Belanger *et al*., 2022). Use of the ceftriaxone and chloramphenicol during the hospital stay was associated with a higher prevalence of ESBL while the use of gentamicin and penicillin was significantly associated with a lower prevalence of ESBL. Similar findings have been reported previously in Burkina Faso (Mahamat & Sore, 2022) on fecal carriage of extended spectrum beta lactamase producing *Enterobactericiae*. Resistance to chloramphenicol may have occurred through modification of antibiotic target sites or efflux pumps extrusion or carriage of plasmid mediated determinants, which reduce the effectiveness of the antibiotic. Chloramphenicol is used as first-line treatment for typhoid in Kenya and other East African countries (Chowdhury *et al*., 2023; Ministry of Health, 2016), and this may have been attributed to high prevalence of resistance to this antimicrobial. Antibiotic resistant bacteria may enter water sources from wastewater released from hospitals, household, or agricultural farmland (Manyi-Loh *et al*., 2018). The finding that children who have access to treated drinking water are at a higher risk of β-lactamase-producing *Klebsiella* spp. is surprising. The current study speculate that households with access to treated drinking water reside in areas where quality of water is assured by the municipality/local government or in areas where water quality is low and therefore requiring treatment at home (Gizachew *et al*., 2020). Most sub-Saharan countries use chlorine in the treatment of water (Sanganyado & Gwenzi, 2019). However, previous studies have reported that drinking chlorination water could contribute to the enrichment of antibiotic resistance bacteria and spread of antibiotic resistance genes, likely induced by underlying mechanism of cross or co-resistance to disinfectants (Jia *et al*., 2015; Shi *et al*., 2013).

Systematic review study in SSA reported that use of treated water by boiling demonstrated lower prevalence of ESBL carriage (Lewis *et al.*, 2020). Alternatively, households that have access to treated drinking water are likely to be those from a higher economic status who are able to access hospital services and antibiotics over

the counter more frequently which may result in an increase in AMR (Simegn & Moges, 2022).

5.4 Genotypic Characterization

ESBL production was detected in 154/245 (62.8%) of the isolates in this study. Similar findings were reported in Ethiopia 265/426 (62.2%) on ESBL production of clinical samples. CTX-M (92.2%) and SHV (92.2%) type ESBL were the most predominant ESBL gene variants detected in this study. This report is consistent with the global increase in CTX-M-type ESBLs witnessed in the last two decades (Badran *et al*., 2025, Saisi *et al*., 2019; Wairimu *et al*., 2021; Woerther *et al.,* 2013). Castanheira *et al.* (2021) reported TEM and SHV to be the most predominant ESBL genes produced in the past. The beta-lactamase genes *bla* CTX-M are responsible for the spread of ESBL-Enterobacterales (ESBL-E) worldwide (Matsumura *et a*l., 2017). *Bla* CTX-M are commonly spread via plasmids, which allows dissemination of multiple resistance genes at one point to Enterobacteriaceae family. The current study reported a high rate prevalence rate of *bla* TEM (57.1%) and this aligns with a previous study (60.1%) done in Egypt (Mohamed *et al*., 2020). Previous study on clinical isolates of *E. coli* and *Klebsiella pneumoniae* in two referral hospitals in Kenya reported TEM as the most predominant ESBL enzyme produced (Maveke *et al.,* 2024). A review of AMR isolates in East Africa found high prevalence rate of *bla* CTX-M genes being the most predominant genes isolated and these findings were consistent with our findings (Katale *et al.,* 2020). High rates of ESBL infections among gram negative isolates with 95% of those presenting *bla* CTX-M genes have also been reported in Ethiopia (Zeynudin *et al.,* 2018), similar findings were reported in Nigeria (Soge *et al.,* 2006), Tanzania (Mshana *et al*., 2013) and Malawi (Musicha *et al*., 2017; Tegha *et al.,* 2021). TEM- and SHV-type β-lactamases, primarily associated with *Klebsiella pneumoniae*, have spread throughout hospital settings, whereas CTX-M enzymes, predominantly associated with *Escherichia coli*, have become predominant in the community (Hijazi *et al*., 2016).

5.5 Co-Carriage of ESBL Encoding Genes

The co-carriage of ESBL genes observed in this study is consistent with findings from other studies (Ge *et al.*, 2022; Negeri *et al.*, 2023; Saisi *et al.*, 2019) . The current study observed isolates that contained up to 4 β-lactamase genes (*bla* CTX M/SHV/ TEM and OXA) inferring circulation of multiple plasmids. These findings align with a study done in Egypt (Badran *et al.*, 2025). Triple combination of *bla* CTX M/SHV/TEM was observed in 45 Klebsiella isolates (29%). However, a study done in Togo contradicts our findings *bla* CTXM/SHV/TEM accounted for (62%) (Diagbouga *et al.,* 2016) . In Tanzania (Mshana *et al.*, 2016) reported *bla* CTXM/TEM accounted for (11.96%) 11/92 and bla CTXM/SHV 10.87 (10/92) respectively. Co-existence of these genes combinations were also reported in this study *bla* CTXM/TEM 1.94 % (3/154), *bla* CTXM/SHV 28.57 % (44/154).

Poor hygiene and inappropriate use of antibiotics could be the key factors attributing to spread and carriage of multiple resistance ESBL genes. These genes are responsible for drug resistance in children under five years of age within Kisii and Homabay county hospitals. These findings are comparable to studies done in Kenya (Tornberg-Belanger *et al.,* 2022) on clinical *E.coli* isolates. The emergence of various resistant determinant ESBL genes with several co-existing genotypes is alarming limiting the available options for treatment thus highlighting the dire effects of AMR on public health.

5.6 Limitations

1. The study was conducted in two counties in Western Kenya and therefore, it is difficult to generalize the level of AMR to other settings.
2. An exhaustive panel of resistance genes was not studied due to limited resources.
3. The study did not account for the polyclonal nature of *Klebsiella spp.,* as only one colony was selected from a single culture plate for analysis.

