PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* IN CLINICAL ISOLATES AT EMBU LEVEL FIVE HOSPITAL AND KENYATTA NATIONAL HOSPITAL, KENYA.

SYLVIA MWELU MAVEKE (BPHARM)

A Thesis Submitted in Partial Fulfilment of the Requirements for the Award of Master of Science Degree in Pharmacology and Toxicology

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY
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DECLARATION OF ORIGINALITY

UNIVERSITY OF NAIROBI

Name of Student: SYLVIA MWELU MAVEKE

Registration Number: J56/11242/2018

Faculty/School/Institute: VETERINARY MEDICINE

Department: PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

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Maveke Sylvia Mwelu (BPHARM)

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Signature……………………………………………………Date 21/11/2023 ……………………………..

Dr. Gabriel O. Aboge (BVM, MSc, Ph.D.),

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

Signature………   …………………Date 21/11/2023 ……………………………..

Dr Laetitia W. Kanja (BSc, MSc, Ph.D.),

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

DEDICATION

To my loving parents, Dr. Richard Musau and Scholastica Maveke, thank you for your endless support. My beloved sister, Rose Ndanu Maveke, I am forever grateful for your encouragement. May God bless you.

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ABBREVIATIONS AND ACRONYMS

AMR	Antimicrobial Resistance
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
CLSI	Clinical Laboratory Standards Institute
CTX-M	Cefotaxime- Munich
DNA	Deoxyribonucleic Acid
ESBL	Extended Spectrum Beta Lactamase
GLASS	Global Antimicrobial Surveillance System
MDR	Multi Drug Resistance
MHA	Mueller Hinton Agar
OXA	Oxacillinase
PCR	Polymerase Chain Reaction
SHV	Sulfhydryl reagent Variable
TEM	Temoneira
UTIs	Urinary Tract Infections
WHO	World Health Organization

ABSTRACT

Escherichia coli (*E. coli*) and *Klebsiella pneumoniae (K. pneumoniae)* are considered by the World Health Organization to be the predominant priority human bacterial pathogens. The pathogens cause blood stream infections, urinary tract infections, and hospital-acquired pneumonia. The production of extended spectrum β-lactamases (ESBLs) among those two pathogens have contributed to the occurrence and transmission of antimicrobial resistance against beta lactam antibiotics and various other categories of antimicrobial agents in hospital settings. These multi drug resistant (MDR) bacterial infections have led to limited therapeutic options, increased healthcare costs and mortalities. Therefore, this study established ESBL producing and multidrug resistant *E. coli* and *K. pneumoniae* isolated from patients in Embu Level 5 and Kenyatta National Hospitals, Kenya. The specific objectives were; (i) To identify the *E. coli* and *K. pneumoniae* infecting patients treated at Embu Level 5 Hospital and Kenyatta National Hospital (ii) To determine the phenotypic antimicrobial resistance profiles of *E. coli* and *K. pneumoniae* infecting patients treated at Embu Level 5 Hospital and Kenyatta National Hospital (iii) To establish the genetic determinants responsible for the resistance phenotypes of *E. coli* and *K. pneumoniae* including MDR isolates.

A cross-sectional laboratory-based study design was adopted whereby 138 *E. coli* and 127 *K. pneumoniae* samples were collected from various clinical specimens at the two health facilities from January 2020 to Feb 2021. The isolates were analyzed at the Pharmacology, Public Health and Toxicology Laboratory, UoN. Phenotypic confirmatory disk diffusion tests for ESBLs and antibiotic susceptibility testing were done according to the Clinical Laboratory Standards Institute guidelines (2020). Molecular analysis was done through conventional Polymerase Chain Reaction (PCR) utilizing primers for *gadA*, *rpoB* and selected ESBL genes. Forty-two representative samples were taken for sequencing and

blasting with accession numbers being assigned through the National Centre for Biotechnology Information genetic sequence database (NCBI GenBank). Data analysis was done using GraphPad prism® version 9.3.1. The diameter zones of inhibition and ESBL genes detected were presented as percentages in Tables 4.3 to 4.10.

Overall, all the *E. coli* and *K. pneumoniae* isolates were positively identified using the *gadA* gene at 373bp and *rpoB* gene at 600bp respectively using PCR. Sequence analysis revealed that only 19% of the *K. pneumoniae* isolates from Embu level 5 Hospital showed 99% nucleotide identity while 100% of the *E. coli* detected 99% nucleotide identity. All the *E. coli* and *K. pneumoniae* isolates from Kenyatta National Hospital showed 99% nucleotide identity with their respective bacteria. Results of the study showed an overall high proportion of ESBL production of 82.6% (p=0.1919) for *E. coli* isolates and 92.9% (p=0.5995) for *K. pneumoniae* isolates. No significant difference was noted between the two health facilities.

Most of the ESBL positive *E. coli* samples at the two health facilities demonstrated resistance to ceftriaxone (69.6%, p=0.3488), levofloxacin (50.7%, p>0.9999) and azithromycin (44.2%, p>0.9999) with no significant difference. Sensitivity was shown towards meropenem (8.7%, p>0.9999), amikacin (2.9%, p>0.9999) and tigecycline (0.72%, p>0.9999). The MDR *E. coli* isolates were 39.9%(p>0.9999). Majority of the ESBL producing *K. pneumoniae* isolates from both facilities were resistant to ceftriaxone (91.3%), amoxicillin/clavulanic acid (70.9%) and cefepime (60.6%). There was no significant difference in level of resistance for the isolates collected from patients seeking treatment at both facilities for the three antibiotics (p>0.5). A few *K. pneumoniae* isolates were resistant to cefoxitin (9.4%), tigecycline (6.2%), meropenem (2.4%) and amikacin (0.8%). This level of sensitivity was not significantly different between the two health facilities. The MDR *K. pneumoniae* isolates were 13.4 % (p>0.9999).

The most frequent ESBL genes among the ESBL producing *E. coli* isolates were bla_{TEM} (42%, p=0.5379), bla_{SHV} (40.6%, p=0.2721) and bla_{OXA} (36.2%, p=0.3192) while the least frequent was $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}2}}$ (13.8%, p=0.2632). Among the ESBL producing *K. pneumoniae* isolates, the most frequent ESBL genes were bla_{TEM} (89%, p>0.9999), bla_{SHV} (82.7%, p>0.9999), bla_{OXA} (76.4%, p>0.9999) and $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}1}}$ (72.5%, p>0.9999) while the least frequent were $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}9}}$ (0.8%, p>0.9999) and $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}2}}$ (2.4%, p>0.9999). There was no $bla_{CTX\text{-}\mathrm{M\text{-}group\text{-}8/25}}$ gene detected in bacterial isolates in this study. The study shows that molecular identification of *E. coli* and *K. pneumoniae* clinical pathogens is crucial in the surveillance of bacterial pathogens in hospital settings for appropriate pharmacotherapy by clinicians.

According to this study's findings, there was a high proportion of ESBL production among both bacterial isolates which could lead an elevated use of last resort antibiotics at the two facilities. Furthermore, the antimicrobial resistance patterns exhibited towards extended spectrum cephalosporins, beta lactam/beta lactamase inhibitor combinations, fluoroquinolones and macrolides show the risk of co-resistance associated with ESBL producing isolates responsible for MDR. This emphasizes the importance of molecular surveillance of ESBL genes in the implementation of antimicrobial stewardship policies and infection prevention control protocols in clinical settings.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Escherichia coli (E. coli) and *Klebsiella pneumoniae (K. pneumoniae)* are classified under family *Enterobacteriaceae*. The bacteria are natural inhabitants of the human gut microbiota with several reservoirs found in the environment, wild and domestic animals. They can be transmitted through food, water or from one person to another through direct or indirect contact (Carattoli, 2008). However, some of these bacterial strains can cause septicaemia, brain abscess, urinary tract infections (UTIs), intra-abdominal infections, wound infections as well as hospital-acquired pneumonia (Paterson and Doi, 2017). Therefore, antibiotics such as tetracyclines, beta lactams, fluoroquinolones and aminoglycosides have been extensively used in hospital settings to treat these infections. In particular, extended spectrum β lactams such as oxyimino cephalosporins have been used for the therapy of life-threatening illnesses brought about by the two pathogens. The use of oxyimino cephalosporins has been necessitated by the therapeutic failure of first and second generation β lactams caused by extended spectrum β-lactamases (ESBLs) originating from *E. coli* and *K. pneumoniae* strains. Moreover, antibiotic misuse has been reported creating selective pressure leading to increased resistance and hence, restricting therapeutic options (Padmini *et al*., 2017).

These ESBL producing bacteria manifest resistant phenotypes to beta lactams like third generation cephalosporins and penicillins. Consequently because of this resistance to ESBLs there have been cases of reduced therapeutic efficacy, high cost of treatment and mortalities. ESBLs are plasmid associated bacterial enzymes which hydrolyse majority of the beta lactam antibiotics like the monobactam (aztreonam), penicillins in addition to cephalosporins thus reducing their clinical efficacy. However, carbapenems and cephamycins are resistant to this degradation. These enzymes can be inhibited by tazobactam, sulbactam or clavulanic acid which are beta-lactamase inhibitors (Paterson and Doi, 2017). ESBL enzymes are encoded by plasmid-mediated genes. These plasmids are known to harbour genes encoding co-resistance to different categories of antimicrobials like fluoroquinolones, aminoglycosides, tetracyclines and sulfamethoxazole-trimethoprim thus giving rise to multidrug resistant organisms (Padmini *et al*., 2017). The predominant ESBLs documented are encoded by *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ which belong to Ambler class A group 2be (Padmini *et al*., 2017; Paterson and Bonomo, 2005). In the past, *K. pneumoniae* was the major cause of global nosocomial outbreaks due to the SHV and TEM genotypes. The CTX-M enzymes evolving from *E. coli* were later implicated in community onset infections, especially the urinary tract isolates (Padmini *et al*, 2017). Presently, the most reported ESBL genotype globally is *bla*$_{\text{CTX-M-15}}$ (Padmini *et al*, 2017).

Surveillance studies carried out globally have documented high proportions exceeding 50% of ESBL producing *K. pneumoniae* and *E. coli* in Asia, Africa including Latin America (WHO, 2014). The Study for Monitoring Antimicrobial Resistance Trends (SMART) reported increased prevalence of the two pathogens including multidrug resistance to the frequently administered antimicrobials from 2005 to 2010 within North America. In the United States, elevated percentages of ESBL producing *K. pneumoniae* and *E. coli* have been documented among inpatients diagnosed with UTIs by the SMART program (Kaye and Pogue, 2015). The European Antimicrobial Resistance Surveillance Network (EARS-Net) documented a significant rise of resistance rates among ESBL producing *K. pneumoniae* and *E. coli* against carbapenems, aminoglycosides, extended spectrum cephalosporins and fluoroquinolones between 2012 and 2015 (Exner *et al*., 2017). A meta-analysis conducted in East African hospitals approximated the total combined ESBL proportion for these hospitals to be at 42 percent. The pooled proportion for Kenyan hospitals was estimated to be 47% while the major genetic variations were those encoding $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} (Sonda *et al.*, 2016).

In Kenya, a recent survey conducted at Kilifi County Hospital documented that over half of the *K. pneumoniae* isolates had ESBL production containing $bla_{\text{SHV-12}}$, $bla_{\text{SHV-2}}$, $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-27}}$ genotypes. Over 50 percent of the isolates demonstrated multidrug resistance (Henson *et al*, 2017). Production of ESBLs was noted in *E. coli* and *K. pneumoniae* isolates obtained at the Aga Khan University Hospital, Nairobi with $bla_{\text{CTX-M}}$, bla_{SHV} and bla_{TEM} being the predominant genotypes (Maina *et al*, 2017). A survey at the Kenyatta National Hospital (KNH) found that the predominant uropathogens were ESBL producing *K. pneumoniae* and *E. coli* from urine specimens at facility (Magale *et al*, 2015). Majority of the samples had resistance to levofloxacin, amoxicillin/clavulanate, cefoperazone, ampicillin, doxycycline, nalidixic acid and gentamicin. However, a major proportion of the isolates had high susceptibility to meropenem and imipenem. Another study at the Aga Khan University Hospital concluded that ESBL production in *K. pneumoniae* and *E. coli* resulted in high proportions of multidrug resistant isolates with high susceptibility towards nitrofurantoin and carbapenems (Maina *et al*, 2013). An analysis of β- lactamase phenotypes and genotypes in *E. coli* strains from clinical isolates over an 18-year period showed that 27% of the isolates had ESBL production (Kiiru *et al*., 2012). There was no resistance to carbapenems among the isolates with 30% being susceptible to piperacillin-tazobactam, cephamycins and cefepime. The findings in all the studies suggested that there was need to conserve the therapeutic benefits of β lactam antibiotics alongside surveillance of ESBLs was necessary to guide pharmacotherapy.

The regional differences in the distribution and proportions of ESBL producing *Enterobacteriaceae* dictates that, therapeutic recommendations should be governed by clinical guidelines obtained from local data (Sonda *et al.*, 2016). Embu Level 5 Hospital is one of the major teaching and referral hospitals serving Embu, Tharaka Nithi and Kirinyaga counties. The Kenyatta National Hospital (KNH) handles countrywide referral cases, has a well-established microbiology laboratory and has evaluated the AMR patterns among the patients served at the facility (Magale *et al.*, 2015). Data on AMR due to the two ESBL producing pathogens was currently not available at the Embu level 5 hospital. It was against this background that the study aimed to establish ESBL distribution patterns at the two hospitals to determine the genetic variation in strains and customise therapeutic interventions.

1.2 Statement of the problem

The WHO considers ESBL producing *E. coli* and *K. pneumoniae* priority human bacterial pathogens which require routine surveillance (WHO, 2017). The occurrence and spread of such bacterial strains have led to an increase in antimicrobial resistance against various classes of antimicrobial agents within clinical settings due to the multidrug resistance associated with these organisms (Alvarez-Uria *et al.*,2018; WHO, 2018). This has culminated in the extensive use of last reserve antibiotics like carbapenems for the management of illnesses arising from ESBL producing bacteria which increases the therapy costs and causes selective pressure against carbapenems (Rodriguez-Bano *et al.*, 2018). In Kenya, studies on the two ESBL producing bacteria depict the same trend in antimicrobial resistance towards the commonly used β lactam antibiotics like cephalosporins and penicillins with increased use of carbapenems being recommended (Magale *et al.*, 2015; Maina *et al.*, 2013). There is inadequate knowledge on antimicrobial resistance patterns brought about by ESBLs produced the two pathogens at Embu Level 5 Hospital despite the extensive usage of carbapenems like meropenem by the various inpatient departments. Kenyatta National Hospital provided additional antimicrobial resistance patterns on the same and offered more insight on therapeutic interventions as a teaching and referral hospital. Therefore, the study determined the antimicrobial resistance profiles and the genotypes of the two pathogens at both facilities.

1.3 Justification of the study

Lack of routine AMR surveillance and antimicrobial stewardship policies may eventually lead to increased health costs, mortalities and fewer treatment options especially in resource limited settings (WHO, 2018; Sonda *et al.*, 2016). The data generated in the study would inform the clinicians on the suitable therapeutic interventions for better clinical outcomes. The data would be used to understand the development and transmission of AMR within the two hospitals.

1.4 Objectives

1.4.1 Overall objective

To assess phenotypic and genetic determinants of antimicrobial resistance of ESBL-producing *Escherichia coli* and *K. pneumoniae* from patients seeking treatment in Embu Level 5 hospital and Kenyatta National Hospital, Kenya.

1.4.2 Specific objectives

1. To identify *E. coli* and *K. pneumoniae* infecting patients treated at Embu Level 5 Hospital and Kenyatta National Hospital.
2. To determine the phenotypic AMR profiles of *E. coli* and *K. pneumoniae* in patients treated at Embu Level 5 Hospital and Kenyatta National Hospital.
3. To establish the genetic determinants responsible for the resistance phenotypes of *E. coli* and *K. pneumoniae* including MDR isolates

1.5 Hypothesis

There is no ESBL production in *E. coli* and *K. pneumoniae* strains including MDR isolates amid patients treated at Embu Level 5 Hospital and Kenyatta National Hospital.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description and Taxonomy of *Escherichia coli* and *Klebsiella pneumoniae*

Escherichia coli and *Klebsiella pneumoniae* are gram negative rods classified under the *Enterobacteriaceae* family of bacteria. The *Escherichia coli* species is from the tribe Escherichieae and the genus Escherichia (Janda and Abbott, 2021; Yu *et al.*, 2021; Parija, 2012). *Escherichia coli* can be categorized as: "A, B1, B2, C, D, E, F, G and clade I phylogenetic groups" (Yu *et al.*, 2021; Sarowska *et al.*, 2019). Due to lactose fermentation, *Escherichia coli* forms pink flat colonies on MacConkey medium and don't form spores. It is motile given the existence of peritrichous flagella and is 1-3 x 0.4-0.7µm in size (Janda and Abbott, 2021; Parija, 2012).

Klebsiella pneumoniae is belongs to the Klebsielleae tribe and the genus Klebsiella. It is non motile, non-sporing, has a polysaccharide capsule and is 1-2 x 0.5-0.8 µm in size. (Janda and Abbott, 2021; Wyres *et al.*, 2020; Parija, 2012). There are distinct phylogroups of the *K. pneumoniae* species complex which have been designated as distinct species, namely, "*K. pneumoniae* (Kp1), *K. quasipneumoniae subspecies quasipneumoniae* (Kp2), *K. variicola subspecies variicola* (Kp3) *Klebsiella quasipneumoniae subspecies similipneumoniae* (Kp4), *Klebsiella variicola subspecies tropica* (Kp5), *Klebsiella quasivariicola* (Kp6) and *Klebsiella africana* (Kp7)" (Wyres *et al.*, 2020; Holt *et al.*, 2015).

2.2 Clinical infections arising from *Escherichia coli* and *Klebsiella pneumoniae* in humans

2.2.1 Clinical infections caused by *Escherichia coli*

Escherichia coli species present in the GIT can lead to endogenous infections such as UTIs and sepsis. Exogenous infections like gastroenteritis and neonatal meningitis can also manifest (Yu *et al.*, 2021; Sarowska *et al.*, 2019; Parija, 2012). UTIs are commonly caused by *E. coli* serotypes found in faeces. Bacteria originating from the GIT contaminate the urethra ascending to the bladder and disseminated to the prostate or kidney. Nephritogenic *E. coli* strains produce adhesins which prevent bacterial elimination in voided urine and hemolysin HlyA which lyses erythrocytes (Yu *et al.*, 2021; Sarowska *et al*., 2019; Parija, 2012).

Septicemia is due to invasion of the bloodstream by strains related with intra-abdominal infections or UTIs. The endotoxin or LPS of the bacteria cause a systemwide response causing disseminated intravascular coagulation and loss of life. Neonatal meningitis is caused by strains possessing the KI capsular antigen frequently found in the GIT of newborn infants and pregnant women (Yu *et al*., 2021; Sarowska *et al*., 2019; Parija, 2012).

Gastroenteritis is caused by exogenous infections arising from contaminated food or water by fecal *E. coli* strains. These strains can be categorized as shown in Table 2.1 (Yu *et al.*, 2021; Sarowska *et al*., 2019; Parija, 2012).

Table 2.1: Gastroenteritis caused by *Escherichia coli* strains

Causative *Escherichia coli* strain	**Disease caused**	**Mechanism of action**
Enteropathogenic *Escherichia coli* (EPEC)	Infant diarrhoea	Adhesion to epithelial cells in small intestine causing microvilli destruction.
Enterotoxigenic *Escherichia coli* (ETEC)	Traveler's diarrhoea	Heat-labile enterotoxins and adhesins in the small intestine.
Enteroinvasive *Escherichia coli* (EIEC)	Dysentery	Invasion and destruction of epithelial cells in large intestine.
Enterohemorrhagic *Escherichia coli* (EHEC)	Hemorrhagic colitis Hemolytic uremic syndrome (HUS)	Shiga toxins
Enteroaggregative *Escherichia coli* (EAEC)	Chronic diarrhoeal disease	Adhesion in small intestine. Heat-stable enterotoxin
Diffusely adherent *Escherichia coli* (DAEC)	Infant diarrhoea	Adhesion in small intestine.