5.7 Conclusion

1. This study observed a high rate of resistance to third generation cephalosporin's including Ceftriaxone (63.2%), cefuroxime (62.8%), and ceftazidime (59.6%). Meropenem and Imipenem were the most susceptible antibiotics in management of *Klebsiella* infections.
2. The current study concluded that the proportion of ESBL carriage among children under five years of age was 62.8% in Homabay and Kisii county hospitals and this contributes to treatment failure leading to serious pathological conditions.
3. The predominant genes detected were *bla* CTX M (92.2% and *bla* SHV (92.2%), highest co-carriage was observed in triple combination of *bla* CTX M/SHV and TEM. These genes encode enzymes that break down beta lactam antibiotics, leading to antimicrobial resistance.
4. Longer duration of hospitalization and prior antibiotic exposure were significant risk factors associated with ESBL carriage among children under five years of age. Longer hospital stay increase exposure to resistant organisms, resulting in higher healthcare costs and increased burden of ESBL producing *Klebsiella.*

5.8 Recommendation

1. Further research is required in Kisii and Homabay county referral hospitals to establish whether the spread of AMR in *Klebsiella Spp* starts at the community level or at the hospital.
2. Develop and enforce legislation on prescriptions for combating AMR by establishing antibiotic prescribing monitoring system and training of healthcare providers on treatment prescribing guidelines.
3. There is need to establish AMR stewardship programs at all levels of the health system due to high prevalence rate of antibiotics resistance observed in this study.
4. The lack of affordable AMR diagnostic tools, stewardship and surveillance programs implies that resistant pathogens will continue spreading from

hospital settings into the community. Adequate resources for equipment, and training of health providers to combat AMR in hospital and communities.

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APPENDICES

Appendix I: Ethical Approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel:(254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website.www.kemri.org

KEMRI/RES/7/3/1 **April 03, 2019**

TO: **DOREEN WANJIRA RWIGI,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CMR,**
NAIROBI

Forwarded & Congradulations
08/04/2019

Dear Madam,

RE: **KEMRI/SERU/CMR/P00105/3813 *(RESUBMISSION II OF INITIAL SUBMISSION)*: PHENOTYPIC AND GENOTYPIC ANTIMICROBIAL RESISTANCE PROFILES OF KLEBIELLA SPECIES FROM CHILDREN UNDER 5 YEARS OF AGE IN KISII AND HOMABAY COUNTY HOSPITALS**

Reference is made to your letter dated March 25, 2019. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on March 28, 2019.

This is to inform you that the Committee notes that the issues raised during the 283rd Committee C meeting of the KEMRI Scientific Ethics Review Unit (SERU) held on **January 31, 2019** have been adequately addressed.

Consequently, the study is **granted approval** for implementation effective this day, **April 03, 2019** for a period of one year. Please note that authorization to conduct this study will automatically expire on **April 02, 2020**. If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval by **February 19, 2020.**

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until a written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advice SERU when the study is completed or discontinued

Yours faithfully,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix II: Publication

BMC Microbiology

RESEARCH

Open Access

Phenotypic and molecular characterization of β-lactamase-producing *Klebsiella* species among children discharged from hospital in Western Kenya

Doreen Rwigi[1,2,3*], Andrew K. Nyerere[3], Mame M. Diakhate[4], Kevin Kariuki[1,2], Kirkby D. Tickell[4,7], Timothy Mutuma[1,2], Stephanie N. Tornberg[4], Olusegun O. Soge[4,5,6], Judd L. Walson[4,5,7,9,10], Benson Singa[1,4], Samuel Kariuki[1,2], Patricia B. Pavlinac[4,8] and Polycarp Mogeni[1,4*]

Abstract

Background The emergence and spread of β-lactamase-producing *Klebsiella* spp. has been associated with a substantial healthcare burden resulting in therapeutic failures. We sought to describe the proportion of phenotypic resistance to commonly used antibiotics, characterize β-lactamase genes among isolates with antimicrobial resistance (AMR), and assess the correlates of phenotypic AMR in *Klebsiella* spp. isolated from stool or rectal swab samples collected from children being discharged from hospital.

Methods We conducted a cross-sectional study involving 245 children aged 1–59 months who were being discharged from hospitals in western Kenya between June 2016 and November 2019. Whole stool or rectal swab samples were collected and *Klebsiella* spp. isolated by standard microbiological culture. β-lactamase genes were detected by PCR whilst phenotypic antimicrobial susceptibility was determined using the disc diffusion technique following standard microbiology protocols. Descriptive analyses were used to characterize phenotypic AMR and carriage of β-lactamase-producing genes. The modified Poisson regression models were used to assess correlates of phenotypic beta-lactam resistance.

Results The prevalence of β-lactamase carriage among *Klebsiella* spp. isolates at hospital discharge was 62.9% (154/245). Antibiotic use during hospitalization (adjusted prevalence ratio [aPR] = 4.51; 95%CI: 1.79–11.4, $p < 0.001$), longer duration of hospitalization (aPR = 1.42; 95%CI: 1.14–1.77, $p < 0.002$), and access to treated water (aPR = 1.38; 95%CI: 1.12–1.71, $p < 0.003$), were significant predictors of phenotypically determined β-lactamase. All the 154 β-lactamase-producing *Klebsiella* spp. isolates had at least one genetic marker of β-lactam/third-generation cephalosporin resistance. The most prevalent genes were bla_{CTX-M} 142/154 (92.2%,) and bla_{SHV} 142/154 (92.2%,) followed by bla_{TEM} 88/154 (57.1%,) and bla_{OXA} 48/154 (31.2%,) respectively.

Conclusion Carriage of β-lactamase producing *Klebsiella* spp. in stool is common among children discharged from hospital in western Kenya and is associated with longer duration of hospitalization, antibiotic use, and access

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to treated water. The findings emphasize the need for continued monitoring of antimicrobial susceptibility patterns to inform the development and implementation of appropriate treatment guidelines. In addition, we recommend measures beyond antimicrobial stewardship and infection control within hospitals, improved sanitation, and access to safe drinking water to mitigate the spread of β-lactamase-producing *Klebsiella* pathogens in these and similar settings.

Keywords Antimicrobial resistance, Beta-lactams, *Klebsiella* spp, Extended Spectrum Beta lactamases, Cephalosporins

Background

Antimicrobial resistance (AMR) is a leading cause of death worldwide, with the highest burden reported in sub-Saharan Africa (SSA) where approximately 1.2 million deaths in 2019 were attributed to bacterial AMR [1, 2]. Nearly all AMR deaths related to under 5-year-old occur in low- or middle-income countries (LMICs) [3] where AMR is associated with a substantial healthcare burden resulting from empirical antimicrobial treatment failure [4]. This translates to a considerable disease burden resulting from limited treatment options, more severe disease leading to longer duration of hospitalization, poorer clinical outcomes, and increased healthcare associated costs [3].

Klebsiella spp. are gram-negative commensal bacteria with pathogenic potential commonly found in the gut. *Klebsiella* spp. bacteremia, for example, has a case fatality rate of at least 30% [5]. Resistance to a commonly used class of antibiotics, beta-lactams, as measured by extended spectrum beta-lactamases (ESBLs), is associated with a 50% higher case fatality rates [5]. ESBLs are of public health concern because they not only suggests resistance to an entire class of antibiotics but can facilitate selection for resistance determinants in other antimicrobial classes, including aminoglycosides and fluoroquinolones [6]. According to Kenyan guidelines [7], initial treatments for suspected severe bacterial infections involve the use of two beta-lactam antibiotics, penicillin, or ampicillin, along with the aminoglycoside antibiotic gentamicin. Subsequently, intravenous ceftriaxone, a cephalosporin antibiotic, is recommended [8]. However, cephalosporin-resistant Klebsiella infections has posed challenges with these antibiotic regimens [8]. ESBL-producing *Klebsiella* spp. is a growing problem in SSA, where antibiotic options are already limited.

Nosocomial spread of *Klebsiella* spp. is prevalent, especially in crowded hospital environments where children are frequently exposed to antibiotics. This hospital settings serves as a particularly significant breeding ground for antibiotic resistant *Klebsiella* spp. Children returning home from hospital who develop infection with AMR *Klebsiella* may have limited treatment options and may spread these AMR bacteria within households and the community.

Although AMR bacterial infections pose a disproportionate public health challenge in SSA, epidemiological data are scarce. We conducted an AMR study that was nested within a clinical trial investigating the impact of azithromycin for prevention of morbidity and mortality in the six months following discharge from hospitals in western Kenya [9]. In this nested study, we sought to describe the proportion of phenotypic resistance to commonly used antibiotics, characterize β-lactamase genes among the phenotypically resistant isolates and assess the correlates of ESBL-producing *Klebsiella* isolates among children discharged from hospital in western Kenya.

Methods

Study design

In the parent trial [9], we systematically recruited children aged 1 – 59 months who were discharged from two county referral hospitals in Western Kenya between June 2016 and Nov 2019. In this nested cross-sectional study, we examine *Klebsiella* isolates collected at enrolment from two county hospitals in Western Kenya. The Kisii Teaching and Referral Hospital is located within the urban center in Kisii town whilst the Homa Bay County Teaching and Referral Hospital is in Homa Bay county. Kisii Teaching and Referral Hospital serves a population of about 1.2 million people with about 220,000 children under five years of age and serves as a major referral hospital in western Kenya [10]. Homa Bay County Teaching and Referral Hospital is classified as a level four healthcare institution, serving a predominantly rural population of around 1.1 million people. Homa Bay county has one of the highest under-five childhood mortality rates and HIV prevalence in the country [11].

Eligibility criteria included children who weighed at least 2 kg, had been hospitalized, recovered, and discharged from hospital, planned to remain in the study area for at least 6 months, had no contradiction to azithromycin, and had not been prescribed any macrolide antibiotics. We excluded children from the study if their hospital admission was solely due to trauma, injury, or birth defect, or if the legal guardian refused consent [9].

Prior to randomization, stool samples were collected from children, processed, and archived. Data

on demographics, medical history, underlying medical conditions, clinical examination, and nutritional anthropometry were collected on standardized paper questionnaires by trained study clinicians. In the current nested cross-sectional study, we utilized a random sample of 245 children whose enrollment stool samples had *Klebsiella* isolated, linked them to clinical data recorded during hospital stay, and demographic and social economic factors collected from the primary caregiver during enrollment.

Sample collection and processing

At enrollment, all children provided whole stool samples, or rectal swabs were used if whole stool collection was not feasible [9]. These samples were preserved in Cary-Blair media to ensure bacterial viability during transportation for microbiological culture. The samples were then promptly shipped to the central laboratory at the Kenya Medical Research Institute-Centre for Microbiology Research (KEMRI-CMR) in Nairobi within a 24-h timeframe. A swab or a sample of stool was streaked on MacConkey (MAC) (Oxoid, United Kingdom) and Eosin Methylene Blue agars (Oxoid, United Kingdom) and incubated in ambient air at 37 °C for 24 h. Morphologically distinct lactose fermenting mucoid colonies were subcultured onto Mueller Hinton (Oxoid, United Kingdom) agar and subjected to API 20E system (bioMérieux, Inc., France) and oxidase reactions for confirmation of *Klebsiella* spp. Confirmed *Klebsiella* spp. isolates were stocked in tryptone soy broth supplemented with 15% glycerol (Oxoid, United Kingdom) and frozen at -80 °C. For this analysis, the *Klebsiella* spp. isolates were thawed, quadrant streaked for isolation onto MAC agar and incubated at 37° C in ambient air to perform antimicrobial susceptibility testing (AST), DNA extraction and genetic characterization.