2.2.2 Clinical infections caused by *Klebsiella pneumoniae*

Community-acquired pneumonia, bacteremia, sepsis, nosocomial infection and liver abscesses are some of the clinical infections caused by *K. pneumoniae* (Paczosa and Mecsas, 2016; Holt *et al.*, 2015; Parija, 2012).

Community acquired pneumonia is usually associated with *K pneumoniae* serotypes 1, 2 and 3 commonly accompanied by acute onset of chills, high fever, flu-like symptoms in addition to productive coughs with mucoid sputum (Paczosa and Mecsas, 2016; Holt *et al.*, 2015; Parija, 2012). UTIs are seen with indwelling catheters while nosocomial infections occur with invasive devices, prolonged hospitalization and poor health status (Paczosa and Mecsas, 2016; Holt *et al.*, 2015; Parija, 2012).

2.3 Virulence mechanisms of the two pathogens

2.3.1 *Escherichia coli* virulence factors

Siderophores remove iron from mammalian iron transport proteins while the endotoxin causes systemic endotoxic shock and prevents phagocytosis of the organism (Yu *et al.*, 2021; Sarowska *et al.*, 2019; Parija, 2012). *Escherichia coli* has the ability to sequester growth factors enabling it to contend for nutrients in host cells. The fimbriae ensure adherence to GIT and hinder serum killing. In *E. coli* strains causing diarrhea surface antigens such as K99 and K88 occur as fimbriae (Yu *et al.*, 2021; Sarowska *et al.*, 2019; Parija, 2012). The capsule comprises of hydrophilic K antigens which repulse the hydrophobic phagocytic cell surface and protects the bacterium from serum killing (Yu *et al.*, 2021; Sarowska *et al.*, 2019; Parija, 2012). The adhesins hinder elimination of the organism by flushing action of voided urine in the urinary tract or intestinal motility in the GIT. There are two types of exotoxins produced by *E. coli* strains; hemolysins and enterotoxins (Yu *et al.*, 2021; Sarowska *et al.*, 2019; Parija, 2012). Hemolysins are critical in uropathogenic *E. coli* strains. The significant enterotoxins are shiga toxins, heat labile and heat stable toxins. Shiga toxins Stx-1 and Stx-2 are cytotoxins (Yu *et al.*, 2021; Sarowska *et al.*, 2019; Parija, 2012). The heat labile toxin LT-I causes increased discharge of fluid into the gut lumen and the heat stable toxin STa triggers an increase in cyclic guanosine monophosphate (cGMP) levels by binding to guanylate cyclase which leads to increased secretion of fluids (Yu *et al.*, 2021; Sarowska *et al.*, 2019; Parija, 2012).

2.3.2 *Klebsiella pneumoniae* virulence factors

ESBL producing *K. pneumoniae* strains are highly virulent, possess capsular type K55 antigen in addition to an exceptional capability to disperse (Wyres *et al.*, 2020; Paczosa and Mecsas, 2016; Holt *et al.*, 2015; Parija, 2012).

The notable virulence factors are the capsule, lipopolysaccharide (LPS), siderophores, fimbriae/pili, porins, outer membrane proteins (OMPs), pumps, transporters and allantion metabolism (Wyres *et al.*, 2020; Paczosa and Mecsas,2016). The capsule is made up of K antigens (K1 to K78) and it protects the bacterium against the host immune response by preventing phagocytosis by immune cells. It also prevents mobilization of the early immune response (Wyres *et al.*, 2020; Paczosa and Mecsas, 2016; Holt *et al.*, 2015; Parija, 2012). The LPS is comprised of a core oligosaccharide, lipid A and an O antigen. It evades the bactericidal activity of cationic antimicrobial peptides and prevents disintegration of the bacteria by the complement membrane attack protein. Type 1 and 3 fimbriae aid in adhesion contributing to intrusion of bladder cells and biofilm production in the organism (Wyres *et al.*, 2020; Paczosa and Mecsas, 2016; Holt *et al.*, 2015; Parija, 2012).

The fimbriae also attach to abiotic surfaces like indwelling catheters or devices increasing the risk of colonization by *K. pneumoniae* strains. Siderophores possess a greater affinity for iron than the host transport proteins (Wyres *et al.*, 2020; Paczosa and Mecsas, 2016; Holt *et al.*, 2015; Parija, 2012). The OMPs inhibit cytokine production and reduce the ability of antimicrobial peptides to act on the organism. The porins are usually downregulated or under detected to decrease the antibiotic entry in ESBL producers and carbapenem-resistant strains (Wyres *et al.*, 2020; Paczosa and Mecsas, 2016; Holt *et al.*, 2015; Parija, 2012). The efflux pump AcrAB aid in the resistance against antimicrobial peptides therefore improving the bacterial fitness in the lungs. Acquisition of iron by the organism is done through the ABC iron transport system denoted *kfu* that is especially present in invasive clinical strains of *K. pneumoniae* originating from liver abscess. Allantoin metabolism is a process for the bacterium to acquire carbon and nitrogen from the host environment (Wyres *et al.*, 2020; Paczosa and Mecsas, 2016; Holt *et al.*, 2015; Parija, 2012).

2.4 Worldwide prevalence in hospital settings

Some reservoirs for outbreaks of multidrug resistant *Enterobacteriaceae* have been identified in hospital settings such as: colonized or infected patients, biofilms on medical devices, hand washing basins, standard toilets with a rim, drainage systems, hospital surfaces and foodstuff (Exner *et al.*, 2017; Paterson and Bonomo, 2005). Risk factors associated with ESBL bacterial infection include: previous use of third-generation cephalosporins, prolonged hospitalization, overcrowding in hospitals, bed sharing, increased age, paramedical use of herbs, insufficient decontamination of medical devices, international travel and inadequate laboratory capacity for routine surveillance (Onduru *et al.*, 2021; Sonda *et al.*, 2016; Paterson and Bonomo, 2005).

2.4.1 Prevalence in Europe, USA, Latin America and Asia-Pacific

Enterobacteriaceae isolates containing ESBLs have increased over the recent years. When it comes to ESBLs, CTX-M has overtaken SHV as the most common in recent years (Rawat and Nair, 2010). An increment in cephalosporin resistance in both pathogens was found in Latin America, Asia, and Europe, caused by the transmission of CTX-M enzymes, according to various studies (Castanheira *et al.*, 2021; Doi *et al.* 2017). The proportions of ESBL production varied between *E. coli* (5.2% to 20.1%) and *K. pneumoniae* (3.2% to 34.7%) isolates in different regions of the United States of America (USA) (McDanel *et al.*, 2017). A retrospective study of urine samples from 322 hospitals in the USA over a decade revealed ESBL phenotypes in *E. coli* (13.1%) and *K. pneumoniae* (12.1%) isolates. These phenotypes appeared to be frequently resistant to fluoroquinolones, cotrimoxazole and nitrofurantoin complicating pharmacotherapy (Aronin *et al.*, 2022).

The CANWARD surveillance study in Canada monitored ESBL producing *K. pneumoniae* and *E. coli* from January 2007 to December 2016 (Denisuik *et al.*, 2019). There was significant increased ESBL production in *E. coli* (3.4% to 11.1%; $p<0.0001$) and *K. pneumoniae* (1.3% to 9.7%; $p<0.0001$). Over 95 percent of the ESBL producing *E. coli* isolates were sensitive towards tigecycline, colistin, carbapenems and amikacin. Similarly, majority of the ESBL *K. pneumoniae* isolates were sensitive towards meropenem and amikacin. The CTX-M-15 gene was noted mostly among *E. coli* (64.2%) and *K. pneumoniae* (51%). A comparable study by Karlowsky *et al.*, (2021) documented that 97.2% of the ESBL producing *E. coli* (671) and *K. pneumoniae* (141) isolates in the CANWARD surveillance from January 2007 to December 2018 had multidrug resistance. However, they were susceptible to meropenem, ceftazidime/avibactam and amikacin and. The major MDR phenotype among ESBL producing *E. coli* was cefazolin-ceftriaxone-ciprofloxacin-cotrimoxazole while ESBL producing *K. pneumoniae* had gentamicin-amoxicillin/clavulanic acid-ceftriaxone-cotrimoxazole. The predominant gene observed was $bla_{\text{CTX-M-15}}$ in *E. coli* (62.3%) and *K. pneumoniae* (48.9%) isolates.

A study done in Brazil found the percentage in ESBL production in *E. coli* and *Klebsiella spp.* as 11% with significantly high resistance to aminoglycosides, cephalosporins, fluoroquinolones, penicillins, carbapenems, nitrofurantoin and tetracycline (Periera *et al.*, 2018). The predominant genes detected were the $bla_{\text{CTX-M-gp-1}}$ (58.3%) and $bla_{\text{CTX-M-gp-8/25}}$ (27.1%). In an analysis of 38 ESBL producing *E. coli* and 24 *K. pneumoniae* isolates in a Northen Portuguese medical centre, the predominant gene detected was $bla_{\text{CTX-M-15}}$ at 84.2% and 66.7% respectively. A diverse combination of AMR phenotypes was also noted with the *K. pneumoniae* isolates having SHV variants (Carvalho *et al.*, 2021).

The prevalence of ESBLs in several European countries, including France, Belgium, Italy, and Poland, has increased significantly (Sepp *et al.*,2019). Only 28% of Bulgaria's *E. coli* and *K. pneumoniae* isolates produce ESBLs, compared to 16% in Cyprus and 11% in Portugal, according to Shakya *et al.* (2017). Between 2013 and 2014, the predominance of *K. pneumoniae* in other *Enterobacteriaceae i*solates increased from 9.0 percent to 13.6 percent. *Escherichia coli* and *K. pneumoniae* isolates that produce ESBLs are fewer within US than in Europe. In 2000, Russia and Poland had the most elevated percentages of ESBL-producing *Enterobacteriaceae* isolates, nearly 50%, and 40%, respectively (Shakya *et al.*,2017). *K. pneumoniae* and *Escherichia coli* isolates which produce ESBL increased in frequency between 2050 and 2017 in both species (Shakya *et al.*,2017). According to Castanheira and colleagues (2021), the predominant ESBL gene in most isolates in the USA (95.1%), Europe (95.1%), Latin America (98.1%) and Asia-Pacific region (85.2%) is CTX-M (especially group 1 and group 9).

Table 2.2: Distribution of ESBL genes in Europe, USA, Latin America and Asia-Pacific regions

	ESBL phenotype			**ESBL genotype**		
Region	***E. coli***	***K. pneumoniae***	**Overall**	***E. coli***	***K. pneumoniae***	**Overall**
Europe	4.8%	19%	**8.6%**	4.5%	18.6%	**8.2%**
USA	16.4%	6.6%	**15.8%**	13.3%	11.8%	**12.8%**
Latin America	25.7%	41.8%	**31.6%**	24.1%	41.1%	**30.3%**
Asia- Pacific	15.2%	22%	**17.4%**	12.8%	21.1%	**15.4%**

Adapted from Castanheira *et al.*, 2021

A 12-year retrospective survey in Madrid, Spain analysed 39,980 urinary samples of *K. pneumoniae* (5422) and *E. coli* (34,564) isolates from hospitalized vs non hospitalized patients (Arana *et al.*, 2016). In hospitalized patients, the MDR proportions increased significantly in both *E. coli* (5.89% to 8.18%) and *K. pneumoniae* (2.38% to 9.35%) isolates. The recurrence of infections caused by MDR ESBL producing isolates was documented for *E. coli* (0.86% to 3.11%) and *K. pneumoniae* (1.09% to 7.72%). In the outpatients, a significant rise in the MDR proportions was documented in both *E. coli* (3.21% to 4.58%) and *K. pneumoniae* (0.08% to 3.78%) isolates. The infections caused by MDR ESBL producing isolates had the same significant trend for *E. coli* (0.18% to 1.08%) and *K. pneumoniae* (0.17% to 2.82%). There was increased resistance to amoxicillin/clavulanate documented. However, fosfomycin and meropenem retained their efficacy against majority of the MDR isolates.

2.4.2 Prevalence in Asia and the Middle East

Previous studies in Thailand done in various hospitals in the Kathmandu region have shown differing ESBL and MDR proportions. According to Nepal *et al.* (2017), 54.2% of the 177 isolates had MDR with 59% in *K. pneumoniae* and 52.9% *in E. coli.* The overall ESBL production in all the isolates was 34.5% distributed among *K. pneumoniae* (38.5%) and *E. coli* (33.3%). High sensitivity to imipenem (94.9%), piperacillin/tazobactam (89.3%), amikacin (89.3%) and cefoperazone/ sulbactam (88.7%) was noted. Another study established a high MDR proportion of 91.3% among 92 *Enterobacteriaceae* isolates with high resistance to fluoroquinolones and cephalosporins (Shilpakar *et al.*, 2021). The isolates were susceptible to carbapenems and polymyxin B. The ESBL producing frequency was documented as 17.4% with the highest proportion being *E. coli* (39.3%). Koirala and colleagues (2021) documented an overall MDR frequency of 232 (67.24%) isolates comprising of *K. pneumoniae* (18.55%) and *E. coli* (48.69%) out of the 345 isolates investigated. The overall ESBL production was noted in 135(58.18%) isolates distributed amid K. *pneumoniae* (18.55%) and *E. coli* (48.69%). The $bla_{\text{CTX-M}}$ gene appeared in 121 (89.62%) isolates encompassing *E. coli* (93.81%) and *K. pneumoniae* (78.94%). It was noted that the ESBL producers had a high resistance to imipenem and gentamicin was the most sensitive antibiotic (Koirala *et al.*, 2021).

A seven-year study on blood stream infections in 2 provinces in Thailand sought to establish the proportion of ESBL production amid isolates from 20 hospitals from 2008 to 2014(Sawatwong *et al.*, 2019). A noteworthy increased production of ESBLs in *E. coli* (19% to 30%) with a p value tend of 0.02 was noted while *K. pneumoniae* (27.4%) had no significant trend. High resistance to oxyimino cephalosporins was observed with susceptibility to carbapenems and amikacin noted. An overall prevalence in ESBL production in *K. pneumoniae* (27.7%) and *E. coli* (22%) was noted. An Iranian study carried out from September 2016 to August 2018 on *E. coli* (113) and *K. pneumoniae* (52) isolates from 5 tertiary care hospitals revealed susceptibility to colistin (90.3%) and tigecycline (82.4%) by the isolates (Sharahi *et al.,* 2021). The frequency of ESBL production was observed for *E. coli* (49.6%) and *K. pneumoniae* (26.6%) with the MDR proportion observed in 16.4% and 3% of the isolates each. The ESBL genes noted in both isolates were bla_{TEM} (59.4%), $bla_{\mathrm{CTX\text{-}M}}$ (46.7%) and bla_{SHV} (32.7%).

2.4.3 Prevalence in North, South and West African regions

A systemic review of the distribution of ESBL producing *Enterobacteriaceae* documented it as being <15% among 16 out of the 26 studies reviewed in 13 African countries (Tansarli *et al.*, 2014). There were extremely high and low percentages of ESBL production observed in Egypt (<75%), Malawi (0.7%) and Algeria (>30%) due to the differences in the clinical specimens used (from bloodstream, urinary tract or surgical site infections) or the antimicrobials available for use in the country. Storberg (2014) conducted a non-systemic analysis of studies reported from 2008 to 2012. In Northern Africa, class A ESBLs were predominant in Algeria (16.4% to 31.4%) and Egypt (11 to 24%) (Saravanan *et al.*, 2018; Sangare *et al.*, 2015). Class D and A ESBLs were noted in Guinea- Bissau (32%), Libya (16%), Morrocco (13% to 7.5%) and Tunisia (11.7% to 77.8%). A hospital-based study in Sudan showed 30.2% ESBL production in *E. coli* isolates (Saravanan *et al.*, 2018). In Eastern Africa, ESBL production was noted in Ethiopia (62.8%), Kenya (37.4%), Rwanda (38.3%), Madagascar (26%) and Tanzania (20% in mothers vs 30% in neonates) where class D and A ESBLs were predominant. In Central Africa class A (55.3%) and class D (82.8%) ESBLs were found in Cameroon with the Central Africa Republic having 11.3% ESBL production.

A study in Gabon observed the ESBL production in various clinical specimens as 15% (2009), 18.9% (2010), 13.5% (2011), 14.2% (2012) from predominant *K. pneumoniae* isolates (Saravanan *et al.*, 2018). In Western Africa, class A ESBLs were found in Ghana (49.4%), Mali (63.4% to 96%) and Niger (40%). The prevalence of class D and A ESBLs was observed in Nigeria (10.3 to 27.5%) and Senegal (10%). In South Africa, class D and A ESBLs had a prevalence of 8.8% to 13.1% in hospital settings. The predominant observed gene was $bla_{\text{CTX-M-15}}$ usually in combination with bla_{SHV} bla_{TEM} and bla_{OXA} (Saravanan *et al.*, 2018; Sangare *et al*., 2015; Storberg, 2014).

According to Ouédraogo *et al.* (2016), ESBL production occurred in *E. coli* (67.5%) and *K. pneumoniae* (26%) isolates obtained at the three main health facilities in Burkina Faso. The ESBL producing isolates had notable resistance ($p<0.05$) to cotrimoxazole (45%), gentamicin (89%), levofloxacin (82%) and ciprofloxacin (80%) while susceptible to imipenem and amikacin. The predominant genes observed were $bla_{\text{CTX-M group-1}}$ (94%) while $bla_{\text{CTX-M group-9}}$ (4%) were the least occurring. In Chad, a survey involving the three main hospitals used MALDI TOF-MS® for identification of 313 bacterial samples (Mahamat *et al.*, 2019). Out of 197 *Enterobacteriaceae* identified, ESBL production was observed in *E. coli* (63.8%) and *K. pneumoniae* (21.2%) isolates. The ESBL genes detected were $bla_{\text{CTX-M group-1}}$ (96.7%) while $bla_{\text{CTX-M group-9}}$ (4.1%). Eighty six percent of the resistant isolates harboured multiple ESBL encoding genes. More than half of the isolates displayed co-resistance to fluoroquinolones, cotrimoxazole and gentamicin while sensitivity was observed to carbapenems and amikacin.

In Côte d'Ivoire, a study done at a university hospital from April 2016 to June 2017 on 107 *K. pneumoniae* isolates documented 84% ESBL production (Müller-Schulte *et al.*, 2020). There was a high resistance observed to sulfonamides (99%), quinolones (81%) and aminoglycosides (79%) with susceptibility to meropenem (100%). It was documented that 71% of the strain harboured *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ gene combination. A study in Nigeria documented ESBL production in *E. coli* (69%) and *K. pneumoniae* (31%) isolates collected from urine samples in two hospitals (Mofolorunsho *et al.*, 2021b). Resistance to ciprofloxacin (83.3%), amoxicillin/clavulanate (83.3%) and ceftazidime (79.6%) was observed in ESBL producing *E. coli* isolates. *Klebsiella pneumoniae* isolates were unsusceptible to ciprofloxacin (75%), amoxicillin/clavulanate (83.3%), imipenem (100%) and cefotaxime (100%).

2.4.4 Prevalence in Eastern Africa

A meta-analysis of ESBL producing *Enterobacteriaceae* among East African hospitals estimated the overall pooled proportion to be 42%, varying in Ethiopia (30%), Kenya (47%), Tanzania (39%) and Uganda (62%) according to available data (Sonda *et al.*, 2016). Sonda and colleagues (2016) further reported the major ESBL genes as *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$. Another meta-analysis of East, Central and Southern African hospitals stated the random pooled prevalence of ESBL producing *Enterobacteriaceae* as 41% with CTX-M, SHV, TEM and OXA genes being detected (Onduru *et al.*, 2021). A survey done in South-west Ethiopia found the overall frequency in ESBL production to be 23% (17 isolates) among the *E. coli* (76.5%, n= 13) and *K. pneumoniae* (23.5%, n=4) isolates collected from urine samples. More than half of the EBSL producing isolates displayed coresistance to tetracycline, fluoroquinolones, cotrimoxazole, aminoglycosides and chloramphenicol. High sensitivity was observed towards amikacin (90.5%) and imipenem (100%) (Abayneh *et al.*, 2018). Teklu and colleagues (2019) carried undertook a study of 426 *Enterobacteriaceae* isolates from 4 labs in Ethiopia whereby the overall MDR was 68.3% with distributed among *E. coli* (35%) and *K. pneumoniae* (20%) isolates. The ESBL producers were mainly19% (*K. pneumoniae*) and

27.9% (*E. coli*) amidst an overall frequency of 57.7% among all the isolates (Teklu *et al.*, 2019).