Antibiotic susceptibility testing

The antibiotic susceptibility profiles of the *Klebsiella* isolates were determined by the Kirby-Bauer disk diffusion method as described by CLSI [12]. The antibiotics panels used included ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), cefoxitin (FOX, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (CN,10 µg), amoxicillin-clavulanate (AMC, 20 µg/10 µg), meropenem (MEM, 10 µg), imipenem (IPM, 10 µg), azithromycin (AZM, 15 µg), and aztreonam (AZT, 30 µg). Zone diameters, measured in millimeters, established by CLSI-2020 M-100 were used to determine susceptibility, resistance, or an intermediate designation [12]. Both intermediate and resistant isolates were classified as non-susceptible [12].

Determination of ESBL–producing *Klebsiella* spp

ESBL production was determined using the double-disc diffusion synergy test, which utilizes cefotaxime and ceftazidime with and without clavulanic acid [12]. The discs were placed 20 mm apart on a lawn culture of *Klebsiella* spp. plated on MH agar and incubated at 37 °C for 24 h as described previously [12, 13]. Quality control was assured by simultaneously plating and testing an ESBL-producing *Klebsiella* strain (ATCC 700603) and an ESBL-negative *E. coli* strain (ATCC 25922) [13]. ESBL-producing *Klebsiella* spp. was confirmed if the difference in the zone size between cefotaxime and the zone size of cefotaxime with clavulanic acid was ≥ 5 mm or if the difference in the zone size between ceftazidime and the zone size of ceftazidime with clavulanic acid was ≥ 5 mm as established previously [12].

Genotypic detection of ESBL genes using conventional PCR

Bacterial DNA was extracted from ESBL-producing colonies of *Klebsiella* spp. using a boiling method. An inoculating loop was placed into bacteria pooled from an overnight culture in MH mixed with 0.5 ml nuclease free water. The cell suspension was heated for 10 min at 100 °C then centrifuged at 15,000 revolutions per minute for 5 min (maintained at 25 °C). The supernatant was used as DNA template for amplification. Extracted DNA was amplified using sets of primers targeting ESBL encoding genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}) as previously described [14–16]. Briefly, a final reaction volume of 25 µl was used in a master mix containing 0.5 µl forward primer (0.2 µM), 0.5 µl reverse primer (0.2 µM), 9.5 µl nuclease free water. A 12.5 µl mix which included Taq DNA polymerase (2.5 units), 1×PCR Buffer, $MgCl_2$ (0.2 µM), and ultrapure dNTPs (200 µM,) followed by addition of 2 µl template DNA was combined with the PCR master mix. Amplification conditions consisted of 30 cycles of 94°C for 30 s, 50°C, 55 C and 60°C for 30 s, initial extension of 68°C for 1 min and with a final extension step of 68 °C for 5 min [16]. Gel electrophoresis of PCR products was carried out at 200 V on a 1.5% agarose gel, stained with gel red stain and visualized on a Vilber E-Box gel documentation system. All PCR reactions were run with both negative and positive DNA control templates.

Definitions

Detailed descriptions of exposure variables and the derived variables has been provided elsewhere [9]. Briefly, we collected data on sex, child age, study site, HIV exposure, nutritional status, history of exclusive breast feeding, childhood vaccination (included pneumonia, rotavirus, measles, DPT, and BCG), length of hospital

stay, antibiotic use during hospitalization, caregiver reported income, caregiver education level, household toilet type, water source and treatment, and household crowding. A household had access to improved water if the caregiver reported access to reliable piped water in the dwelling or community, or if the household primarily used water from a borehole, a protected spring, a well with a pump, bottled water, or rainwater from storage tanks for household chores. Access to treated drinking water was defined as a household whose drinking water is filtered, boiled, or chlorinated before use. Household crowding was defined as a household with more than two individuals sharing a room. The 2006 WHO growth references for children age < 5-years were used to construct anthropometric z-scores. We defined underweight as weight-for-age z-score (WAZ) less than -2SD, stunting as a height-for-age z-score (HAZ) less than -2SD, and wasting as weight-for-height/length z-score (WHZ) less than -2SD. Data on vaccination was derived from childhood vaccination cards if the cards were available at the hospital. However, if the cards were not available, the caregiver provided a report on the child's vaccination status including the doses taken thus far. We derived an overall vaccine variable that defined children who had completed all essential age-appropriate vaccines, herein referred to "complete age-appropriate vaccination".

Statistical analysis

We reported the proportion of *Klebsiella* spp. isolates resistant to each tested antibiotic and carrying ESBL. To evaluate correlates of ESBL-producing *Klebsiella* spp., we constructed univariate and multivariable Poisson regression models with a robust variance for various child, hospital, and household factors, adjusting for key a priori confounders (age, sex, and site). Associations were considered statistically significant at an alpha < 0.05. All statistical analyses were performed in Stata (Version 17.0, Stata Corp, College Station, TX, USA).

Results

Baseline characteristics

Out of 1400 children enrolled in the parent trial, 461/1400 (32.9%) had *Klebsiella* spp. isolated from their stool samples and 245 of those with *Klebsiella* spp. isolated, were randomly chosen to be included in this substudy (Fig. 1). The 245 children had a median age of 15 months (IQR 8–30), 144/245 (58%) were from the Kisii site, 140/245 (57%) were male, 20/245 (8%) had severe wasting, and 18/245(7%) had moderate wasting. Diagnosis at discharge included 57/245 (23%) pneumonia, 19/245 (8%) diarrhea and 41/245 (17%) malaria cases. During hospitalization, 219/245 (89%) children had taken at least one antibiotic and 172/245 (70%) had taken more than 1 antibiotic: 77/245 (31%) received ceftriaxone, 2/245 (1%) received ciprofloxacin, 5/245 (2%) received cefuroxime, 142/245 (58%) received gentamicin, 19/245 (8%) received chloramphenicol, and 165/245 (67%) received penicillin. Further detailed description of the participants characteristics is shown by site in (Table S1).

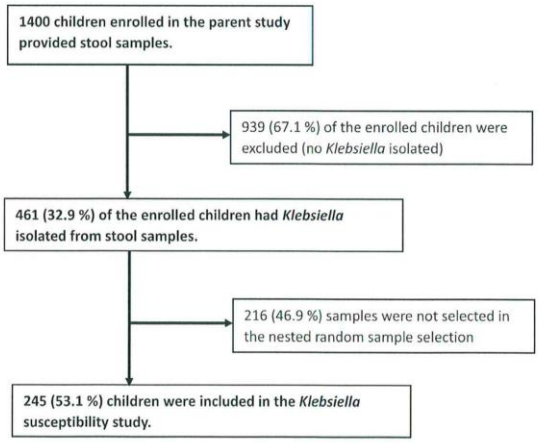

Fig. 1 Participant flow chart

Phenotypic and genetic AMR

Overall, 231/245 (94.3%) were *K. pneumoniae* and 14/245 (5.7%) were *K. oxytoca*. A total of 154/245 (62.8%) isolates were phenotypically ESBL positive, 148/231 (64.1%) *K. pneumoniae* and 6/14 (42.9%) *K. oxytoca*. More than half, 152/245 (62%) harbored *Klebsiella* spp. isolates that were resistant to a third-generation cephalosporin; more specifically, 155/245 (63.2%) were resistant to ceftriaxone, 154/245 (62.8%) were resistant to cefotaxime and 146/245 (59.6%) were resistant to ceftazidime. A total of 152/245 (62%) *Klebsiella* isolates were non susceptible to at least three categories of antimicrobials considered as multidrug resistant (MDR). Resistance to cefoxitin was only 12/154 (4.9%) and 143/245 (58.0%) were resistant to gentamicin. Furthermore, among the less commonly prescribed antibiotics in Kenyan hospitals, 103/245 (42%) were resistance to chloramphenicol, 79/245 (32%) to ciprofloxacin and 59/245 (24%) to azithromycin. In contrast, only 4 isolates were resistant carbapenem antibiotics (meropenem or imipenem) (Fig. 2).

At least one ESBL-conferring gene was detected in all the 154 ESBL-producing *Klebsiella* isolates genotyped. The $bla_{\text{CTX-M}}$ 142/154 (92.2%) and bla_{SHV} 142/154 (92.2%) were the most prevalent ESBL-conferring gene followed by bla_{TEM} 88/154 (57.1%) and bla_{OXA} 48/154 (31.2%). Over 90% (141/154) of the ESBL positive samples had multiple genetic markers, further details on co-carriage of genetic markers of resistance are shown in Table 1 segregated by species (Table 1). A total of 32/154 (20.8%) isolates co-carried all the 4 *bla* genes screened while majority of the isolates 61/154 (39.6%) had co-carriage of 3 β-lactamase genes. Only 13 isolates had either $bla_{\text{CTX-M}}$ or bla_{SHV} as the only β-lactamase genes present (Fig. 3). A further description of the phenotypic resistance against gene carriage among isolates that were positive for ESBL is shown in Table 2.

Table 1 Co-carriage of resistance genes among ESBL positive Klebsiella isolates segregated by *Klebsiella* species

Only one ESBL encoding gene	*K. pneumoniae* (*N* = 148)	*K. oxytoca* (*n* = 6)	Total
$bla_{\text{CTX-M}}$	4	0	4/154
bla_{SHV}	9	0	9/154
bla_{TEM}	0	0	
*bla*OXA	0	0	
Combination of 4 encoding gene			
$bla_{\text{CTX-M+SHV+TEM+OXA}}$	30	2	32/154
Combination of 3 encoding gene			
$bla_{\text{CTX-M+SHV+TEM}}$	45	0	45/154
$bla_{\text{SHV+TEM+OXA}}$	2	0	2/154
$bla_{\text{CTX-M+TEM+OXA}}$	4	1	5/154
$bla_{\text{CTX-M+SHV+OXA}}$	7	2	9/154
Combination of 2 ESBL encoding Genes			
$bla_{\text{CTX-M+SHV}}$	43	1	44/154
$bla_{\text{CTX-M+TEM}}$	3	0	3/154
$bla_{\text{SHV+TEM}}$	1	0	1/154

Risk factors of ESBL carriage among *Klebsiella* spp. isolates

In the multivariable regression model adjusted for a priori confounders (age, site, and sex), any antibiotic administration during hospitalization was associated with a

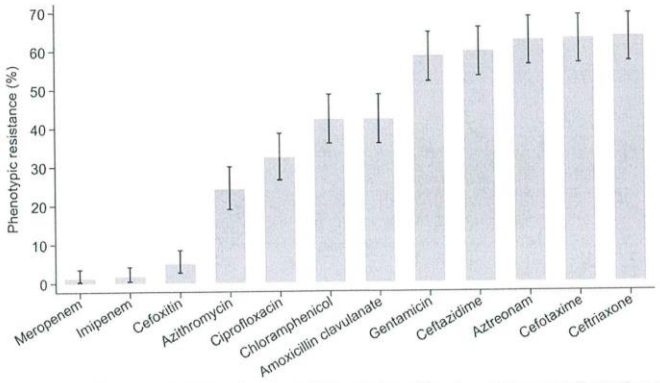

Fig. 2 Prevalence of phenotypic resistance in *Klebsiella* isolates from children discharged from hospital in western Kenya. The error bars represent 95% CI