In Rwanda, the production of ESBLs occurred in *Klebsiella spp.* (82.8%) and *E. coli* (68.5%) isolates collected at a referral hospital (Sutherland *et al.*, 2019). An elevated proportion of resistance to oxyimino cephalosporins noted. A survey done in Tanzania observed ESBL production in *E. coli* (22%) and *K. pneumoniae* (4%) isolates amid paediatric patients in three health facilities. More than 50% of both isolates were unsusceptible to gentamicin, cotrimoxazole and ciprofloxacin. Sensitivity was demonstrated towards amikacin, piperacillin/tazobactam and meropenem (Letara *et al.*, 2021). A comparable survey conducted in Tanzania by Silago and colleagues (2021) documented multiple ESBL genes in 75 *E. coli* and *K. pneumoniae* isolates producing ESBLs. At least one gene appeared in 93.3% of the isolates while 62.9% had a combination of bla_{TEM}, $bla_{\mathrm{CTX\text{-}M}}$ and bla_{SHV}. Majority of the genes observed were $bla_{\mathrm{CTX\text{-}M}}$ (98.6%), bla_{TEM} (85.7%) and bla_{SHV} (71.4%).

A previous study in Uganda documented the antimicrobial resistance to cefoxitin (22%), piperacillin/tazobactam (27%), amoxicillin/clavulanate (36%) as well as cotrimoxazole (70%) amidst *K. pneumoniae* and *E. coli* isolates acquired at different outpatient departments in the region. However, all the isolates were sensitive to imipenem. The production of ESBLs was found in 5.3% of the isolates while multidrug resistance was noted in *E. coli* (34.7%) and *K. pneumoniae* (60%) (Najjuka *et al.*, 2016). According to Baguma *et al.* (2017), there was ESBL production in *K. pneumoniae* (92%) and *E. coli* (87%) isolates obtained at a regional health facility in Uganda. A survey among pastoralist communities in Uganda documented high sensitivity to carbapenems among *E. coli* (99.4%) and *K. pneumoniae* (100%) isolates collected (Iramiot *et al.*, 2018). Production of ESBLs was observed in *E. coli* (12%) and *K. pneumoniae* (23%) in addition to elevated resistance noted towards cotrimoxazole, amoxicillin/clavulanate and ciprofloxacin. Multi drug resistance was noted in *K. pneumoniae* (82%) and *E. coli* (57%). A retrospective survey carried out at a teaching hospital in Kampala documented that 56.2% of *K. pneumoniae* (13.1%) and *E. coli* (43.1%) produced ESBLs (Mbyemeire *et al.*, 2021). The predominant genes harboured included *bla*$_{\text{CTX-M-15}}$ (8.4%), *bla*$_{\text{CTX-M-1}}$ (22.4%), *bla*$_{\text{TEM}}$ (27.3%) and *bla*$_{\text{SHV}}$ (42%).

2.4.5 Prevalence in Kenya

Previous studies in Kenya done at KNH revealed a significant ESBL production in *K. pneumoniae* (79%) and *E. coli* (61%) isolates obtained there. Significant resistance was noted towards amoxicillin/clavulanic acid, levofloxacin and ampicillin (Magale *et al.*, 2015). A retrospective review of VITEK 2® records at the same facility showed resistance to ceftriaxone (75%) and ceftazidime (66%) amidst *E. coli* isolates. A similar elevated resistance in *K. pneumoniae* isolates to ceftriaxone (82%) and ceftazidime (83%) was observed. High susceptibility was exhibited towards meropenem (76-87%) and amikacin (91-97%) by both isolates (Wangai *et al.*, 2019). The Kenya Medical Research Institute (KEMRI) carried out an 18-year survey from 1992 to 2010 whereby *E. coli* isolates from 13 health centres were analysed (Kiiru *et al.*, 2012). There was ESBL production observed in 27% of the isolates with *bla*$_{\text{CTX-M-15}}$ (24%) and *bla*$_{\text{CTX-M-14}}$ (29%) detected.

A tertiary private hospital in Nairobi, the Aga Khan University Hospital, conducted a review of *K. pneumoniae* and *E. coli* isolates whereby over 80% multidrug resistance was recorded with ESBL production exhibited by 159/336 (47.3%) isolates (Maina *et al.*, 2013). There was low carbapenem resistance and nitrofurantoin was recommended for uncomplicated urinary tract infections. A previous study at the same facility had documented the genotypes in24 *K. pneumoniae* and 28 *E. coli* ESBL producing isolates being *bla*CTX-M (88.5%), *bla*TEM (34%) and *bla*SHV (25%). There was significant association noted between the *bla*SHV and ceftazidime resistance (Maina *et al.*, 2011). Maina and colleagues (2017) further analyzed fifty-four multidrug resistant *E. coli* (35) and *K. pneumoniae* (19) isolates acquired at the same hospital for ESBL genes. The most common genotypes observed were bla_{TEM} (24%), bla_{SHV} (33%) and $bla_{\text{CTX-M}}$ (74%).

A study done in Eldoret at the Moi Teaching and Referral Hospital on *K. pneumoniae* isolates collected over 11 years from blood cultures revealed high resistance to ceftriaxone (87.2%), cefepime (85.4%), ceftazidime (69.7%), macrolides (87%) and tetracyclines (95%). There was a high prevalence of over 80% multidrug resistance recorded with susceptibility documented towards meropenem and amikacin (Apondi *et al.*, 2016). At the Kilifi County hospital, there was a high proportion ESBL production (79%) in *K. pneumoniae* isolates accumulated over 10 years from the nosocomial infections. There was notable detection of $bla_{\text{SHV-27}}$, $bla_{\text{SHV-12}}$, $bla_{\text{SHV-2}}$ as well as $bla_{\text{CTX-M-15}}$ ESBL genes. There was multi drug resistance in more than 50 percent among the isolates but sensitivity to carbapenems noted (Henson *et al.*, 2017).

Taitt and colleagues (2017) analyzed 87 *K. pneumoniae* isolates collected at 8 different locations through KEMRI and observed 36.7% multi drug resistance among them. Only 7 percent of the isolates were ESBL producers with bla_{SHV} (87%), bla_{TEM} (58%) and $bla_{\text{CTX-M-1}}$ (9%) detected. A private hospital in Nairobi conducted an analysis of the bacterial isolates collected in its microbiology laboratory between 2012 and 2016 (Lord *et al.*, 2021). There was a high proportion of AMR observed towards beta lactam/beta lactamase inhibitors (91.2%), cotrimoxazole (83.7%), penicillins (67.6%) and extended spectrum cephalosporins (52.9%) A study of ninety-five uropathogenic *E. coli* isolates from 6 hospitals through KEMRI showed 24.2% multi drug resistance with 24% ESBL production observed (Muriuki *et al.*, 2022). The ESBL genes observed were *bla*$_{\text{TEM}}$ (21.7%), *bla*$_{\text{SHV}}$ (95.6%) and *bla*$_{\text{CTX-M-1}}$ (95.6%).

2.5 Laboratory bacterial identification and characterization

Various techniques are usually deployed to identify bacterial strains based on microscopic observations, macroscopic properties of the colonial morphology, biochemical tests, mass spectrometry analyzing the bacterial proteome and genetic analysis through PCR (Książczyk *et al.*,2016). Selective culture media like eosin methylene blue (EMB) agar is used to pinpoint *E. coli* due to the formation of a green metallic sheen by the colonies. Lactose-rich MacConkey Agar has traditionally been used to cultivate *E. coli* and *K. pneumoniae* (Parija, 2012). Unique biochemical properties like lactose fermentation, utilization of citrate as a source of carbon, hydrogen sulphide gas production, degeneration of urea and conversion of tryptophan into indole can be investigated by use of selective or differential media (Książczyk *et al.*,2016). Phenotypic techniques are tedious and laborious compared to molecular tools which are faster, have higher specificity, sensitivity and reproducibility.

Automated commercial methods like Vitek® 2 compact and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDITOF MS) are used in identification. Vitek® 2 compact system is based on the biochemical properties whereby compact plastic cards consisting of minute amounts of selective or differential media are combined with a fluorescent and/or colorimetry-based system (Książczyk *et al.*,2016). The MALDI-TOF MS uses ionization and vaporization of huge stable biological fragments like ribosomal proteins or peptides which are highly conserved and abundant in various bacterial strains (Van Belkum *et al.*, 2017; Angeletti *et al.*, 2017b; Książczyk *et al.*, 2016; De Carolis *et al.*, 2014). Assorted algorithms are used to compare the protein fingerprint signatures generated to a catalogue of cross-reference spectra. Major disadvantages are the low distinction of closely related microbial isolates in mixed culture samples and the unavailability of mass spectra in the database (Van Belkum *et al*.,2017; Angeletti *et al.*, 2017b; Książczyk *et al*.,2016).

Molecular analysis is used to distinguish strains of the same species based on magnification of house-keeping genes, distinct to particular pathogens like those encoding elongation factors, ribosomal DNA or RNA polymerase genes, coupled with unearthing of distinct sequences for species in the PCR amplicons (Książczyk *et al*.,2016). PCR-based assays can be used to identify *E. coli* isolates using specific primers for the *gadA* gene as outlined by Doumith *et al*. (2012) and *K. pneumoniae* identification using the *rpoB* gene as designed for this study (Table 2.3).

Table 2.3: Biochemical tests for identification

Test	***Escherichia coli***	***K. pneumoniae***
Catalase	+	+
Oxidase	-	-
Indole	+	-
Methyl Red (MR)	+	-
Voges Proskauer (VP)	-	+
Citrate	-	+
Triple Sugar Iron (TSI)	+ Acid slant and butt	+ Acid slant and butt
Urea hydrolysis	-	+

Table 2.4: PCR assays for identification of *E. coli* and *K. pneumoniae*

Targeted gene	**PCR step**	**Temperature (°C)**	**Time**	**Cycle**
gad A	Initial denaturation	94	4 minutes	1
	Denaturation	94	30 seconds	
	Annealing	65	30 seconds	30
	Elongation	72	30 seconds	
	Final elongation	72	5 minutes	1
rpoB	Initial denaturation	95	7 minutes	1
	Denaturation	94	40 seconds	
	Annealing	57	40 seconds	35
	Elongation	72	90 seconds	
	Final elongation	72	7 minutes	1

2.6 Definition and Classification of ESBLs

ESBLs are plasmid associated bacterial enzymes which hydrolyze “penicillins, first-, second-, and oxyimino-generation cephalosporins as well as aztreonam, although they are susceptible to cephamycins and carbapenems. Such enzymes are inactivated by beta lactamase inhibitors like clavulanic acid, sulbactam and tazobactam” (Paterson and Bonomo, 2005). ESBLs are categorised in the molecular class A based on the “Ambler classification and functional group 2be as per the Bush-Jacoby-Medeiros system” (Paterson and Bonomo, 2005). The Ambler system defines the resemblance of the amino acid, but not the phenotypic characteristics, into four major classes (A to D). Metallo-β-lactamases are Class B are while serine β lactamases include classes A, C and D. According to the Bush-Jacoby-Medeiros scheme the inhibitor as well as substrate profiles represented within four groups inclusive of multiple subgroups (Table 2.4). This scheme is considered to be more clinically relevant (Rawat and Nair, 2010). ESBLs are disseminated in plasmids harbouring genes encoding co-resistance to diverse antimicrobials like tetracyclines, aminoglycosides, fluoroquinolones, cotrimoxazole and chloramphenicol (Paterson and Bonomo, 2005; Padmini *et al*., 2017).

Table 2.5: Classification of beta lactamases

Ambler class	Bush Jacoby Medeiros group	Active site	Enzyme type	Inhibition by Clavulanic acid	Substrates
A	2b, 2be, 2br, 2c. 2e, 2f	Serine	Broad spectrum β lactamases (TEM,SHV) ESBL(TEM, SHV,CTX-M) carbapenemases (KPC,GES, SME)	Yes, except 2br	Ampicillin, cephalothin Penicillins,3rd generation cephalosporins All β lactams
B	3	Zinc binding thiol group	carbapenemases (VIM,IMP NMD)	No	All β lactams
C	1	Serine	AmpC cephamycinases (AmpC)	No	Cephamycins, 3rdgeneration cephalosporins
D	2d	Serine	AmpC cephamycinases (CMY, DHA, FOX, ACC) Broad spectrum β lactamases (OXA) ESBL(OXA) carbapenemases (OXA)	Yes	Cephamycins, 3rdgeneration cephalosporins Oxacillin, ampicillin, Cephalothin Penicillins, 3rdgeneration cephalosporins All β lactams

Adapted from Padmini *et al.*,2017

2.6.1 SHV

Originally discovered in *K. pneumoniae* as chromosomally encoded enzymes, SHV lactamases were named for the sulfhydryl reagent variable due to inhibition by chloromercuribenzoate (Castanheira *et al.*, 2021). SHV-2, which originated from a point mutation in SHV-1, was the first ESBL detected in *K. ozaenae* in Germany (Rahman *et al.*, 2018). In many of the SHV type lactamases, mutations occur at Ambler positions 238(Gly to Ser) as well as 240 (Lys to Glu). Hydrolysis of ceftazidime occurs due to replacement of serine at position 238 while that of cefotaxime arises from substitution of lysine at position 240 (Castanheira *et al.*, 2021). Antibiotics like tigecycline, piperacillin and ampicillin, may be resistant to SHV-1 because it confers resistance on bacteria to tigecycline as well as piperacillin but not oxyimino substituted cephalosporins (Rawat and Nair, 2010). Clinical isolates of *K. pneumoniae, Pseudomonas aeruginosa* in addition to other *Enterobacterales* have been discovered to contain SHV-type ESBLs. The most frequent ESBL variants encountered in Enterobacterales have been SHV-5 and SHV-12. (Castanheira *et al.*, 2021).

2.6.2 TEM

TEM was detected from patient called Temoneira in an *Escherichia coli* isolate from Athens, Greece in 1965 (Shaikh *et al.*, 2015). TEM-1 enzymes have spread around as a result of the plasmids and transposons, and can currently be identified in a vast range of Enterobacteriaceae species, in addition to *H. influenzae* and *N. gonorrhoeae* (Rawat and Nair, 2010). Only penicillins and first-generation cephalosporins can be hydrolyzed by the TEM-1 enzyme (Rahman *et al.*, 2018). TEM-3 was originally reported to show elevated activity against oxyimino cephalosporins (Shaikh *et al.*, 2015). The amino acid residues that bestow the ESBL phenotype to TEM-type enzymes "include Gly238 and Glu240 found on the b3 β-pleated sheet; Glu104 positioned directly across from Gly238 Glu240 at the opening of the active-site cavity; and Arg164 stationed on the neck of the X loop" (Castanheira *et al.*, 2021). Notably, the changes to Gly238Ser and Glu240Lys seem to possess the greatest significance in the production of the ESBL phenotype.

2.6.3 CTX-M

CTX-M enzymes are plasmid encoded cefotaximases with higher affinity for cefotaxime hydrolysis compared to ceftazidime (Rahman *et al.*, 2018). These enzymes have enhanced sensitivity to tazobactam in comparison to sulbactam and clavulanate (Shaikh *et al.*,2015). The CTX-M enzymes occurred through horizontal gene transfer of chromosomal β lactamases from the *Kluyvera* spp. into conjugative plasmids or transposons (Shaikh *et al.*,2015). However, point mutations around the active site, the serine residue stationed at position 237 in every CTX-M enzyme, may have enhanced their capability to significantly hydrolyze ceftazidime (Rahman *et al.*, 2018; Peirano and Pitout, 2019). This is especially prevalent in the enzymes classified under the CTX-M-9 group (CTX-M-27) and CTX-M-1 group (CTX-M-15). Currently, there are 220 CTX-M-β-lactamases enzymes categorised as five groups depending on their amino acid sequences: "CTX-M-25, CTX-M-9, CTX-M-8, CTX-M-2 and CTX-M-1" (Rahman *et al.*, 2018; Peirano and Pitout, 2019). The 'CTX-M pandemic' signified the worldwide dissemination of $\textit{bla}_{\text{CTX-M}}$ genes in clinical isolates making them predominant ESBL types in the 2000s (Castanheira *et al.*, 2021).

2.6.4 OXA

These enzymes can hydrolyze cloxacillin and oxacillin at rates higher than 50% compared to benzylpenicillin (Shaikh *et al.*,2015). They are classified as functional group 2d and Ambler class D under the Bush-Jacoby-Medeiros scheme (Castanheira *et al.*,2021). *P. aeruginosa* has been found to be the most common carrier although numerous gram-negative bacteria have been implicated (Rawat and Nair, 2010). However, OXA-1 was the most frequent OXA-type beta-lactamase found in 1 to10% of *E.coli* isolates (Paterson and Bonomo, 2005; Shaikh *et al.*, 2015). Majority of the ESBL OXA enzymes are acquired from OXA-2 and OXA-10 (Castanheira *et al.*, 2021). It is noteworthy that microorganisms can become resistant towards all beta-lactam drugs when simultaneous production of a metalloenzyme capable of hydrolyzing a carbapenem occurs and an OXA enzyme capable of hydrolyzing aztreonam (Paterson and Bonomo, 2005; Rawat and Nair, 2010). Currently, 27 extended spectrum OXA enzymes have been described to hydrolyze penicillins and oxyimino cephalosporins (Castanheira *et al.*, 2021). *Bla*$_{\text{OXA-10}}$ weakly breaks down aztreonam, ceftriaxone and cefotaxime. Besides, OXA-11, -14, -15, -16, -17, -18, -19, -28, -31, -32, -35 and -45 bestow genuine resistance to cefotaxime, ceftazidime and aztreonam (Paterson and Bonomo, 2005; Rawat and Nair, 2010).

2.7 Phenotypic and molecular confirmation of ESBLs

Phenotypic testing for ESBLs involves screening isolates using an indicator cephalosporin to indicate resistance or diminished susceptibility while confirmation is done by testing for synergy between clavulanic acid and an oxyimino cephalosporin (CLSI, 2020). Disk-diffusion techniques can be applied for screening for ESBL production and antibiotic susceptibility testing in bacterial strains (Rawat and Nair, 2010). Specific zone diameters are used to detect the levels of ESBL production using aztreonam, cefotaxime, ceftazidime, cefpodoxime or ceftriaxone disks with two or more agents being used for increased sensitivity (CLSI, 2020). Broth microdilution methods can also be used with the same agents whereby growth at or over the screening antibiotic concentration is interpreted as an indication of ESBL production (CLSI, 2020). Quality control is done by concurrently testing using an ESBL-positive bacterial strain (*K. pneumoniae* ATCC 700603) and the ESBL-negative bacterial strain (*E. coli* ATCC 25922).

The combined disk technique recommends the application of ceftazidime (30 µg), cefotaxime (30 µg) disks coupled with clavulanic acid (10 µg) on Mueller-Hinton Agar (MHA) for ESBL confirmation in *K. pneumoniae* and *E. coli* strains (CLSI,2020). Confirmation of ESBL production is defined as a disparity of ≥5 mm amongst the zone diameters of whichever of the cephalosporin disks alongside its corresponding cephalosporin/ clavulanate disks. According to the CLSI (2020), broth microdilution assays can alternatively be conducted using predetermined concentrations of ceftazidime, cefotaxime and their combinations with clavulanic acid. Phenotypic confirmation is determined as ≥ twofold serial-dilution reduction in minimum inhibitory concentration (MIC) of whichever cephalosporin coupled with clavulanic acid contrasted with its MIC during separate analysis. The Double-disk synergy test uses oxyimino cephalosporins and amoxicillin/clavulanate are placed 30 mm apart on inoculated MHA. ESBL production is interpreted as a visible expansion of the margin in regards to the inhibition zone of the cephalosporin approaching amoxicillin/clavulanate disk (Rawat and Nair, 2010; CLSI, 2020).

Various commercially available methods are: the E test, Vitek ESBL test, MicroScan panels and BD Phoenix automated microbiology system (Rawat and Nair, 2010; Paterson and Bonomo, 2005). The Vitek ESBL analysis applies ceftazidime as well as cefotaxime alone or coupled with clavulanate. Automatic examination of every well is carried out when the growth control well has achieved a set baseline of about 4-15 hours of incubation. Comparison of the level of growth in the two wells indicates presence of ESBL as per a predetermined reduction (Paterson and Bonomo, 2005). Two gradients are contained within an E test ESBL strip (AB Biodisk, Solna, Sweden) including ceftazidime plus clavulanic acid on one end alongside ceftazidime at the opposite end. ESBL presence is considered when there is a difference in MIC is equal to or greater than 8 (Rawat and Nair, 2010).