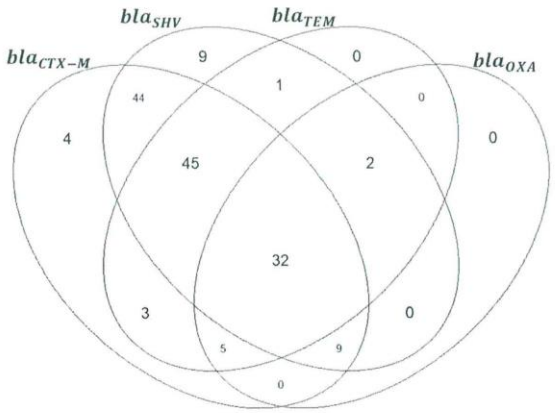

Fig. 3 Venn diagram showing co-carriage of resistance genes among ESBL positive *Klebsiella* isolates

significant increase in the likelihood of *ESBL*-producing *Klebsiella* spp. (aPR = 4.51; 95%CI: 1.79–11.37, $p < 0.001$). Among children who received at least one antibiotic during hospitalization, ESBL-producing *Klebsiella* spp. isolates were more likely to be found in children who received ceftriaxone (aPR = 1.42; 95%CI: 1.19–1.71, $p < 0.001$) and chloramphenicol (aPR = 1.28; 95%CI: 1.01–1.62, $p = 0.042$) but not gentamicin (aPR = 0.72; 95%CI: 0.60–0.87, $p < 0.001$) or penicillin (aPR = 0.77; 95%CI: 0.62–0.95, $p = 0.014$) during hospital admission (Table S2). Compared to children who stayed in the hospital for 3 days or less, a longer hospital stay (> 3 days) was associated with an increased risk of ESBL-producing *Klebsiella* spp. (aPR = 1.42; 95% CI: 1.14–1.77, $p = 0.002$). Surprisingly, children residing in households with access to treated water were significantly associated with a higher likelihood of ESBL carriage (aPR = 1.38; 95%CI: 1.12–1.71, $p < 0.003$). Living in a crowded household, open defecation, nutritional status, HIV status and household reported income were not significantly associated with β-lactamase-producing *Klebsiella* spp. (Table S2).

Discussion

Among children discharged from hospital in western Kenya with *Klebsiella* spp. isolated in stool samples, resistance to commonly used antibiotics was common, and over 60% were ESBL-producing. ESBL-conferring genes of high epidemiologic significance were found in ESBL-producing *Klebsiella* spp. isolates, most commonly $bla_{\text{CTX-M}}$ and bla_{SHV}. These findings highlight the burden of commensal AMR bacteria with the ability to cause infections in children discharged from hospitals in western Kenya and suggest the potential for disease and/or transmission during the period following discharge from hospital.

Antibiotics are an essential, and often life-saving tool for hospitalized children in settings of high infectious-disease related morbidity and mortality. Phenotypic AMR was associated with longer period of hospitalization and antibiotic use during hospitalization consistent with our previous findings [17, 18] and findings from studies conducted elsewhere [19]. We observed a high prevalence of non-susceptibility to 3rd generation cephalosporins (cefotaxime, ceftriaxone and ceftazidime) in our study sites, as has been described elsewhere in SSA [20–22]. These antibiotics are widely used during hospitalization in the management of bacterial infections [23]. Empiric antibiotic treatment is critical to pediatric hospital care, particularly where access to diagnostic tools such as bacterial culture and antimicrobial susceptibility testing are limited or unavailable. Therefore, while antibiotic use is often unavoidable during a hospital stay, it could be that limiting time in hospital could reduce the likelihood of carriage of ESBL-producing *Klebsiella* spp. that could pose problems during the discharge period.

Children leaving the hospital may be carrying ESBL producing *Klebsiella* acquired during their hospital stay. The high rates of AMR acquisition in *Klebsiella* spp. during hospitalization are well established. For instance, 55% of the neonates admitted without ESBL in Kilifi, Kenya, acquired ESBL during hospitalization whilst only 10% had ESBL at admission to inpatient care [24]. Children may acquire ESBL-producing pathogens from nosocomial infections during hospital

Table 2 Descriptive data on antimicrobial susceptibility testing and Beta lactamase genes among isolates that tested positive for ESBL