MicroScan panels consist of anhydrous panels containing cefotaxime or ceftazidime plus ß-lactamase inhibitors combinations. The Becton Dickinson (BD) Phoenix Automated Microbiology System utilizes growth response to cefotaxime, ceftazidime, ceftriaxone, cefpodoxime alongside their combinations with clavulanic acid, for the observation of ESBLs (Rawat and Nair, 2010).

ESBL detection in MALDI-TOF MS involves the identification of cleavage products generated through hydrolysis of the β-lactam rings by these bacterial enzymes. This is after an incubation period of one to three hours of the antibiotic under investigation alongside the bacteria. Production of an alteration in the quantity of the antibiotic compound can be determined by the mass spectrometer thereby establishing the existence of ESBLs (Van Belkum *et al*.,2017; Angeletti *et al.*, 2017b; De Carolis *et al*., 2014). Molecular confirmation of commonly encountered ESBLs using multiplex PCR is an economical, fast and reliable technique which may be adapted to monitor their emergence and spread in hospital set ups or epidemiological surveys (Dallenne *et al*, 2010).

Table 2.6: PCR assay for identification of ESBLs

Targeted genes	**PCR step**	**Temperature (°C)**	**Time**	**Cycle**
$bla_{\text{CTX-M}}$	Initial denaturation	94	10 minutes	1
bla_{TEM}	Denaturation	94	40 seconds	
bla_{SHV}	Annealing	60	40 seconds	30
bla_{OXA}	Elongation	72	1 minutes	
	Final elongation	72	7 minutes	1

2.8 Antibiotic Resistance Mechanisms

2.8.1 Beta Lactams

These bactericidal antibiotics interfere with the cell wall structure through covalent binding with vital penicillin-binding proteins (PBPs) required in the cross-linking of peptidoglycans in gram-positive and gram-negative bacteria (Bush and Bradford, 2016). The resistance towards drugs is likely caused by one of four mechanisms: (1) blocking the drug's ability to bind to its target PBP, which is usually accomplished by altering the drug's existing PBPs or acquiring new PBPs; (2) the extruding of beta-lactam drugs due to efflux pumps; (3) drug hydrolysis by β-lactamase enzymes; and (4) absence or reduced expression of outer membrane proteins (Peterson and Kaur, 2018; Huletsky and Bergeron, 2017).

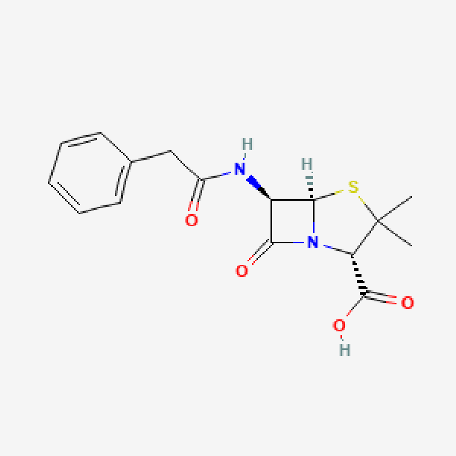

Figure 1: Structure of Penicillin (NCBI, 2021a).

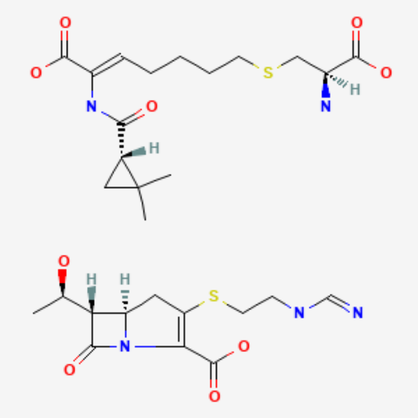

Figure 2: Structure of Imipenem (NCBI, 2021b).

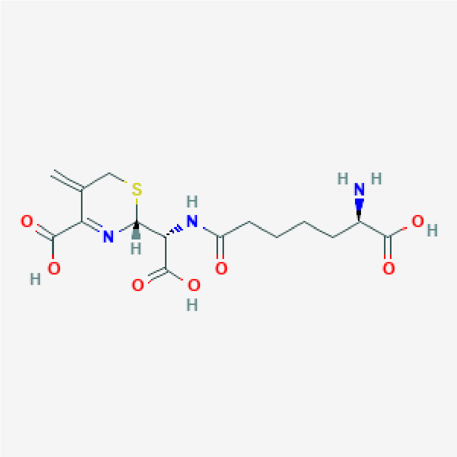

Figure 3: Structure of Cephalosporin (NCBI, 2021c).

2.8.2 Tetracyclines

Tetracyclines are bacteriostatic antibiotics that bind to the 16S rRNA reversibly near the ribosomal acceptor A position. This inhibits connection of aminoacyl tRNA to this position hindering the extension phase of protein synthesis. The most common resistance mechanisms include: (1) active efflux depleting the drug in the cytoplasm; (2) ribosomal preservation preventing ribosomal binding; (3) inactivation of the drug molecule and (4) rRNA mutations preventing binding (Peterson and Kaur, 2018; Huletsky and Bergeron, 2017).

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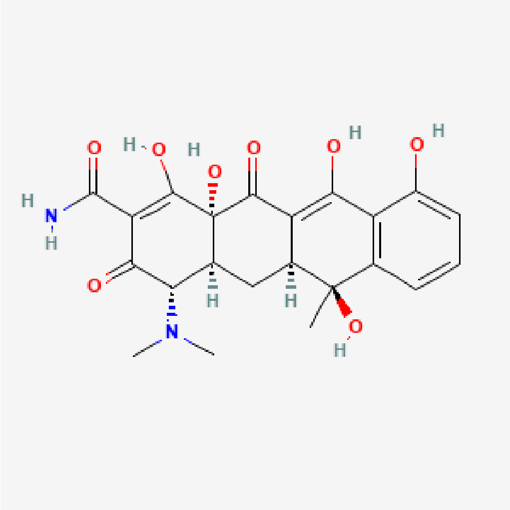

Figure 4: Structure of Tetracyclines (NCBI, 2021d).

2.8.3 Fluoroquinolones

Fluoroquinolones interact with topoisomerase IV as well as DNA gyrase which are crucial for bacterial DNA replication. These drugs combine with the structure comprised of the DNA and one of the 2 enzymes causing inhibition of this phase (Huletsky and Bergeron, 2017). Resistance to these antibiotics can be caused by; (1) chromosomal mutations in the drug target; (2) modified permeation or enhanced drug efflux causing reduced drug access to target enzymes; (3) qnr proteins which protect DNA gyrase against quinolone activity; (4) aac encoding aminoglycoside acetyltransferase encoded (5) acetylation of quinolones by 1b-cr, and (6) efflux pumps encoded by qepA (Peterson and Kaur, 2018; Huletsky and Bergeron, 2017).

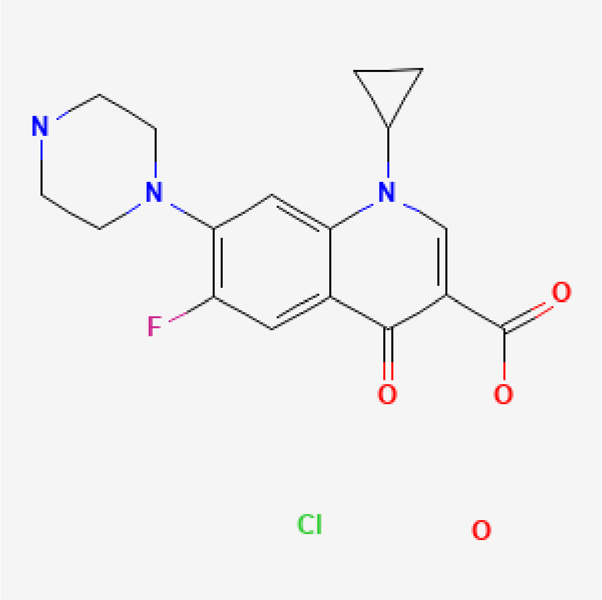

Figure 5: Structure of Ciprofloxacin (NCBI, 2021e).

2.8.4 Nitrofurans

Nitrofurantoin exhibits several antimicrobial mechanisms like inhibition of bacterial acetyl COA synthesis interfering in carbohydrate metabolism (McOsker and Fitzpatrick, 1994). There are nitro reductases found in *E. coli* which reduce nitrofurans into their active metabolites leading to antibacterial activity causing DNA lesions, inhibition of the RNA and protein biosynthesis plus oxidative stress (McOsker and Fitzpatrick, 1994; Van Hugh Le and Rakonjac, 2021). Antimicrobial resistance is caused by mutations which disrupt product activation by nitro reductase and *bla*$_{\text{CTX-M-14}}$ mutations which catalyzes nitrofuran hydrolysis (Van Hugh Le and Rakonjac, 2021). Recent synergistic interactions have been proposed whereby nitrofurantoin has been used in combination with deoxycholate and vancomycin for pharmacotherapy (Van Hugh Le and Rakonjac, 2021). Another strategy is collateral sensitivity whereby tigecycline resistance caused by loss of function mutation results in hypersensitivity to nitrofurantoin. Therefore, switching to nitrofurantoin from tigecycline in consecutive therapies eliminates resistant strains (Van Hugh Le and Rakonjac, 2021).

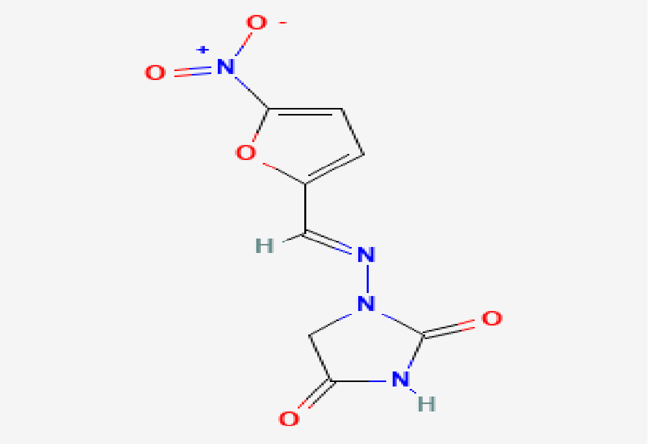

Figure 6: Structure of Nitrofurantoin (NCBI, 2021f).

2.8.5 Macrolides

These drugs reversibly attach to the 50S ribosomal subunit through peptidyl tRNA area, causing detachment of the peptidyl-tRNA molecule from the ribosome throughout elongation thus inhibiting protein synthesis (Huletsky and Bergeron, 2017; Fyfe *et al.*, 2016). The resistance pathways include modifications in the ribosomal target site via different transmitted erythromycin ribosomal methylases (erm) which methylate the adenine residues within 23S RNA or chromosomal gene mutations. Other mechanisms are active drug efflux through mef (A) and destruction of the drug by different enzymes like esterases, phosphorylases, lyases and transferases (Peterson and Kaur, 2018; Huletsky and Bergeron, 2017; Fyfe *et al.*, 2016).

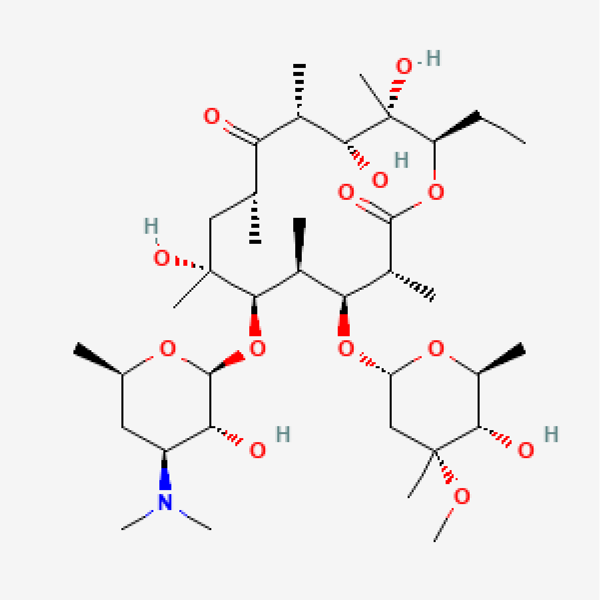

Figure 7: Structure of Azithromycin (NCBI, 2021f).

2.8.6 Aminoglycosides

These antibiotics hinder the translation process through attachment to the16S rRNA of the 30S ribosomal subunit (Huletsky and Bergeron, 2017). Enzymatic inactivation of these antibiotics by aminoglycoside modification enzymes (AMEs) can cause resistance through O-phosphotransferases (APH), O-adenyl transferases (ANT) and N-acetyl transferases (AAC) (Peterson and Kaur, 2018). The aminoglycoside antibiotic is phosphorylated, acetylated, or adenylated by each of these enzymes. Other resistance pathways encompass; (1) modifications in the ribosomal target site preventing binding; (2) decreased permeability of the cell membrane; (3) removal by efflux pumps and (4) methylation of the target site on the 16S rRNA (Peterson and Kaur, 2018; Huletsky and Bergeron, 2017).

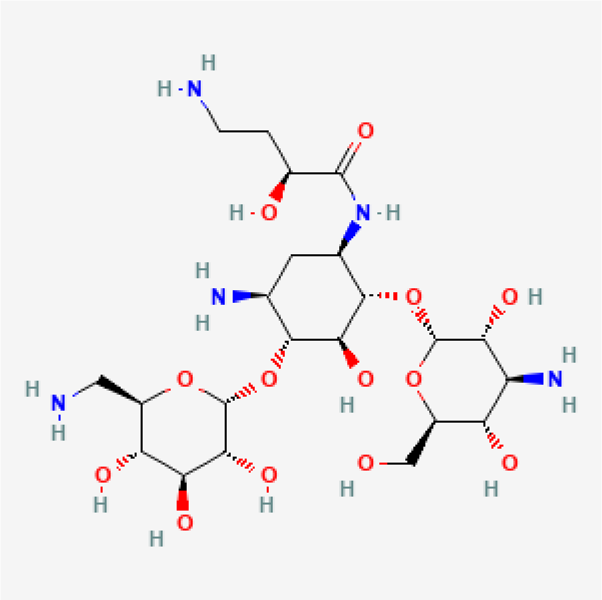

Figure 8: Structure of Amikacin (NCBI, 2021g).

2.8.7 Glycylcyclines

A variety of efflux pumps are found in the majority of bacteria, which are often the target sites for glycylcycline resistance (Fair and Tor, 2014). A tigecycline efflux system capable of pumping tigecycline out of the cytoplasm of microorganisms, thus, causing resistance to glycylcyclines (Figure 9).

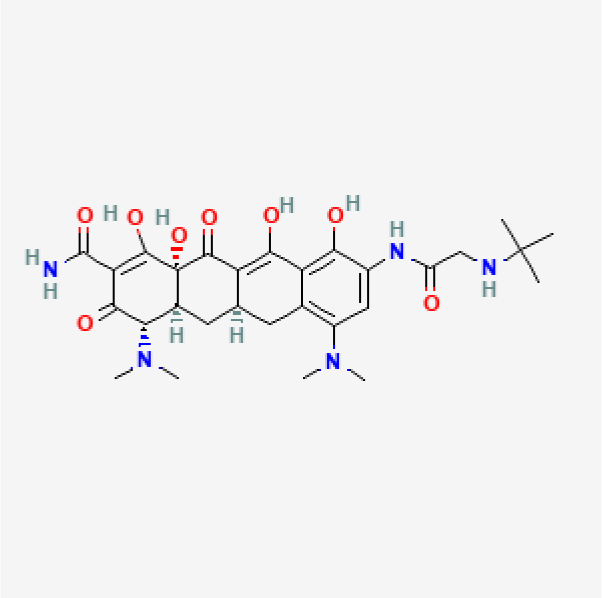

Figure 9: Structure of tigecycline (NCBI, 2021h).

2.9 Antimicrobial Susceptibility testing

The disk diffusion and broth microdilution methods can be applied for AST using specified antimicrobial disks or dilutions according to the bacterial isolates under investigation as described in the CLSI (CLSI, 2020). The zones of inhibition or minimum inhibitory concentrations are then utilized to classify the isolates as resistant, susceptible or intermediate as per the guidelines set (CLSI, 2020). The Vitek 2 is an automated method which determines the minimum inhibitory concentration of the antimicrobials under investigation versus the pure colonies of the microorganisms of interest using disposable AST cards and software. The findings are then described as resistant, susceptible or intermediate as per the CLSI protocols (CLSI, 2020). MALDI-TOF MS is a newer method currently applied in antimicrobial sensitivity testing. The most involving process is the sample preparation which involves in-house lysis filtration and lysis centrifugation. Other significant types of tests can be visualized: a) investigation of antibiotic alteration or destruction according to variations in mass of the antibiotic; (b) identification of proteomic differences in resistant against susceptible bacterial species in the with or without the presence of the antimicrobial agents; (c) semi quantitation of intracellular or extracellular antibiotics; and (d) distinguishing of altered antibiotic target sites (Van Belkum *et al.*,2017; Angeletti *et al.*, 2017b).

2.10 Current therapy against ESBL producing *E. coli* and *K. pneumoniae*

Pharmacotherapy against ESBL production in bacterial isolates impedes antibiotic therapy notably in patients ailing from complicated infections like bloodstream-associated infections due to the MDR phenotypes of produced by the microorganisms (Peirano and Pitout, 2019). Moreover, these infections consequently hinder the initiation of appropriate pharmacotherapy culminating in extended hospitalization, inflated costs as well as greater risks of patient fatalities (Peirano and Pitout, 2019).

Carbapenems like meropenem, imipenem, doripenem and ertapenem are resistant to hydrolysis by ESBLs and spread-out into distinct body tissues at significant concentrations. They are advantageous because there is a reduction of the inoculum effect (Peirano and Pitout, 2019). However, there are major limitations like the increased cost of the antibiotics in addition to selective pressure of carbapenem-resistant bacteria. Therefore alternative carbapenem-sparing therapies like β-Lactam/β-lactamase inhibitor combinations are generally preferred (Peirano and Pitout, 2019).

Piperacillin-tazobactam and amoxicillin-clavulanic acid have proven effectiveness against illnesses arising from production of ESBLs by the two pathogens in addition to being non-inferior when compared to carbapenems in UTI as well as biliary tract infections (Rodriguez-Baño *et al.*, 2018). Amoxicillin-clavulanic acid has proven to have no inoculum effect and is convenient for oral switch. It however has more than 40% resistance rates among ESBL producers and may not be readily accessible for intravenous use in many regions (Rodriguez-Baño *et al.*, 2018). However, piperacillin-tazobactam shows erroneous susceptibility with some computerized systems, has the inoculum effect and shows 5 to 30% resistance proportions among ESBL producers (Rodriguez-Baño *et al.*, 2018).

Ceftazidime–avibactam and ceftolozane–tazobactam display activity against higher proportions of susceptible isolates. Nonetheless, both are considered reserve drugs for OXA-48 or KPC producing *Enterobacteriaceae* and MDR *P. aeruginosa* infections respectively(Rodriguez-Baño *et al.*, 2018). Ceftolozane–tazobactam exhibits 10–30% resistance rates amidst ESBL producers (Rodriguez-Baño *et al.*, 2018).

The oxyimino cephalosporins like cefotaxime, ceftriaxone, ceftazidime and cefepime can be used for susceptible ESBL producers. However, there is an increased resistance rate, the inoculum effect alongside scarce and contradictory data (Rodriguez-Baño *et al*., 2018). Cephamycins like flomoxef, cefotetan, moxalactam, cefmetazole and cefoxitin are effective against large proportions of susceptible ESBL producing isolates, in the lack of other resistance mechanisms, especially in UTIs (Rodriguez-Baño *et al*., 2018). On the other hand, the limitations are the inoculum effect and development of resistance during therapy.

Temocillin, plazomicin and tigecycline are newer agents which are active against ESBL producers but they not widely available in most countries and comparative studies may be lacking (Rodriguez-Baño *et al*., 2018; Peirano and Pitout, 2019).Gentamicin, amikacin and tobramycin continue to be useful in the therapy of UTIs arising from ESBL producing isolates but are highly nephrotoxic, cause heterogeneous resistance rated and are less efficacious in non UTIs (Rodriguez-Baño *et al*., 2018).

Fosfomycin is considered just as effective as piperacillin-tazobactam in complicated UTIs. On the other hand, it is not accessible in most regions, there is sparse knowledge and a danger of development of resistant subpopulations with monotherapy(Rodriguez-Baño *et al*., 2018). Ciprofloxacin and levofloxacin are suitable for oral use and susceptible isolates. Notwithstanding, there have been high resistance rates reported (Rodriguez-Baño *et al*., 2018).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Design

The study was an experimental design as previously described by Periera *et al.* (2018) and Ouédraogo *et al.* (2016). The ESBL production in bacterial isolates was phenotypically verified using the combined disk method and antimicrobial susceptibility testing was done through the Kirby Bauer disk diffusion. The experimental guidelines were followed as described by on the Clinical Laboratory Standard Institute (CLSI) of the year 2020. The multiplex PCR was carried out as previously described by Dallene *et al.* (2020).

3.2 Study areas

Embu Level 5 Hospital is the referral hospital for Embu County which is located 130 kilometres North-East of Nairobi along the Embu-Meru highway. The hospital has a 580-bed capacity comprising of specialised departments comprising of the paediatric, surgical, internal medicine, psychiatry, maternity, renal, obstetrics/gynaecology and intensive care units. The Kenyatta National Hospital handles countrywide referral cases having a bed capacity of 1800 and has a well-established microbiology lab which handles a high volume of clinical specimens. KNH acts as a teaching and referral hospital for several medical learning institutions and is located in Nairobi County. As a level 6 hospital KNH has highly specialised departments which regularly carry out medical research and contributes to national treatment guidelines due to its referral status.

3.3 Sample size determination

The formula for comparing proportions was used (Dohoo *et al.*, 2003)

$$n = \frac{[\, Z\alpha \sqrt{(2pq)} - Z\beta \sqrt{p_1q_1 + p_2q_2}\,]^2}{[p_1 - p_2\,]^2}$$

Where n = sample size,

$Z\alpha$ = the statistic confidence level (1.96)

$Z\beta$ = type 2 error at 20% (–0.84)

p = 0.47 which was the estimated pooled proportion of ESBL producing *Enterobacteriaceae* established at East African hospitals (Sonda *et al.*, 2016).

p1= proportion at risk in the case group which was assumed to be 60% (0.6) for this study

p2= proportion at risk in the control group which was assumed to be 40% (0.4) for this study

p1-p2 = effect size which was assumed to be 20% (0.2) for this study

Hence by substitution

$$n = \frac{[1.96\sqrt{(2\times0.47\times0.53)} - -0.84 \sqrt{(0.6\times0.4) + (0.4\times0.6)}]^2}{[0.6- 0.4]^2}$$

$$n = \frac{[(1.96 \times 0.7058) + (0.84 \times 0.6928)]^2}{[0.2]^2}$$

$$n = \frac{[(1.3834 + 0.582)]^2}{0.04} = \frac{3.8628}{0.04}$$

$n = 96.57 \approx 97$ samples of bacterial isolates were ideal.

Therefore 100 samples of each bacterial isolate were collected.

3.4 Sampling procedure

Overall, 265 clinical specimens consisting of: urine (82), blood (77), pus swabs (55), tracheal aspirates (17), cerebrospinal fluid (11), stool (9), high vaginal swabs (3) and pleural fluid (1), were collected from patients seeking treatment at both facilities by medical lab technologists. Patients from whom the specimens were collected were classified based on the gender and type of admission according to the laboratory registers. The specimens were properly handled and stored according to the prescribed laboratory procedures. The specimen collection containers were properly sealed, labelled and coded. If any of the above criteria were not met, the bacteria isolates were precluded from the study.

A total of 138 *E. coli* and 127 *K. pneumoniae* isolates were cultured from the respective clinical specimens. The Embu level 5 hospital lab technologists used the conventional IMVIC method to identify the bacteria. The bacterial isolates were inoculated in nutrient slant tubes and stored at room temperature for 30 days before transportation to the Public Health, Pharmacology and Toxicology Department laboratory. The KNH microbiology lab technologists used Vitek® 2 compact and MALDITOF® MS methods to identify the bacteria isolates. The isolates were stored in cryovials comprising of Brain Heart Infusion Broth with 20% glycerol and transported at 4°C to the Department of Public Health, Pharmacology and Toxicology laboratory. Patient details were obtained from the hospital lab registers. Biosafety guidelines and sampling procedures prescribed by both the hospital microbiology laboratory were followed.

3.5 Identification of *E. coli* and *K. pneumoniae* isolates

3.5.1 Culturing of the Isolates

A loopful of every bacterial isolate was inoculated on MacConkey agar plates (Oxoid, England) then afterwards incubated at 37°C aerobically over 24 hours. Every presence of large non mucoid colonies with an even pink colour indicated *E. coli* while large mucoid colonies with colourless peripheries confirmed *K. pneumoniae*.

3.5.2 Confirmation by PCR and Sequencing

A loopful of a single bacterial colony was boiled after suspension in 100µl of distilled water in sterile eppendorf tubes at 100°C for 30 minutes. Centrifugation of the mixture was done at 15,000 rpm for 5 minutes and the DNA extracted from the supernatant portion was transferred to fresh sterile eppendorf tubes. The *gadA* gene was utilized in molecular detection of the *E.coli* isolates using forward primer (5'GATGAAATGGCGTTGGCGCAAG-3') and reverse primer (5'GGCGGAAGTCCCAGACGATATCC-3'). Amplification was done using this protocol: an initial denaturation step at 94°C for 4 min; 30 cycles of 30s at 94°C, 30s at 65°C and 30s at 72°C; and final elongation at 72°C for 5 min (Doumith *et al*, 2012). The PCR assays for *Escherichia coli* were carried out in 40 µl reaction mixtures comprising of 20 µl of 1X OneTaq® Master Mix (New England BioLabs, Ipswich, MA, USA) 10µM concentration of each primer, 5 µl of the DNA template including 9.88µl of nuclease free water.

The *rpoB* gene was used for the *K. pneumoniae* isolates with primers designed for this study: the forward primer (5'-GTCGTCACGGTAACAAGGGT-3') and reverse primer (5'-GACCACCGAACTGTGCCTTA-3'). The PCR protocol used was: an initial denaturation at 95°C for 7 min; then 35 cycles of 40s at 94°C, 40s at 57°C and 90s at 72°C; and final elongation step at for 7min at 72°C. The 96 well T100™ thermal cycler was used for amplification (Bio-Rad Laboratories, CA, USA). The PCR assays for *K. pneumoniae* were performed in 32 µl reaction mixtures containing 16 µl of 1X OneTaq® Master Mix (New England BioLabs, Ipswich, MA, USA) 10µM concentration of each primer, 5 µl of the DNA template and 7.8 µl of nuclease free water.

Preparation of the gel was done by dissolving 1.5% agarose gel powder in 1X Tris-acetate EDTA (TAE) solution through boiling. About 0.5µg/ml of ethidium bromide was incorporated to the solution then poured onto gel trays with the comb placed appropriately to form the wells upon solidifying. About 12 µl of the PCR products and 2 µl of the GeneRuler™ 100 bp DNA ladder (ThermoScientific, Waltham, Massachusetts, USA) were loaded onto the wells. Electrophoresis was done at 100V for 45 minutes. The gels were captured using the UVP GelMax® 125 imager (Upland, CA, USA). The positive controls used were the DNA extracted from *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922. Nuclease free water served as the negative control throughout.

3.6 ESBL Detection methods

3.6.1 Phenotypic confirmation of ESBL production

The standard disk diffusion technique was applied where ceftazidime (30 µg), cefotaxime (30 µg), ceftazidime/clavulanic acid (30 µg/10 µg) and cefotaxime-clavulanic acid (30 µg/10 µg) discs were positioned on an MHA plate at a gap of 20mm (CLSI, 2020). Incubation of the plate was done at 37°C for 16 to 18 hours in circulating air with eventual examination based on the zone of inhibition. A ≥ 5mm zone diameter increment for any antibiotic with clavulanic acid versus its individual antibiotic zone was viewed as positive for ESBL production (CLSI, 2020). *K. pneumoniae* ATCC® 700603 and *Escherichia coli* ATCC® 25922 were employed for quality control. The agar plates were disposed of by sterilization for 30 minutes in an autoclave at 121°C and later placed in a designated bin for incineration as per the laboratory biosafety guidelines.

3.6.2 Antibiotic Susceptibility Testing

This was conducted adopting the Kirby-Bauer disk diffusion technique. Normal saline was employed to suspend a single bacterial colony to register an Opacity Equivalent (OE) similar to 0.5 McFarland. A thin suspension of the bacterial colony was applied onto MHA plates (Oxoid Ltd) by use of an aseptic cotton swab. *Escherichia coli* ATCC 25922 was employed for quality control for each of the *E. coli* samples while *K. pneumoniae* ATCC® 700603 was utilized for the *K. pneumoniae* isolates (Table 3.2). The antibiotic discs were radially placed on different inoculated plates at 37° C for 16 to 18 hours in circulating air. The panel for the antibiogram constituted: ceftriaxone (30µg), piperacillin/tazobactam (100/10µg), cefoxitin (30µg), cefepime (30µg), amoxicillin/clavulanic acid (20/10µg), meropenem (10 µg), tigecycline (15 µg), levofloxacin (5µg), amikacin(30µg), nitrofurantoin(300µg), azithromycin (15µg) and minocycline (30µg). Categorization of the isolates categorised them as being resistant, susceptible or intermediate with reference to Clinical and Laboratory Standards Institute as shown in Table 3.1(CLSI, 2020). The agar plates were disposed of by sterilization for 30 minutes in an autoclave at 121°C and later placed in a designated bin for incineration as per the laboratory biosafety guidelines.

3.7 Molecular Characterization of ESBL Genes

Those ESBL strains which were phenotypically confirmed to produce ESBL were analysed using multiplex PCR to identify $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} and bla_{OXA} as shown in Table 3.3 (Dallenne *et al.*, 2010). Preparation of DNA was done through heating a loopful of colonies for 30 minutes at 95°C in 100µl distilled water in eppendorf tubes. The cell suspension was centrifuged in the next step. The PCR mixture was subjected to amplification: an initial denaturation for 10minutes at 94^{o}C; 30 cycles of 40 seconds at 94^{o}C, 40 seconds at 60^{o}C, and 1 minute at 72^{o}C; and the final elongation for 7 minutes at 72^{o}C (Dallenne *et al*., 2010). UV visualization of the amplicons was done by running the amplicons at 100V in 1.5% agarose gel incorporated with Ethidium Bromide (EDTA) for 45 minutes. The molecular size marker used was a 100-base pair GeneRuler™ DNA ladder (ThermoScientific, Waltham, Massachusetts, USA). The agarose gels were disposed of in a designated bin containing activated charcoal and later taken to the incinerator while following the laboratory biosafety guidelines to avoid contamination.

3.8 Nucleic Acid Sequencing

A representative sample of 42 DNA isolates were sent for purification and Sanger sequencing at Macrogen Europe. This comprised of 21 *E. coli* and 21 *K. pneumoniae* isolates from both facilities which presented with the ESBL genes during PCR. The selected sequences were deposited on the NCBI website for allocation of accession numbers after blasting as shown in the Appendices 1-3.

3.9 Data Analysis

Data entry and management was done on MS excel spread sheets. The Graph Pad prism® version 9.3.1 software was utilised for statistical analysis. The diameters of the zones of inhibition and the proportions of ESBL genes was determined in frequency tables. Association between bacterial susceptibility and the respective antibiotic was determined through chi square at 95% confidence level.

3.10 Ethical approval

Ethical approval was requested from the Kenyatta National Hospital- University of Nairobi Ethics and Research Committee (KNH-UoN ERC) and issued as REF P866/10/2019. The National Commission for Science Technology and Innovation (NACOSTI) issued research approval under License No: NACOSTI/P/20/4019. Research authorisation was also granted by the Embu County Director of Medical Services and the KNH microbiology laboratory.

3.11 Blast analysis of the DNA sequences

Representative samples which harboured the $\mathit{bla}_{\text{TEM}}$, $\mathit{bla}_{\text{SHV}}$, $\mathit{bla}_{\text{OXA}}$, $\mathit{bla}_{\text{CTXMgp1}}$, $\mathit{bla}_{\text{CTXMgp2}}$ and $\mathit{bla}_{\text{CTXMgp9}}$ resistant genes were analysed using the BLASTn tool. Accession numbers were allocated by the NCBI as shown in the appendices.

Table 3.1: Zone diameter interpretive standard break points

	Zone diameter (nearest whole mm)				
Antimicrobial agent	Disk content (µg)	Susceptible	Susceptible dose dependent	Intermediate	Resistant
Amoxicillin/clavulanate	20/10	≥18	-	14 -17	≤13
Piperacillin/tazobactam	100/10	≥21	-	18 -20	≤17
Cefoxitin	30	≥18	-	15 -17	≤14
Ceftriaxone	30	≥23	-	20 -22	≤19
Cefepime	30	≥25	19 - 24	-	≤18
Meropenem	10	≥23	-	20 -22	≤19
Amikacin	30	≥17	-	15 -16	≤14
Azithromycin	15	≥13	-	-	≤12
Minocycline	30	≥16	-	13 -15	≤12
Nitrofurantoin	300	≥17	-	15 -16	≤14
Levofloxacin	5	≥21	-	17 -20	≤16
⁕Tigecycline	15	≥18	-	-	<18

Source: Clinical and Laboratory Standards Institute (CLSI, 2020)

⁕ FDA guidelines used (Shankar *et al.*, 2017).

Table 3.2: Quality control ranges of *Escherichia coli* ATCC® 25922 and *Klebsiella pneumoniae* ATCC® 700603 for the selected antimicrobial agents

		Zone diameter QC range(mm)	
		Escherichia coli ATCC® 25922	*Klebsiella pneumoniae* ATCC® 700603
Antimicrobial agent	Disk content (µg)		
Amoxicillin/clavulanate	20/10	18 - 24	-
Piperacillin/tazobactam	100/10	24 - 30	-
Cefoxitin	30	23 - 29	-
Ceftriaxone	30	29 - 35	16 - 24
Cefepime	30	31 - 37	23 - 29
Meropenem	10	28 -35	-
Amikacin	30	19 - 26	-
Azithromycin	15	-	-
Minocycline	30	19 - 25	-
Nitrofurantoin	300	20 - 25	-
Levofloxacin	5	29 - 37	-
Tigecycline	15	20 - 27	-

Source: Clinical and Laboratory Standards Institute (CLSI, 2020)

Table 3.3: Primers utilized in amplifying antimicrobial resistant genotypes

Primer name	Sequence (5’- 3’)	ESBL targeted	Amplicon size (bp)
MultiTSO-T_for	CATTTCCGTGTCGCCCTTATTC	TEM including	
MultiTSO-T_rev	CGTTCATCCATAGTTGCCTGAC	TEM -1 and TEM-2	800
MultiTSO-S_for	AGCCGCTTGAGCAAATTAAAC	SHV including	713
MultiTSO-S_rev	ATCCCGCAGATAAATCACCAC	SHV -1	
MultiTSO-O_for	GGCACCAGATTCAACTTTCAAG	OXA-1, OXA-4 and	564
MultiTSO-O_rev	GACCCCAAGTTTCCTGTAAGTG	OXA-30	
MultiCTXMGp1_for	TTAGGA AATGTGCCGCTGCA		
MultiCTXMGp1-2_rev	CGATATCGTTGGTGGTGC CAT	CTX-M-1,	688
		CTX-M-3 and CTX-M-15	
MultiCTXMGp2_for	CGTTAACGGCACGATGAC		
MultiCTXMGp1-2_rev	CGATATCGTTGGTGGTGCCAT	CTX-M-2	404
MultiCTXMGp9_for	TCA AGCCTGCCGATCTGGT		
MultiCTXMGp9_rev	TGATTCTCGCCGCTGAAG	CTX-M-9 and CTX-M-14	561
CTX-Mg8/25_for	AACACACAGACGCTCTAC	CTX-M-8, CTX-M-25,	
CTX-Mg8/25_rev	TCGAGCCGGAAGGTGTTAT	CTX-M-26 and CTX-M-39 to CTX-M-41	326

Source: Dallenne *et al.* 2010

CHAPTER FOUR

4.0 RESULTS

4.1 Classification of patients and the collected clinical isolates

The patients from whom the clinical specimens were obtained in the Kenyatta National Hospital (KNH) and Embu Level-5 Hospital (ELVH) were characterised by gender and patient admission type. Out of the 138 *E. coli* isolates, 57 (41.3%) isolates were collected from men, 71 (51.4%) from women and the gender was not indicated in 10 (7.2%) isolates. Thirty-four (24.6%) isolates were collected from the outpatient department while 91 (65.9%) were from the inpatient department. However, it was unclear whether the remaining 13 (9.4%) isolates were from outpatient or inpatient departments. Six (17.6%) *E. coli* isolates originating from male patients, while 26 (76.5%) *E. coli* samples were acquired from female patients at ELVH. Notably, the gender of patients from 2 (5.9%) *E. coli* isolates were obtained was not captured in the laboratory records in ELVH. Twenty-four (70.6%) *E. coli* isolates were collected from patients treated in the outpatient clinics, while 10 (29.4%) *E. coli* were obtained from patients admitted in the wards in the ELVH (Table 4.1).

Fifty-one (49%) *E. coli* isolates were from male patients, 45 (43.3%) were obtained from female patients in the KNH and 8 (7.7%) *E. coli* isolates had no gender indicated in the KNH microbiology laboratory records. Moreover, 10 (9.6%) *E. coli* isolates were from the outpatient clinics, while 81 (77.9%) *E. coli* isolates were from inpatient wards in the KNH and13 (12.5%) isolates had no admission type indicated in the microbiology laboratory records in the KNH (Table 4.1).

Among the 127 *K. pneumoniae* isolates, a total of 57 (44.9%) isolates were collected from men, 63 (49.6%) from women and the gender was not indicated in 7 (5.5%) isolates. The isolates collected at the outpatient department were 10 (7.9%) and 117 (92.1%) from the inpatient department. Two (16.7%) *K. pneumoniae* samples were obtained from male patients, 5 (41.6%) from female patients and 5 (41.6%) isolates had no gender indicated at ELVH. Six (50%) isolates were obtained outpatient clinics and 6 (50%) *K. pneumoniae* isolates from inpatient wards (Table 4.1). Fifty-five (47.8%) of *K. pneumoniae* isolates originated from male patients, 58 (50.4%) were from female patients and no gender was indicated in 2 (1.8%) isolates at the KNH. Four (3.5%) isolates originated at the outpatient clinics and 111 (96.5%) isolates were retrieved at the inpatient wards at the KNH (Table4.1).

Table 4.1: Classification of patients from whom the clinical isolates were obtained

Cadre	Sub-cadre	ELVH		KNH	
		E. coli	***K. pneumoniae***	***E. coli***	***K. pneumoniae***
		n= 34(%)	n= 12 (%)	n= 104 (%)	n= 115 (%)
Gender	Male	6 (17.6)	2 (16.7)	51 (49)	55 (47.8)
	Female	26 (76.5)	5 (41.6)	45 (43.3)	58 (50.4)
	*Unknown	2 (5.9)	5 (41.6)	8 (7.7)	2 (1.8)
Admission type	Outpatient	24 (70.6)	6 (50)	10 (9.6)	4 (3.5)
	Inpatient	10 (29.4)	6 (50)	81 (77.9)	111 (96.5)
	*Unknown	0 (0)	0 (0)	13 (12.5)	0 (0)

KNH: Kenyatta National Hospital; ELVH: Embu Level V Hospital; n: Sample size.
*Unknown: gender or type of admission not indicated in the departmental records

In this research, clinical samples of both pathogens obtained from varied biological specimens at ELVH and KNH, respectively, were determined. Out of the 46 clinical isolates obtained from ELVH, 34 (73.91%) were *E. coli* and 12 (26.09%) were *K. pneumoniae* bacterial strains. Among the 219 isolates collected at KNH, 104 (47.49%) were *E. coli* and 115 (52.51%) were *K. pneumoniae* isolates (Table 4.2).