	ESBL Genes							
	bla_{CTX-M}		bla_{SHV}		bla_{TEM}		bla_{OXA}	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
	N = 12	N = 142	N = 12	N = 142	N = 66	N = 88	N = 106	N = 48
Chloramphenicol								
Susceptible	5 (42%)	63 (44%)	6 (50%)	62 (44%)	19 (29%)	49 (56%)	41 (39%)	27 (56%)
Non susceptible	7 (58%)	79 (56%)	6 (50%)	80 (56%)	47 (71%)	39 (44%)	65 (61%)	21 (44%)
Ciprofloxacin								
Susceptible	4 (33%)	71 (50%)	6 (50%)	69 (49%)	36 (55%)	39 (44%)	61 (58%)	14 (29%)
Non susceptible	8 (67%)	71 (50%)	6 (50%)	73 (51%)	30 (45%)	49 (56%)	45 (42%)	34 (71%)
Gentamicin								
Susceptible	2 (17%)	17 (12%)	1 (8%)	18 (13%)	4 (6%)	15 (17%)	14 (13%)	5 (10%)
Non susceptible	10 (83%)	125 (88%)	11 (92%)	124 (87%)	62 (94%)	73 (83%)	92 (87%)	43 (90%)
Ceftriaxone								
Susceptible	1 (8%)	0 (0%)	0 (0%)	1 (1%)	1 (2%)	0 (0%)	1 (1%)	0 (0%)
Non susceptible	11 (92%)	142 (100%)	12 (100%)	141 (99%)	65 (98%)	88 (100%)	105 (99%)	48 (100%)
Cefoxitin								
Susceptible	11 (92%)	133 (94%)	10 (83%)	134 (94%)	62 (94%)	82 (93%)	99 (93%)	45 (94%)
Non susceptible	1 (8%)	9 (6%)	2 (17%)	8 (6%)	4 (6%)	6 (7%)	7 (7%)	3 (6%)
Amoxicillin/ clavulanate								
Susceptible	6 (50%)	54 (38%)	6 (50%)	54 (38%)	29 (44%)	31 (35%)	42 (40%)	18 (38%)
Non susceptible	6 (50%)	88 (62%)	6 (50%)	88 (62%)	37 (56%)	57 (65%)	64 (60%)	30 (62%)
Aztreonam								
Susceptible	1 (8%)	3 (2%)	0 (0%)	4 (3%)	1 (2%)	3 (3%)	2 (2%)	2 (4%)
Non susceptible	11 (92%)	139 (98%)	12 (100%)	138 (97%)	65 (98%)	85 (97%)	104 (98%)	46 (96%)
Ceftazidime								
Susceptible	2 (17%)	6 (4%)	1 (8%)	7 (5%)	4 (6%)	4 (5%)	7 (7%)	1 (2%)
Non susceptible	10 (83%)	136 (96%)	11 (92%)	135 (95%)	62 (94%)	84 (95%)	99 (93%)	47 (98%)
Cefotaxime								
Susceptible	1 (8%)	1 (1%)	0 (0%)	2 (1%)	2 (3%)	0 (0%)	1 (1%)	1 (2%)
Non susceptible	11 (92%)	141 (99%)	12 (100%)	140 (99%)	64 (97%)	88 (100%)	105 (99%)	47 (98%)
Azithromycin								
Susceptible	7 (58%)	103 (73%)	8 (67%)	102 (72%)	53 (80%)	57 (65%)	83 (78%)	27 (56%)
Non susceptible	5 (42%)	39 (27%)	4 (33%)	40 (28%)	13 (20%)	31 (35%)	23 (22%)	21 (44%)
Imipenem								
Susceptible	11 (92%)	139 (98%)	12 (100%)	138 (97%)	64 (97%)	86 (98%)	103 (97%)	47 (98%)
Non susceptible	1 (8%)	3 (2%)	0 (0%)	4 (3%)	2 (3%)	2 (2%)	3 (3%)	1 (2%)
Meropenem								
Susceptible	11 (92%)	141 (99%)	12 (100%)	140 (99%)	66 (100%)	86 (98%)	105 (99%)	47 (98%)
Non susceptible	1 (8%)	1 (1%)	0 (0%)	2 (1%)	0 (0%)	2 (2%)	1 (1%)	1 (2%)

admission or through colonization of ESBL-producing bacteria resulting from antimicrobial selection pressure [18, 25, 26]. There was no significant association between ESBL-producing *Klebsiella* spp. and social-demographic factors including age, sex and site consistent with findings from other studies [27, 28]

The WHO recommends tailoring therapy to local AMR patterns in SSA, however, this is usually hindered by the lack of data about local antimicrobial susceptibility profiles due to a lack of reliable and consistent testing due to insufficient laboratory capacities [22]. As a result, hospitals in SSA rely on clinical syndromes and

the administration of broad-spectrum antibiotics in the treatment of serious bacterial infections [22]. Evidence shows that while syndromic diagnosis has a high sensitivity of detection, it is associated with low specificity therefore driving higher than necessary consumption of antibiotics, a key factor in the selection of antimicrobial-resistant bacteria [29]. In this African setting, most children received antibiotic during hospitalization which may explain the high rates of ESBL carriage. This is a cause of concern considering that beta-lactams are the mainstay in the management of bacterial illnesses in SSA. Cephalosporins mediate co-selection pressure conferring resistance to facilitating horizontal transfer of resistance determinants non-beta lactam antibiotics that include aminoglycosides and quinolones [18, 30].

Phenotypic AMR was associated with antibiotic use and duration of hospitalization, suggesting either selection for antibiotic-resistant bacteria or exposure to ESBL-producing bacteria during hospitalization. The association between ceftriaxone use and ESBL carriage has been previously described in the same settings [18]. Use of ceftriaxone and chloramphenicol during the hospital stay was associated with a higher prevalence of ESBL while the use of gentamicin and penicillin was significantly associated with a lower prevalence of ESBL, likely due to ceftriaxone exposure in the comparison group. Resistance to chloramphenicol may have occurred through modification of antibiotic target sites or efflux pumps extrusion or carriage of plasmid mediated determinants which reduce the effectiveness of the antibiotic. Chloramphenicol is used as first-line treatment for typhoid in Kenya and other East African countries [19, 31]. Carbapenems are broad spectrum members of the β-lactam antimicrobials with an additional β-ring which makes the antibiotic potent against beta-lactamases, attaching to the penicillin binding proteins of the cell wall thereby resulting in bacterial cell death [32] and are therefore effective in the treatment of ESBL producing *Klebsiella*. Additionally, carbapenems are expensive and not commonly prescribed, especially in public hospitals hence they are less likely to develop resistance.

The percentage of decreased susceptibility to gentamicin (58.0%), a common first-line antibiotics recommended by the WHO in the treatment and management of severe bacterial infections in resource limited settings [21], was consistent with previous studies conducted in SSA [29]. We observed a high degree of susceptibility to cefoxitin likely because cephamycin antibiotics are less likely to be hydrolyzed by ESBLs [18, 33]. Bla_{CTX-M} and bla_{SHV}-type ESBL were the most predominant ESBL gene variants detected in this area consistent with the global increase in bla_{CTX-M} type ESBLs witnessed in the last two decades [33–35]. The co-carriage of bla genes observed in this study is consistent with findings from other studies [34, 36, 37]. Intrestingly, we obaserved isolates that contained upto upto 4 β-lactamase genes inferring circulation of multiple plasmid [36] within the same genetic environment medaiting multidrug resistance phenotype. This phenomena has previouly been reported in other studies within the SSA and other parts of the world [34, 36–38].