Most *E. coli* isolates (25=76.5%) at ELVH were from urine specimens, others were derived from stool (7=20.6%) and pus swabs (1=2.9%) only. At the KNH most *E. coli* strain isolates obtained from KNH were from pus swabs (51=49%), urine (34= 32.7%), blood cultures (10= 9.6%), tracheal aspirates (1=1%), cerebrospinal fluid (CSF) (6= 5.8%) and high vaginal swabs (2= 1.9%). Notably, no *E. coli* isolate was derived from the stool and pleural fluid specimens in KNH (Table 4.2).

Out of the 12 *K. pneumoniae* isolates obtained from ELVH, most (9=75%) were derived from urine specimens, pleural fluid (1=8.3%) and stool (2= 16.7%) only. Most clinical isolates of *K. pneumoniae* (67=58.3%) obtained from KNH were derived from blood cultures, pus swabs (13=11.3%), urine (13= 11.3%), tracheal aspirates (16= 13.9%), SCF (5=4.3%), and one (0.9%) high vaginal swab (Table 4.2).

Table 4.2: Classification of clinical specimens providing the *E. coli* and *K. pneumoniae* isolates

Specimen type	**Total (N)**	**ELVH**		**KNH**	
		***E. coli* n (%)**	***K. pneumoniae* n (%)**	***E. coli* n (%)**	***K. pneumoniae* n (%)**
Urine	82	26 (76.5)	9 (75)	34 (32.7)	13 (11.3)
Blood cultures	77	0 (0)	0 (0)	10 (9.6)	67 (58.3)
Pus swabs	65	1 (2.9)	0 (0)	51 (49)	13 (11.3)
Tracheal aspirates	17	0 (0)	0 (0)	1 (1)	16 (13.9)
Cerebral Spinal Fluid	11	0 (0)	0 (0)	6 (5.8)	5 (4.3)
Stool	9	7 (20.6)	2 (16.7)	0 (0)	0 (0)
High vaginal swab	3	0 (0)	0 (0)	2 (1.9)	1 (0.9)
Pleural fluid	1	0 (0)	1 (8.3)	0 (0)	0 (0)
Total (N)	265	34	12	104	115

KNH: Kenyatta National Hospital; ELVH: Embu Level V Hospital; n: Sample size.

4.2 Molecular confirmation of by PCR and sequencing

Overall, 265 DNA samples including 138 from *E. coli* and 127 from *K. pneumoniae* were identified by PCR using the *gadA* (373bp) and *rpoB* (600bp) genes respectively as seen in Figure 4.1. Therefore, the confirmed isolates could be used in antibiotic susceptibility testing. The 21 representative samples of *E. coli* and *K. pneumoniae* each, obtained from amplified DNA, which harboured selected ESBL genes were taken for sequencing as well as BLAST analysis. Out of the 12 *K. pneumoniae* DNA samples from ELVH only 4(4/21, 19%) were identified as *K. pneumoniae* after sequencing. On the other hand, all the 9 *K. pneumoniae* DNA samples from the KNH were confirmed to be *K. pneumoniae*. All the 21 *E. coli* DNA samples (100%) from both facilities were confirmed as *E. coli*. The sequences were submitted to the NCBI database (GenBank) and accession numbers are as shown in Appendix 1 and 2.

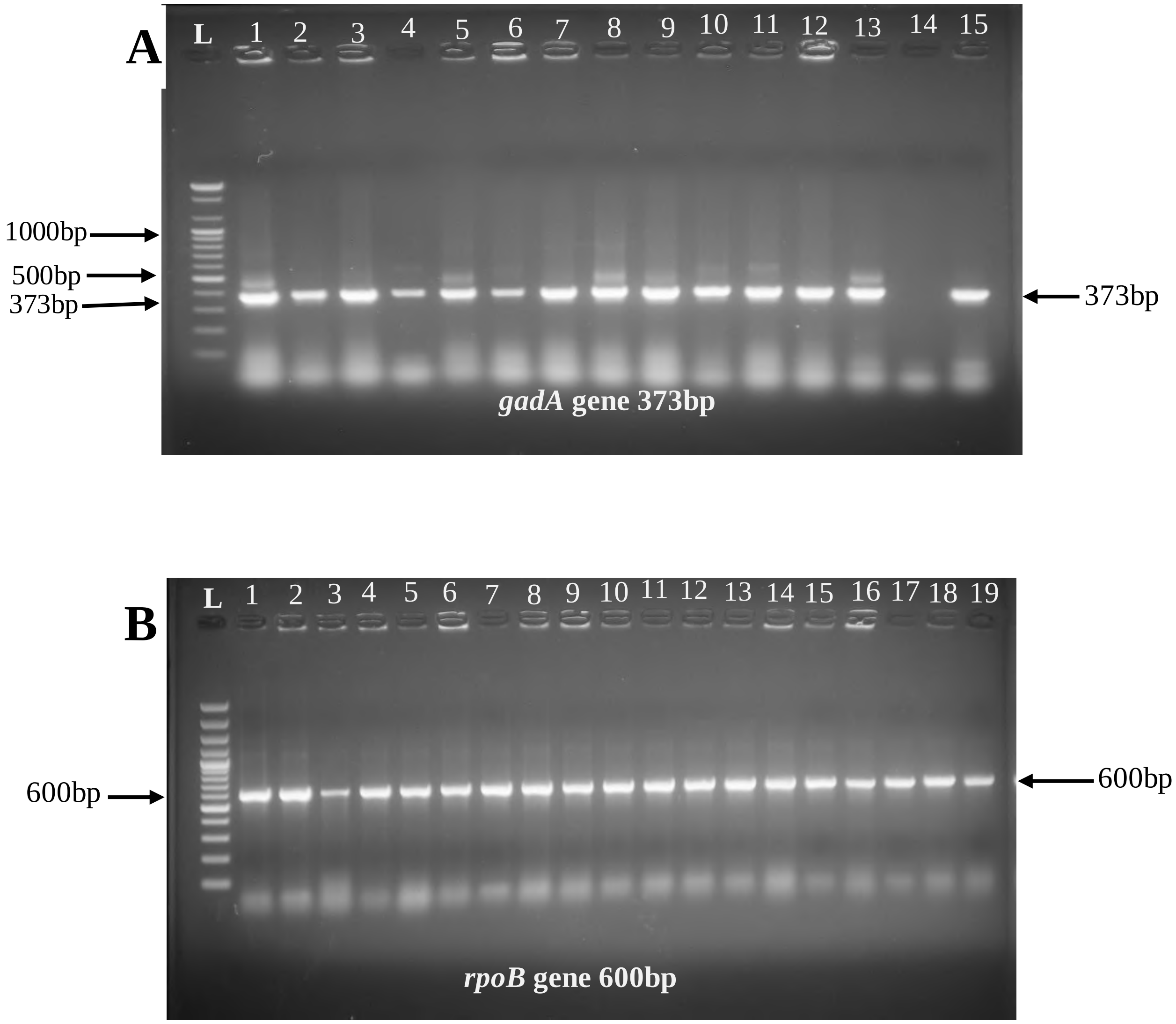

FIGURE 4.1: 1.5% w/v Agarose gel electrophoresis of *E. coli* and *K. pneumoniae* PCR products**.** For Panel (A) L-100bp molecular ladder, lanes 1 to 13- *Escherichia coli* positive samples, lane 14- nuclease free water (negative control) and lane 15- *Escherichia coli* ATCC 25922(positive control). For Panel (B) L-100bp molecular ladder, lanes 1 to 18- *K. pneumoniae* positive samples and lane 19- *K. pneumoniae* ATCC 700603 (positive control).

4.3 ESBL production in the clinical isolates from the health facilities

To establish ESBL production for both bacterial isolates the diameter zone of inhibition for the cephalosporin disks on MHA were determined as shown in Figure 4.2. Overall ESBL production was observed in 232 isolates where 118 were *K. pneumoniae* isolates while 114 were *E. coli* isolates (Table 4.3). There was higher ESBL production observed in *K. pneumoniae* isolates than *E. coli* isolates. Majority of the *E. coli* (82.6 %) and *K. pneumoniae* (92.9%) isolates obtained from patients seeking treatment at both facilities were ESBL producers. The differences in ESBL production for the isolates from the two health facilities was not significant (p>0.05).

Table 4.3: ESBL production at ELVH and KNH

Isolate	ESBL trait	ELVH n (%)	KNH n (%)	*P*-value	Total (%)
E. coli	ESBL +ve	31 (91.2)	83 (79.8)	0.1919	114 (82.6)
	ESBL-ve	3 (8.8)	21 (20.2)		24 (17.4)
K. pneumoniae	ESBL +ve	12 (100)	106 (92.2)	0.5995	118 (92.9)
	ESBL-ve	0(0)	9 (7.8)		9 (7.1)

Fisher's exact test at $\alpha_{0.05}$; ESBL: Extended-Spectrum β-Lactamase; +ve: Positive; -ve: Negative n: sample size; KNH: Kenyatta National Hospital; ELVH: Embu Level V Hospital.

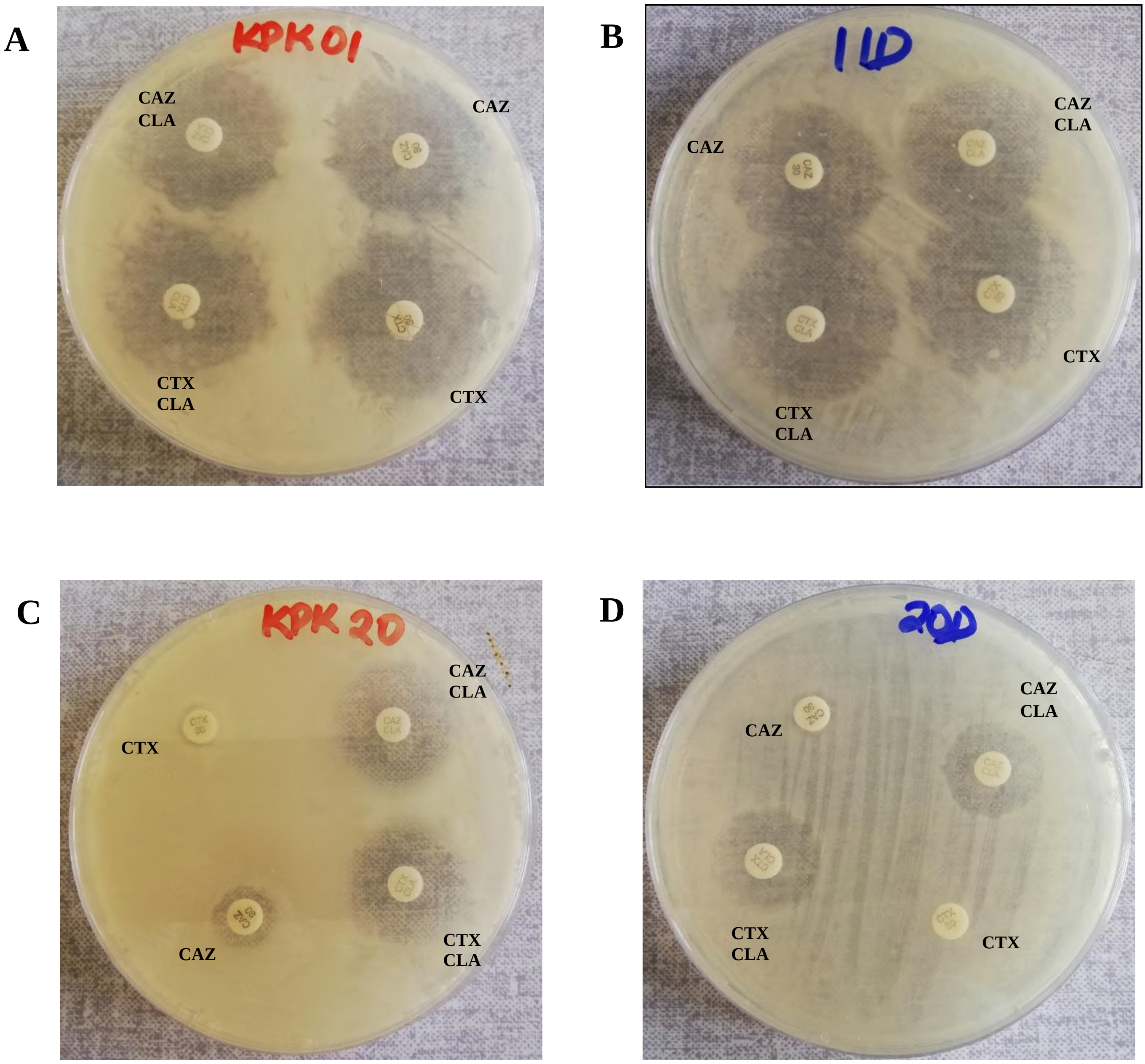

FIGURE 4.2: Phenotypic confirmation of ESBL production using ceftazidime 30µg (CAZ), ceftazidime30µg/clavulanate10µg(CAZ/CLA), cefotaxime30µg(CTX) and cefotaxime30µg /clavulanate10µg (CTX/CLA) on Mueller Hinton agar (MHA). Panels A and C show *K. pneumoniae* and *Escherichia coli* isolates respectively demonstrating no ESBL production. Panels B and D show *K. pneumoniae* and *Escherichia coli* isolates respectively exhibiting ESBL production.

4.4 ESBL-associated antimicrobial resistance of the bacterial isolates

This study characterised the antimicrobial resistance associated with ESBL production among the collected isolates of *E. coli* and *K. pneumoniae* to some conventional antimicrobial drugs.

4.4.1 ESBL-associated antimicrobial resistance of *E. coli* isolates

More than a third of the ESBL producing *E. coli* isolates at both facilities were resistant to ceftriaxone (69.6%), levofloxacin (50.7%), azithromycin (44.2%) and cefepime (34.1%). Only 3 (2.9%) of the non ESBL producing were resistant to levofloxacin at the KNH and none at ELVH. The ESBL producing *E. coli* isolates were also resistant to amoxicillin/clavulanic acid (26.1%), piperacillin/tazobactam (17.4%), cefoxitin (15.9%) and minocycline (14.5%) at both facilities. Only 3 (2.9%) of the non ESBL producing *E. coli* isolates exhibited resistance towards amoxicillin/clavulanic acid and minocycline at the KNH and none at the ELVH. Low resistance level was observed towards nitrofurantoin (10.3%), meropenem (8.7%), amikacin (2.9%) and tigecycline (0.72%) in both the ESBL as well as non ESBL producing isolates from patients seeking treatment at both health facilities. The MDR isolates were observed at 39.9% among the ESBL producing *E. coli* isolates (Table 4.4). There was no significant difference noted between the antimicrobial resistance associated with the production of ESBLs in *E. coli* isolates at both health facilities (p>0.05).

Table 4.4: ESBL-associated antimicrobial resistance of *E. coli* samples at ELVH and KNH

Antimicrobial agent	ESBL status	ELVH n = 34 (%)	KNH n = 104 (%)	P value	Total n= 138 (%)
Ceftriaxone	Positive	16 (47.1)	80 (76.9)	0.3488	96 (69.6)
	Negative	0 (0.0)	0 (0.0)		
Levofloxacin	Positive	13 (38.2)	54 (51.9)	>0.9999	70 (50.7)
	Negative	0 (0.0)	3 (2.9)		
Azithromycin	Positive	12 (35.3)	49 (47.1)	>0.9999	61 (44.2)
	Negative	0(0.0)	0(0.0)		
Cefepime	Positive	14 (41.2)	33 (31.7)	>0.9999	47 (34.1)
	Negative	0(0.0)	0(0.0)		
Amoxicillin/clavulanic acid	Positive	3 (8.8)	30 (28.8)	>0.9999	36 (26.1)
	Negative	0(0.0)	3 (2.9)		
Piperacillin/ Tazobactam	Positive	4 (11.8)	20 (19.2)	>0.9999	24 (17.4)
	Negative	0(0.0)	0(0.0)		
Cefoxitin	Positive	5 (14.7)	17 (16.3)	>0.9999	22 (15.9)
	Negative	0(0.0)	0(0.0)		
Minocycline	Positive	2 (5.9)	15 (14.4)	>0.9999	20 (14.5)
	Negative	0(0.0)	3 (2.9)		
Meropenem	Positive	4 (11.8)	8 (7.7)	>0.9999	12 (8.7)
	Negative	0(0.0)	0(0.0)		
Amikacin	Positive	4 (11.8)	0 (0)	>0.9999	4 (2.9)
	Negative	0(0.0)	0(0.0)		
Tigecycline	Positive	1 (2.9)	0 (0)	>0.9999	1(0.72)
	Negative	0(0.0)	0(0.0)		
⁎Nitrofurantoin	Positive	1 (3.1) [a]	5 (19.2) [b]	>0.9999	6(10.3) [c]
	Negative	0(0.0)	0(0.0)		
MDR	Positive	12(35.3)	43(41.3)	>0.9999	55(39.9)
	Negative	0 (0.0)	0(0.0)		

Fisher's exact test at $\alpha_{0.05}$; ESBL: Extended-Spectrum β-Lactamase; +ve: Positive; -ve: Negative; NC: not computed; n: sample size; KNH: Kenyatta National Hospital; ELVH: Embu Level V Hospital; ⁎: Only urine isolates were tested against Nitrofurantoin according to the CLSI 2020 guidelines; [a]: the total urine isolates from Embu Level V Hospital; [b]: Total number urine isolates from KNH; [c]: the overall number of urine isolates; MDR: Multi-drug Resistance.

4.4.2 ESBL-associated antimicrobial resistance of *K. pneumoniae* isolates

More than half of the ESBL producing *K. pneumoniae* isolates were resistant to ceftriaxone (91.3%), amoxicillin/clavulanic acid (70.9%) and cefepime (60.6%) at both health facilities. Six non ESBL producing *K. pneumoniae* isolates collected from patients seeking treatment at the KNH had low level resistance to ceftriaxone (0.9%), amoxicillin/clavulanic acid (2.6%) and cefepime (1.7%). All the non ESBL producing *K. pneumoniae* isolates from patients seeking treatment at ELVH were sensitive to the tree antibiotics above.

Less than a third of the ESBL producing *K. pneumoniae* isolates exhibited resistance against azithromycin (22%), levofloxacin (12.6%), piperacillin/tazobactam (12.6%) and minocycline (11.8%) at both health facilities. Eight non ESBL producing *K. pneumoniae* isolates from patients treated at KNH exhibited low resistance to azithromycin (0.9%), levofloxacin (2.6%), piperacillin/tazobactam (1.7%) and minocycline (1.7%). No resistance observed among the non ESBL producing *K. pneumoniae* isolates from patients at ELVH.

Less than ten percent resistance was observed towards cefoxitin (9.4%), tigecycline (6.2%), meropenem (2.4%) and amikacin (0.8%) in ESBL producing *K. pneumoniae* isolates collected at both facilities. Only one non ESBL producing isolate (0.9%) at the KNH had resistance to cefoxitin while all the non ESBL producing showed sensitivity to the above-named antibiotics. The ESBL producing *K. pneumoniae* isolates collected from the 22 urinary samples exhibited resistance to nitrofurantoin (45.5%) at both health facilities. The MDR proportion among the ESBL producing *K. pneumoniae* was 13.7% and only one non ESBL producing isolate (0.9%) from a patient at KNH (Table 4.5). As shown in Table 4.5, no significant difference was observed between the ESBL-associated resistance of *K. pneumoniae* isolates obtained from the ELVH and KNH in this study (P>0.05).

Table 4.5: ESBL-associated antimicrobial resistance by *K. pneumoniae* isolates

Antimicrobial agent	**ESBL status**	**ELVH n = 12 (%)**	**KNH n = 115 (%)**	**P-value**	**Total n= 127 (%)**
Ceftriaxone	Positive	12 (100)	103 (89.5)	>0.9999	116 (91.3)
	Negative	0 (0.0)	1 (0.9)		
Amoxicillin/clavulanic acid	Positive	7 (58.3)	80 (69.6)	>0.9999	90 (70.9)
	Negative	0 (0.0)	3 (2.6)		
Cefepime	Positive	2 (16.7)	73 (63.5)	>0.9999	77 (60.6)
	Negative	0 (0.0)	2 (1.7)		
Azithromycin	Positive	5 (41.7)	22 (19.1)	>0.9999	28 (22)
	Negative	0 (0.0)	1 (0.9)		
Levofloxacin	Positive	2 (16.7)	11 (9.6)	>0.9999	16 (12.6)
	Negative	0 (0.0)	3 (2.6)		
Piperacillin/tazobactam	Positive	1 (8.3)	13 (11.3)	>0.9999	16 (12.6)
	Negative	0 (0.0)	2 (1.7)		
Minocycline	Positive	3 (25.0)	10 (8.7)	>0.9999	15 (11.8)
	Negative	0 (0.0)	2 (1.7)		
Cefoxitin	Positive	4 (33.3)	7 (6.1)	>0.9999	12 (9.4)
	Negative	0 (0.0)	1 (0.9)		
Tigecycline	Positive	4 (33.3)	4 (3.48)	>0.9999	8 (6.2)
	Negative	0 (0.0)	0 (0.0)		
Meropenem	Positive	1 (8.3)	2 (1.7)	>0.9999	3 (2.4)
	Negative	0 (0.0)	0 (0.0)		
Amikacin	Positive	0 (0.0)	1(0.79)	>0.9999	1 (0.8)
	Negative	0 (0.0)	0 (0.0)		
⁎Nitrofurantoin	Positive	7 (77.8) [a]	3 (23.1) [b]	>0.9999	10 (45.5) [c]
	Negative	0 (0.0)	0 (0.0)		
MDR isolates	Positive	6 (50.0)	10 (8.7)	>0.9999	17 (13.4)
	Negative	0 (0.0)	1 (0.9)		

Fisher's exact test at $\alpha_{0.05}$; ESBL: Extended-Spectrum β-Lactamase; NC: not computed; n: sample size; KNH: Kenyatta National Hospital; ELVH: Embu Level V Hospital; ⁎: Only urine isolates were tested against Nitrofurantoin according to the CLSI 2020 guidelines; [a]: the total urine isolates from Embu Level V Hospital; [b]: the total urine isolates from Kenyatta National Hospital; c: Total number of urine isolates; MDR; Multi-drug Resistance.

4.5 Multi-drug Resistant (MDR) phenotypes of ESBL producing clinical isolates

The bacterial isolates exhibiting resistance to three or more antimicrobial agent categories were phenotypically classified as multidrug-resistant.

4.5.1 Multi-drug Resistant (MDR) phenotypes of *E. coli* isolates

Cumulatively, all the 55 (39.9 %) ESBL producing *E. coli* isolates from ELVH and KNH showed MDR phenotypes. Overall, MDR phenotypes were observed in 12 (35.3 %) *E. coli* isolates in ELVH. The ceftriaxone-cefepime-azithromycin-levofloxacin MDR phenotype was the most frequent in 4(11.8%) *E. coli* isolates. The second most-frequent MDR phenotypes observed in the other 8 isolates was cefoxitin-ceftriaxone-cefepime-amoxicillin/clavulanic acid-piperacillin/tazobactam-meropenem-azithromycin-levofloxacin-tigecycline-minocycline-nitrofurantoin-amikacin (Table 4.6).

The MDR phenotypes were present in 43 (41.3 %) *E. coli* isolates from KNH patients. The ceftriaxone-azithromycin-levofloxacin MDR phenotype was the most frequent in 8 (7.7 %) of the *E. coli* obtained from the KNH patients. The other remaining isolates had a varied combination of cefoxitin-ceftriaxone-cefepime-amoxicillin /clavulanic acid-piperacillin/tazobactam-meropenem-azithromycin-levofloxacin-minocycline-nitrofurantoin (Table 4.6).

Table 4.6: Proportions of the ESBL-associated MDR phenotypes in the *E. coli* isolates

Multi-drug Resistance phenotypes	ELVH n (%)	KNH n (%)	Total N (%)
FOX-CRO-FEP-MEM-AZM-LVX-MI-TGC-AN	1 (2.9)	0 (0)	1 (0.7)
FOX-CRO-FEP-AMC-TZP-AZM-LVX-MI-FM	0 (0)	1 (1)	1 (0.7)
FOX-CRO-FEP-AMC-TZP-AZM-LVX-FM	1 (2.9)	1 (1)	2 (1.4)
FOX-CRO-FEP-AMC-TZP-MEM-AZM-LVX	0 (0)	6 (5.8)	6 (4.3)
FOX-CRO-FEP-AMC-TZP-AZM-LVX-MI	0 (0)	4 (3.8)	4 (2.9)
FOX-CRO-FEP-AMC-TZP-LVX-MI	0 (0)	2 (1.9)	2 (1.4)
FOX-CRO-FEP-MEM-AZM-TGC-AN	1 (2.9)	0 (0)	1 (0.7)
FOX-CRO-FEP-AMC-TZP-MEM-LVX	0 (0)	1 (1)	1 (0.7)
CRO-FEP-AMC-TZP-AZM-LVX-FM	0 (0)	1 (1)	1 (0.7)
CRO-FEP-AMC-TZP-MEM-AZM-LVX	0 (0)	1 (1)	1 (0.7)
FOX-CRO-FEP-MEM-AZM-AN	1 (2.9)	0 (0)	1 (0.7)
FOX-FEP-AMC-AZM-LVX-MI	0 (0)	1 (1)	1 (0.7)
FOX-CRO-FEP-AMC-LVX-MI	0 (0)	1 (1)	1 (0.7)
CRO-FEP-AMC-TZP-AZM-LVX	1 (2.9)	0 (0)	1 (0.7)
CRO-FEP-TZP-MEM-AN	1 (2.9)	0 (0)	1 (0.7)
CRO-FEP-AMC-AZM-LVX	0 (0)	3 (2.9)	3 (2.2)
CRO-FEP-AZM-LVX-MI	1 (2.9)	1 (1)	2 (1.4)
CRO-FEP-AZM-LVX	4 (11.8)	1 (1)	5 (3.6)
CRO-FEP-LVX-FM	0 (0)	1 (1)	1 (0.7)
CRO-FEP-LVX-MI	0 (0)	1 (1)	1 (0.7)
CRO-TZP-AZM-LVX	0 (0)	1 (1)	1 (0.7)
CRO-TZP-AZM-TGC	1 (2.9)	0 (0)	1 (0.7)
CRO-AMC-AZM-LVX	0 (0)	3 (2.9)	3 (2.2)
CRO-AZM-LVX-MI	0 (0)	5 (4.8)	5 (3.6)
CRO-AZM-LVX	0 (0)	8 (7.7)	8 (5.8)
Total number of the MDR phenotypes	12(35.3)	43(41.3)	55(39.9)

All the isolates were ESBL-positive. FOX: Cefoxitin; CRO: Ceftriaxone; FEP: Cefepime; AMC: Amoxicillin/Clavulanic acid; TZP: Piperacillin/Tazobactam; MEM: Meropenem; AN: Amikacin; AZM: Azithromycin; LVX: Levofloxacin; MI: Minocycline; TGC: Tigecycline (TGC); FM: Nitrofurantoin; ELVH: Embu Level V Hospital; KNH; Kenyatta National Hospital; n: Sample size; N: Total number of isolates.

4.5.2 Multi-drug Resistant (MDR) phenotypes of *K. pneumoniae* isolates

Generally, MDR phenotypes were observed in 6 (50%) *K. pneumoniae* isolates from the ELVH and 11 (9.6%) *K. pneumoniae* isolates from the KNH with an overall MDR proportion of 13.4% in this study. Majority were ESBL producers with one non ESBL producing isolate observed from KNH patients. The most predominant MDR phenotype noted in two isolates at the ELVH was cefoxitin-ceftriaxone-amoxicillin/clavulanic acid-minocycline-tigecycline-nitrofurantoin (16.7%). The remaining 4 isolates had MDR phenotypes comprising cefoxitin-ceftriaxone-cefepime-amoxicillin/clavulanic acid-meropenem-azithromycin-minocycline-levofloxacin-tigecycline-nitrofurantoin (Table 4.7).

The most frequent MDR phenotypes observed in 2 *K. pneumoniae* isolates each was cefoxitin- ceftriaxone-cefepime-amoxicillin/clavulanic acid-piperacillin/tazobactam-azithromycin-levofloxacin-minocycline and ceftriaxone- amoxicillin/clavulanic acid-piperacillin/tazobactam-azithromycin- minocycline from KNH patients. Notably, the cefoxitin- ceftriaxone-cefepime-amoxicillin/clavulanic acid- piperacillin/tazobactam-azithromycin-levofloxacin-minocycline phenotype was observed in both the ESBL-producing as well as the non ESBL producing isolates from KNH patients (Table 4.7).

Table 4.7: Proportions of the MDR phenotypes in the *K. pneumoniae* isolates

Multi-drug resistance phenotype	**ELVH n (%)**	**KNH n (%)**	**Total N (%)**
AN-CRO-FEP-AMC-TZP-MEM-AZM-LVX-TGC	0 (0)	1 (0.9)	1 (0.8)
*FOX-CRO-FEP-AMC-TZP-AZM-LVX-MI	0 (0)	2 (1.7)	2 (1.6)
FOX-CRO-FEP-AMC-TZP-MEM-AZM-LVX	0 (0)	1 (0.9)	1 (0.8)
FOX-CRO-AMC-AZM-MI-TGC-FM	1 (8.3)	0 (0)	1 (0.8)
FOX-AMC-TZP-AZM-LVX-FM	0 (0)	1 (0.9)	1 (0.8)
CRO-FEP-AZM-LVX-TGC-FM	0 (0)	1 (0.9)	1 (0.8)
FOX-CRO-AMC-MEM-TGC-FM	1 (8.3)	0 (0)	1 (0.8)
FOX-CRO-AMC-MI-TGC-FM	2 (16.7)	0 (0)	2 (1.6)
CRO-AMC-TZP-AZM-MI	0 (0)	2 (1.7)	2 (1.6)
CRO-FEP-AMC-AZM-LVX	1 (8.3)	0 (0)	1 (0.8)
CRO-FEP-AMC-AZM-MI	0 (0)	1 (0.9)	1 (0.8)
FOX-CRO-FEP-MI-TGC	0 (0)	1 (0.9)	1 (0.8)
CRO-FEP-AZM-LVX	0 (0)	1 (0.9)	1 (0.8)
CRO-AZM-LVX	1 (8.3)	0 (0)	1 (0.8)
Total number of MDR phenotypes	6(50)	11(9.6)	17(13.4)

FOX: Cefoxitin; CRO: Ceftriaxone; FEP: Cefepime; AMC: Amoxicillin/Clavulanic acid; TZP: Piperacillin/Tazobactam; MEM: Meropenem; AN: Amikacin; AZM: Azithromycin; LVX: Levofloxacin; MI: Minocycline; TGC: Tigecycline (TGC); FM: Nitrofurantoin; ELVH: Embu Level V Hospital; KNH; Kenyatta National Hospital; n: Sample size; N: Total number of isolates; *: Observed in both the ESBL-positive and ESBL-negative isolates.

4.6 Genetic determinants of the ESBL-associated antimicrobial resistance in the clinical isolates

The ESBL genes were investigated using multiplex PCR whereby specific primers were utilised to identify *bla*$_{\mathrm{TEM}}$, *bla*$_{\mathrm{SHV}}$, *bla*$_{\mathrm{OXA}}$, *bla*$_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$, *bla*$_{\mathrm{CTX\text{-}M\text{-}gp\text{-}2}}$, *bla*$_{\mathrm{CTX\text{-}M\text{-}gp\text{-}9}}$ and *bla*$_{\mathrm{CTX\text{-}M\text{-}gp\text{-}8/25}}$ genes in the 265 bacterial isolates as shown in Figures 4.3 and 4.4.

4.6.1 ESBL genes detected in *E. coli* clinical isolates

Overall, the *bla*$_{\mathrm{TEM}}$ gene was found in 58 (42%) out of the 138 *E. coli* isolates from patients treated at the two facilities. Some patients at the ELVH had only 4 (11.8 %) ESBL-producing and 1 (2.9 %) non ESBL-producing *E. coli* isolates detected. The *bla*$_{\mathrm{TEM}}$ gene was detected in 46 (44.2 %) ESBL-producing and 7 (6.8 %) non ESBL-producing *E. coli* isolates from KNH patients. As shown in Table 4.8 there was no significant difference in the detection of the *bla*$_{\mathrm{TEM}}$ in the *E. coli* samples from patients treated at the two hospitals (P>0.05).

The *bla*$_{\mathrm{SHV}}$ gene was observed in 56(40.6%) *E. coli* isolates from patients treated at both health facilities. Twenty-eight (82.4 %) ESBL-producing and 3 (8.8 %) non ESBL producing *E. coli* isolates were obtained from ELVH patients. The patients treated at KNH had 19 (18.3 %) ESBL-producing and 6 (5.7 %) non ESBL-producing *E. coli* isolates harbouring the *bla*$_{\mathrm{SHV}}$ gene (Table 4.8). Additionally, no significant disparity between the *bla*$_{\mathrm{SHV}}$ gene detection in the *E. coli* samples acquired at ELVH and KNH (P>0.05).

In general, the *bla*$_{\mathrm{OXA}}$ gene was found in 47 (34.1%) isolates whereby only 13 (17.6 %) ESBL-producing *E. coli* isolates from patients treated at the ELVH. The *E. coli* isolates from KNH patients showed 31 (29.8 %) were ESBL-producers while 6 (5.8 %) were non ESBL-producers. However, the contrast between the *bla*$_{\mathrm{OXA}}$ gene detected amidst the *E. coli* isolates at ELVH and KNH patients was insignificant (P>0.05).

The lowest observed gene was *bla*$_{\text{CTX-M-group-2}}$ in 19 (13.8%) out of the total *E. coli* isolates from patients at the two health facilities. This comprised of 7 (20.6 %) ESBL producing *E. coli* isolates from ELVH patients, 9 (8.7 %) ESBL producing isolates and 3 (2.8 %) non ESBL producers from patients treated at KNH. As shown in Table 4.8, no significant difference was found between the *bla*$_{\text{CTX-M-group-2}}$ gene harboured in the *E. coli* isolates from patients at both facilities ($P>0.05$).

However, the *bla*$_{\text{CTX-M-group-8/25}}$ gene was not observed in any *E. coli* isolates from patients treated at ELVH and KNH. Notably, 6(4.3%) samples didn't harbour any of the ESBL genes.

Table 4.8: Proportions of the ESBL genes in the *E. coli* isolates

ESBL gene	**ESBL status**	**ELVH n (%)**	**KNH n (%)**	**Total N (%)**
bla_{TEM}	Positive	4 (11.8)	46 (44.2)	58 (42.0)
	Negative	1 (2.9)	7 (6.8)	
bla_{SHV}	Positive	28 (82.4)	19 (18.3)	56 (40.6)
	Negative	3 (8.8)	6 (5.7)	
bla_{OXA}	Positive	13 (17.6)	31 (29.8)	50 (36.2)
	Negative	0 (0.0)	6 (5.8)	
$bla_{\text{CTX-M-gp-1}}$	Positive	5 (14.7)	23 (22.1)	28 (20.3)
	Negative	0(0.0)	0 (0.0)	
$bla_{\text{CTX-M-gp-9}}$	Positive	3 (8.8)	21 (20.2)	24 (17.4)
	Negative	0 (0.0)	0 (0.0)	
$bla_{\text{CTX-M-gp-2}}$	Positive	7 (20.6)	9 (8.7)	19 (13.8)
	Negative	0 (0.0)	3 (2.8)	
$bla_{\text{CTX-M-gp-8/25}}$	Positive	0 (0.0)	0 (0.0)	0 (0.0)
	Negative	0 (0.0)	0 (0.0)	
No genes detected	Positive	1(2.9)	4 (3.8)	6 (4.3)
	Negative	0(0.0)	1 (0.0)	

Fisher's exact test at $\alpha_{0.05}$; ESBL: Extended-Spectrum β-Lactamase; NC: not computed; n: sample size; KNH: Kenyatta National Hospital; ELVH: Embu Level V Hospital; n: Sample size; N: Total number of isolates.

*All p values >0.05

4.6.2 ESBL genes detected in *K. pneumoniae* clinical isolates

In general, the *bla*$_{\text{TEM}}$ gene was found in 113 (89%) of the 127 *K. pneumoniae* isolates from patients treated at the two facilities. The gene was harboured in each of the 12(100%) ESBL-producing *K. pneumoniae* isolates from ELVH patients and 94 (81.7 %) from KNH patients. Only 7 (6.1%) of the non ESBL-producers *K. pneumoniae* isolates from KHN patients harboured the *bla*$_{\text{TEM}}$ gene at KNH. As shown in Table 4.9, no significant difference was found between the *bla*$_{\text{TEM}}$ gene detected in the *K. pneumoniae* isolates from patients treated at the two hospitals found (P>0.05).

The *bla*$_{\text{SHV}}$ gene was detected in 105 (82.7%) *K. pneumoniae* isolates from patients treated at both health facilities. Ninety-one (79.1 %) ESBL-producing *K. pneumoniae* isolates from KNH patients and 6(50%) ESBL-producing *K. pneumoniae* isolates from ELVH patients harboured the *bla*$_{\text{SHV}}$ gene. Only 8 (7.0 %) of the non ESBL-producing *K. pneumoniae* isolates from patients treated at KNH harboured the *bla*$_{\text{SHV}}$ gene. However, no significant difference was found between the detection of the *bla*$_{\text{SHV}}$ gene in the *K. pneumoniae* isolates from patients treated at both facilities as shown in Table 4.9 (P>0.05).

Overall, the *bla*$_{\text{OXA}}$ gene was found in 97 (76.4%) isolates form patients treated at both facilities. Eighty-eight (76.5%) ESBL-producing *K. pneumoniae* isolates from KNH patients and 2 (16.7 %) ESBL-producing *K. pneumoniae* isolates from ELVH patients harboured the *bla*$_{\text{OXA}}$ gene. Only 7 (6.1 %) of the non ESBL-producing *K. pneumoniae* isolates from KNH patients harboured the *bla*$_{\text{OXA}}$ gene. As shown in Table 4.9, there was no significant difference found between the *bla*$_{\text{OXA}}$ gene detected in the *K. pneumoniae* isolates from patients treated at ELVH and KNH (P>0.05).

Ninety-two (72.5%) *K. pneumoniae* isolates from patients treated at the two health facilities harboured the *bla*$_{\text{CTX-M-group-1}}$ gene. The ESBL producers were 86 (74.8 %) *K. pneumoniae* isolates from KNH patients and 4 (33.3 %) *K. pneumoniae* isolates from ELVH patients. Only 2 (1.7 %) of the non ESBL-producing *K. pneumoniae* isolates at KNH harboured the *bla*$_{\text{CTX-M-group-1}}$ gene. As shown in Table 4.9, no significant difference was found between the *bla*$_{\text{CTX-M-group-1}}$ detected in the *K. pneumoniae* isolates from patients treated at each hospital (P>0.05).

There was only one (8.3 %) of the ESBL-producing *K. pneumoniae* isolate from a patient treated at ELVH which harboured the *bla*$_{\text{CTX-M-group-9}}$ gene. Moreover, the *bla*$_{\text{CTX-M-group-8/25}}$ gene was not found in any of the *K. pneumoniae* isolates obtained from patients treated at both hospitals. Notably, 1(0.85%) ESBL producing and 1 (0.85%) non ESBL producing *K. pneumoniae* isolates didn't have any ESBL genes from patients treated at KNH (Table 4.9).

Table 4.9: Proportions of the ESBL genes in the *K. pneumoniae* isolates

ESBL gene	ESBL status	ELVH n (%)	KNH n (%)	Total N (%)
bla_{TEM}	Positive	12 (100.0)	94 (81.7)	113 (89.0)
	Negative	0 (0.0)	7 (6.1)	
bla_{SHV}	Positive	6 (50.0)	91 (79.1)	105 (82.7)
	Negative	0 (0.0)	8 (7.0)	
bla_{OXA}	Positive	2 (16.7)	88 (76.5)	97 (76.4)
	Negative	0 (0.0)	7 (6.1)	
$bla_{\text{CTX-M-gp-1}}$	Positive	4 (33.3)	86 (74.8)	92 (72.5)
	Negative	0 (0.0)	2 (1.7)	
$bla_{\text{CTX-M-gp-9}}$	Positive	1 (8.3)	0 (0.0)	1 (0.8)
	Negative	0 (0.0)	0 (0.0)	
$bla_{\text{CTX-M-gp-2}}$	Positive	1 (8.3)	1 (0.85)	3 (2.4)
	Negative	0 (0.0)	1 (0.85)	
$bla_{\text{CTX-M-gp-8/25}}$	Positive	0 (0.0)	0 (0.0)	0 (0.0)
	Negative	0 (0.0)	0 (0.0)	
No genes detected	Positive	0 (0.0)	1 (0.85)	2 (1.6)
	Negative	0 (0.0)	1(0.85)	

Fisher's exact test at $\alpha_{0.05}$; ESBL: Extended-Spectrum β-Lactamase; NC: not computed; n: sample size; KNH: Kenyatta National Hospital; ELVH: Embu Level V Hospital; n: Sample size; N: Total number of isolates.