Antibiotic resistant bacteria may enter water sources from wastewater released from hospitals, household, or agricultural farmland [39]. The finding that children who have access to treated drinking water are at a higher risk of β-lactamase-producing *Klebsiella* spp. is surprising. We hypothesize that households that reported access to treated drinking water were probably situated in areas linked to water supply systems that are vulnerable to contamination [40], particularly in urban or peri-urban settings. Alternatively, whilst most countries in SSA recommend the use of chlorine in the treatment of water [41], studies have reported that chlorinated drinking water may contribute to the enrichment of antibiotic resistance bacteria and therefore aid the spread of antibiotic resistance genes [42–44].

An important strength of our study is that few studies provide a comprehensive assessment of AMR among children discharged from hospital who are at a high risk of morbidity and mortality. The study was conducted in a rural and peri-urban setting in western Kenya and therefore provides important data on AMR from a low-income country with a high burden of bacterial infection. Our study also had important limitations. In recruiting children discharged from hospital, we excluded those that died during hospitalization, a population potentially at the highest risk of AMR carriage. Therefore, the burden of ESBL in *Klebsiella* spp. isolates that was observed is likely an underestimate of the true burden among hospitalized children. This analysis included only children with *Klebsiella* isolated thus selecting for patients with high amounts of healthcare exposure introducing a potential limitation on generalizability. We only collected stool samples at the point of hospital discharge, making it difficult to distinguish between AMR acquired during hospitalization and that acquired before admission. Furthermore, we are not able to definitively determine how resistance to the antibiotic classes was acquired. To do this, targeted PCR analysis or whole genome sequencing would be required. Our study was conducted in two counties of western Kenya and therefore difficult to generalize to other settings. Finally, we cannot exclude the possibility that the identified risk factors may be explained by confounders not accounted for in the analysis. Potential confounders may include unmeasured sociodemographic factors.

Conclusion

Carriage of β-lactamase producing *Klebsiella* spp. is common among children discharged from hospital in western Kenya and is associated with the duration of hospitalization and antibiotic use. The findings emphasize the need for continued monitoring of antimicrobial susceptibility profiles to inform the development and implementation of appropriate treatment guidelines. Measures beyond antimicrobial stewardship that include infection control within hospitals are needed to mitigate the spread and impact of β-lactamase-producing *Klebsiella* on the public health system. Efforts to improve diagnosis and detection of AMR pathogens within the healthcare system is needed to inform personalized therapeutics in these settings. Furthermore, research is needed to investigate the quality of treated drinking water and examine the effectiveness of the technologies used in the treatment of drinking water in these settings to inform public health policy change.

Abbreviations

AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
Bla	Beta Lactamase
CLSI	Clinical Laboratory Standard Institute
ESBL	Extended Spectrum Beta Lactamase
PCR	Polymerase Chain Reaction
WHO	World Health Organization
SSA	Sub Saharan Africa
LMIC	Low- and Middle-Income Countries
KEMRI	Kenya Medical Research Institute

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

Conceptualization and design: DR, OS, ST, BS, SK, JW, PP. Performed the experiments: DR, OS, ST, TM, KK, BS, PP. Data curation: DR, KT, MD, PM. Funding acquisition: JW, PP. Project administration and investigation: DR, AN, MD, KK, TM, OS, KT, ST, BS, SK, PP, PM. Formal data analysis: DR, PM. Writing original draft: DR. Writing review and editing: DR, AN, MD, OS, KT, TM, ST, KK, BS, SK, JW, PP, PM. All authors reviewed the manuscript.

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Availability of data and materials

All datafiles are available from Harvard Dataverse (https://doi.org/ https://doi.org/10.7910/DVN/CEJVKZ).

Declarations

Ethics approval and consent to participate

Ethical clearance for the main study was obtained from the Institution Review Boards (IRB) of the University of Washington and the Kenya Medical Research Institute (SERU 3086). Sample analysis and archiving was approved by the Kenya Medical Research Institute (SERU P001105/3813). Prior to recruitment, written informed consent was obtained from parents/caregivers of all participating children.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Publisher's Note

Appendix III: JKUAT Approval

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

OFFICE OF THE DIRECTOR, BOARD OF POSTGRADUATE STUDIES

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REF: **JKU/2/11/HSB321-0003/2017** 8TH NOVEMBER, **2022**

DOREEN WANJIRA RWIGI
C/o SOBMS
JKUAT

Dear, Rwigi,

RE: APPROVAL OF RESEARCH PROPOSAL AND APPOINTMENT OF SUPERVISORS

Kindly note that your MSc. research proposal entitled: **"PHENOTYPIC AND GENOTYPIC ANTIMICROBIAL RESISTANCE PROFILES OF KLEBSIELLA SPECIES FROM CHILDREN UNDER FIVE YEARS OF AGE IN KISII AND HOMABAY COUNTY HOSPITAL."** has been approved. The following are your approved supervisors:-

1. Prof. Samuel Kariuki
2. Dr. Andrew K. Nyerere

Yours sincerely,

PROF. LOSENGE TUROOP
DIRECTOR, BOARD OF POSTGRADUATE STUDIES
Copy to: Dean, SOBMS
/cao

Appendix IV: CLSI Guidelines

Clinical and Laboratory Standards Institute (2020) Guidelines on performance standards for Antimicrobial Susceptibility Testing.

ANTIBIOTIC	SENSITIVE	INTERMEDIATE	RESISTANT
Cefotaxime	≤ 26	23-25	≥ 22
Ceftriaxone	≤ 23	20-22	≥ 19
Ceftazidime	≤ 21	18-20	≥ 17
Cefoxitin	≤ 18	15-17	≥ 14
Aztreonam	≤ 21	18-20	≥ 17
Imipenem	≤ 23	20-22	≥ 19
Meropenem	≤ 23	20-22	≥ 19
Gentamicin	≤ 15	13-14	≥ 12
Azithromycin	≤ 13	-	≥ 12
Ciprofloxacin	≤ 26	22-25	≥ 21
Chloramphenicol	≤ 18	13-17	≥ 12
Amoxicillin-Clavulanate	≤ 18	14-17	≥ 13