*All the p values were >0.05

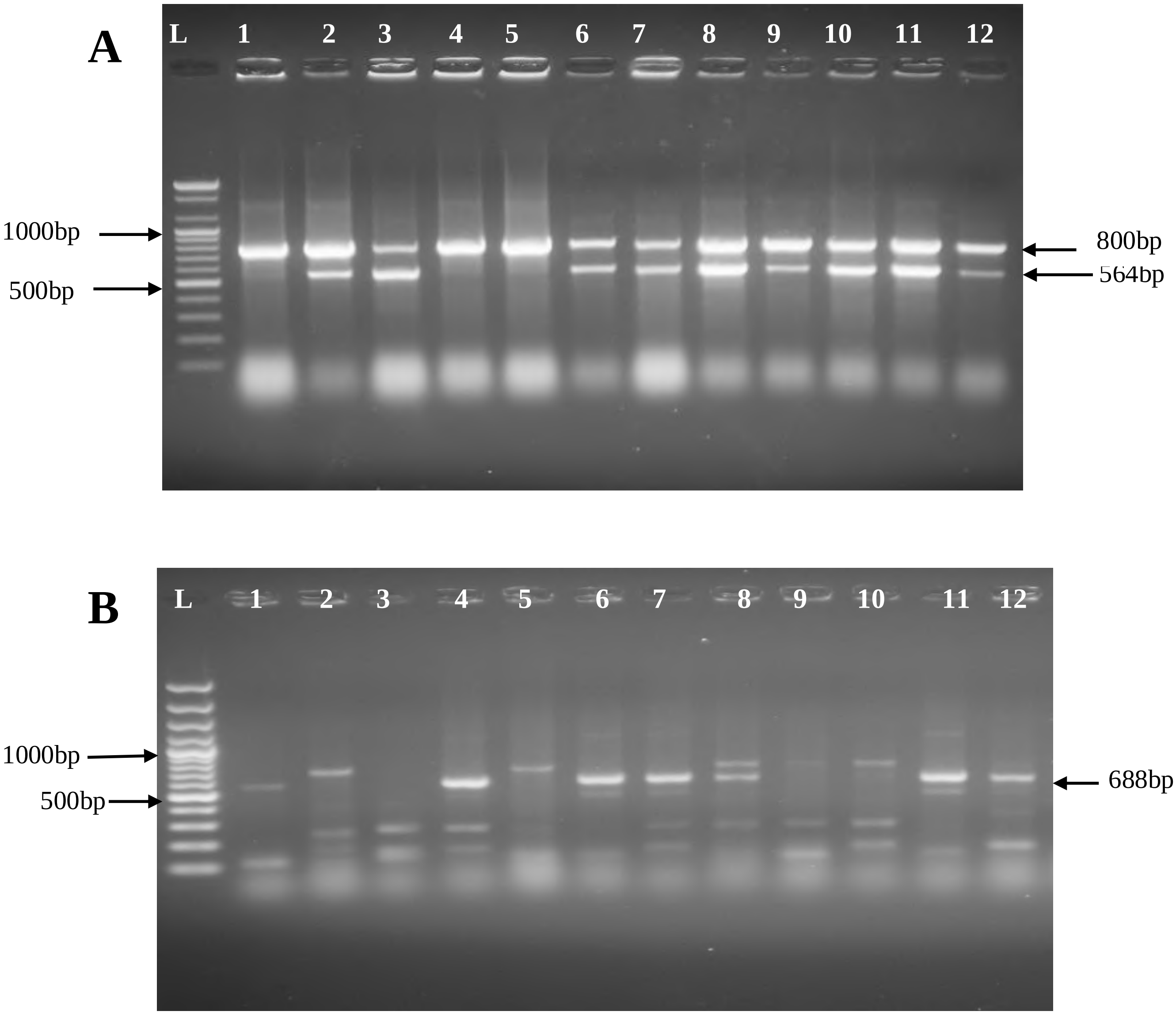

FIGURE 4.3. 1.5% w/v Agarose gel electrophoresis of *Escherichia coli* PCR products. For Panel (A) L-100bp molecular ladder, lanes 1 to 12- *E. coli* samples with bla$_{TEM}$ and bla$_{OXA}$ genes. For Panel (B) L-100bp molecular ladder, lanes 1 to 12 *E. coli* samples with *bla*$_{CTXM\ group2}$ genes.

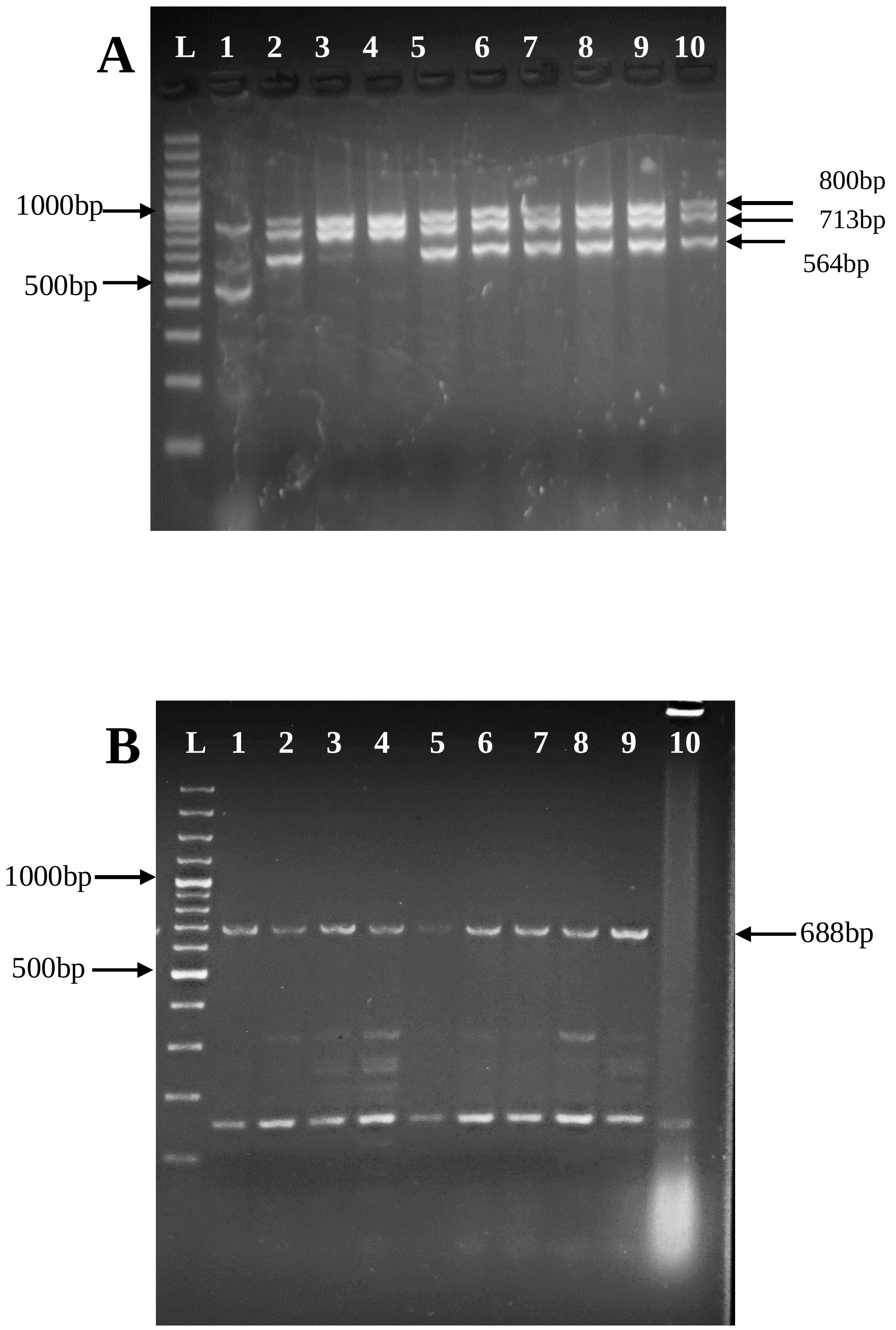

FIGURE 4.4. **1.5% w/v Agarose gel electrophoresis of *K. pneumoniae* PCR products**. For Panel (A) L-100bp molecular ladder, lanes 1 to 10- *K. pneumoniae* samples with bla$_{\mathrm{TEM,}}$ bla$_{\mathrm{SHV}}$ and bla$_{\mathrm{OXA}}$ genes. For Panel (B) L-100bp molecular ladder, lanes 1 to 10- *K. pneumoniae* samples with *bla*$_{\mathrm{CTXMgroup2}}$ genes.

4.7 The ESBL genes associated with MDR phenotypes in the clinical isolates

The frequency of the ESBL gene families detected by the MDR isolates of both pathogens from ELVH and KNH was determined.

4.7.1 ESBL genes associated with MDR phenotypes in *E. coli* isolates

All the 55 (39.9%) MDR *E. coli* isolates from patients seeking treatment at both facilities had ESBL production. The predominant resistance phenotype was ceftriaxone with 13 (30.2%) *E. coli* isolates from patients treated at KNH harbouring bla_{TEM}, bla_{SHV}, bla_{OXA}, $bla_{\text{CTX-M-group-1}}$, $bla_{\text{CTX-M-group-2}}$, and $bla_{\text{CTX-M-group-9}}$ genes. Eight (18.6%) *E. coli* isolates harboured the bla_{TEM}, bla_{SHV}, bla_{OXA}, $bla_{\text{CTX-M-group-1}}$ and $bla_{\text{CTX-M-group-9}}$ genes responsible for the cefoxitin-ceftriaxone-cefepime-amoxicillin/clavulanic acid-piperacillin/tazobactam MDR phenotype (Table 4.10).

Five (41.7%) *E. coli* isolates from patients treated at ELVH harboured the bla_{TEM}, bla_{SHV}, bla_{OXA}, $bla_{\text{CTX-M-group-1}}$ and $bla_{\text{CTX-M-group-9}}$ genes responsible for the ceftriaxone-cefepime MDR phenotype. Three (25%) *E. coli* isolates from ELVH patients harboured bla_{SHV} and bla_{OXA} genes responsible for cefoxitin-ceftriaxone-cefepime-meropenem MDR phenotype (Table 4.10).

Table 4.10: Proportions of the ESBL genes associated with MDR phenotypes in the *E. coli* isolates

ESBL-associated MDR phenotype	**ESBL genes**	**ELVH n=12 (%)**	**KNH n =43(%)**	**Total N=55 (%)**
FOX-CRO-FEP-AMC-TZP-MEM	bla_{TEM} + $bla_{\text{CTX-M-gp-9}}$	0 (0)	1 (2.3)	1(1.8)
	bla_{TEM}	0 (0)	2 (4.7)	2 (3.6)
	bla_{TEM} + bla_{OXA}	0 (0)	2 (4.7)	2 (3.6)
	bla_{SHV} + bla_{OXA}	0 (0)	1 (2.3)	1(1.8)
	bla_{OXA}	0 (0)	1 (2.3)	1(1.8)
FOX-CRO-FEP-AMC-TZP	bla_{SHV} + bla_{OXA} + $bla_{\text{CTX-M-gp-1}}$	1(8.3)	0 (0)	1(1.8)
	bla_{OXA}	0 (0)	2 (4.7)	2 (3.6)
	bla_{TEM}	0 (0)	1 (2.3)	1(1.8)
	bla_{TEM} + bla_{OXA}	0 (0)	1 (2.3)	1(1.8)
	bla_{SHV} + bla_{OXA}	0 (0)	2 (4.7)	2 (3.6)
	bla_{SHV} + bla_{OXA} + $bla_{\text{CTX-M-gp-1}}$ + $bla_{\text{CTX-M-gp-9}}$	0 (0)	1 (2.3)	1(1.8)
	bla_{TEM} + bla_{OXA} + $bla_{\text{CTX-M-gp-1}}$	0 (0)	1 (2.3)	1(1.8)
CRO-FEP-AMC-TZP-MEM	bla_{SHV}	0 (0)	1 (2.3)	1(1.8)
FOX-CRO-FEP-MEM	bla_{SHV}	2 (16.7)	0 (0)	2 (3.6)
	$bla_{\text{CTX-M-gp-1}}$	1 (8.3)	0 (0)	1(1.8)
FOX-CRO-FEP-AMC	bla_{TEM} + bla_{OXA} + $bla_{\text{CTX-M-gp-1}}$	0 (0)	1 (2.3)	1(1.8)
CRO-FEP-AMC-TZP	bla_{TEM}	0 (0)	1 (2.3)	1(1.8)
	bla_{SHV} + bla_{OXA}	1 (8.3)	0 (0)	1(1.8)
CRO-FEP-TZP-MEM	bla_{SHV}	1 (8.3)	0 (0)	1(1.8)
FOX-FEP-AMC	bla_{TEM} + $bla_{\text{CTX-M-gp-1}}$	0 (0)	1 (2.3)	1(1.8)
CRO-FEP-AMC	bla_{TEM} + bla_{OXA}	0 (0)	1 (2.3)	1(1.8)
	bla_{SHV} + $bla_{\text{CTX-M-gp-9}}$	0 (0)	1 (2.3)	1(1.8)
	bla_{TEM}	0 (0)	1 (2.3)	1(1.8)
CRO-AMC	bla_{OXA}	0 (0)	1 (2.3)	1(1.8)
	$bla_{\text{CTX-M-gp-9}}$	0 (0)	1 (2.3)	1(1.8)
	bla_{TEM} + bla_{SHV} + $bla_{\text{CTX-M-gp-1}}$	0 (0)	1 (2.3)	1(1.8)
CRO-FEP	bla_{SHV}	1 (8.3)	0 (0)	1(1.8)
	bla_{TEM} + $bla_{\text{CTX-M-gp-1}}$ + $bla_{\text{CTX-M-gp-9}}$	0 (0)	1 (2.3)	1(1.8)
	bla_{TEM}	1 (8.3)	0 (0)	1(1.8)
	bla_{TEM} + bla_{SHV} + $bla_{\text{CTX-M-gp-1}}$ + $bla_{\text{CTX-M-gp-9}}$	1 (8.3)	0 (0)	1(1.8)
	bla_{OXA} + $bla_{\text{CTX-M-gp-1}}$	0 (0)	1 (2.3)	1(1.8)
	bla_{OXA}	2 (16.7)	0 (0)	2 (3.6)
	bla_{TEM} + $bla_{\text{CTX-M-gp-1}}$	0 (0)	1 (2.3)	1(1.8)
	$bla_{\text{CTX-M-gp-1}}$	0 (0)	1 (2.3)	1(1.8)
CRO-TZP	bla_{TEM} + $bla_{\text{CTX-M-gp-9}}$	0 (0)	1 (2.3)	1(1.8)
	bla_{OXA}	1 (8.3)	0 (0)	1(1.8)
CRO	bla_{TEM} + bla_{OXA}	0 (0)	1 (2.3)	1(1.8)
	bla_{TEM} + bla_{SHV} + $bla_{\text{CTX-M-gp-1}}$ + $bla_{\text{CTX-M-gp-9}}$	0 (0)	1 (2.3)	1(1.8)
	bla_{TEM}	0 (0)	3 (7.0)	3 (5.5)
	bla_{TEM} + $bla_{\text{CTX-M-gp-9}}$	0 (0)	1 (2.3)	1(1.8)
	bla_{OXA} + $bla_{\text{CTX-M-gp-2}}$	0 (0)	1 (2.3)	1(1.8)

ESBL-associated MDR phenotype	**ESBL genes**	**ELVH n=12 (%)**	**KNH n =43(%)**	**Total N=55 (%)**
	bla$_{TEM}$ + *bla*$_{SHV}$ + *bla*$_{OXA}$ + *bla*$_{CTX\text{-}M\text{-}gp\text{-}1}$	0 (0)	1 (2.3)	1(1.8)
	bla$_{OXA}$	0 (0)	1 (2.3)	1(1.8)
	bla$_{SHV}$ + *bla*$_{CTX\text{-}M\text{-}gp\text{-}1}$	0 (0)	1 (2.3)	1(1.8)
	bla$_{TEM}$ + *bla*$_{CTX\text{-}M\text{-}gp\text{-}1}$	0 (0)	1 (2.3)	1(1.8)
	bla$_{CTX\text{-}M\text{-}gp\text{-}1}$	0 (0)	1 (2.3)	1(1.8)
	bla$_{SHV}$	0 (0)	1 (2.3)	1(1.8)

FOX: Cefoxitin; CRO: Ceftriaxone; FEP: Cefepime; AMC: Amoxicillin/Clavulanic acid; TZP: Piperacillin/Tazobactam; MEM: Meropenem; n: sample size; ELVH: Embu Level V Hospital; KNH: Kenyatta National Hospital.

4.7.2 ESBL genes associated with MDR phenotypes in *K. pneumoniae* isolates

The predominant MDR phenotypes observed in ESBL producing *K. pneumoniae* isolates from patients treated at KNH harbored bla_{TEM}, bla_{SHV}, bla_{OXA} and $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}1}}$ genes responsible for the cefoxitin-ceftriaxone-cefepime-amoxicillin/clavulanic acid-piperacillin/tazobactam MDR phenotype (18.2%) and ceftriaxone-cefepime (18.2%) MDR phenotype. It was noteworthy that one non ESBL-producing *K. pneumoniae* isolate from a patient at KNH harboured bla_{TEM}, bla_{SHV} and bla_{OXA} genes responsible for cefoxitin-ceftriaxone-cefepime-amoxicillin/clavulanic acid-piperacillin/tazobactam MDR phenotype (Table 4.11).

Three ESBL producing *K. pneumoniae* isolates from patients treated at ELVH harboured bla_{TEM}, bla_{SHV}, $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}1}}$ or $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}2}}$ genes responsible for the cefoxitin-ceftriaxone-amoxicillin/clavulanic acid MDR phenotype (Table 4.11).

Table 4.11: Proportions of the ESBL genes associated with MDR phenotypes in the *K. pneumoniae* isolates

ESBL-associated MDR phenotype	ESBL genes	ELVH n=6 (%)	KNH n=11 (%)	Total N=17 (%)
FOX-CRO-FEP-AMC-TZP-MEM	$bla_{\mathrm{SHV}} + bla_{\mathrm{OXA}}$	0 (0)	1 (9.1)	1 (5.9)
FOX-CRO-FEP-AMC-TZP	$bla_{\mathrm{SHV}} + bla_{\mathrm{OXA}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$	0 (0)	1 (9.1)	1 (5.9)
	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}} + bla_{\mathrm{OXA}}$	0 (0)	1 (9.1)	1 (5.9)
CRO-FEP-AMC-TZP-MEM	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}} + bla_{\mathrm{OXA}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$	0 (0)	1 (9.1)	1 (5.9)
FOX-CRO-AMC-MEM	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}}$	1 (16.7)	0 (0)	1 (5.9)
FOX-AMC-TZP	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}} + bla_{\mathrm{OXA}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$	0 (0.)	1 (9.1)	1 (5.9)
FOX-CRO-AMC	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$	1 (16.7)	0 (0)	1 (5.9)
	$bla_{\mathrm{TEM}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$	1 (16.7)	0 (0)	1 (5.9)
	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}2}}$	1 (16.7)	0 (0)	1 (5.9)
FOX-CRO-FEP	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$	0 (0)	1 (9.1)	1 (5.9)
CRO-AMC-TZP	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$	0 (0)	2 (9.1)	2 (11.8)
CRO-FEP-AMC	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}}$	1 (16.7)	1 (9.1)	2 (11.8)
CRO-FEP	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$	0 (0)	1 (9.1)	1 (5.9)
	$bla_{\mathrm{TEM}} + bla_{\mathrm{CTX\text{-}M\text{-}gp\text{-}1}}$	0 (0)	1 (9.1)	1 (5.9)
CRO	$bla_{\mathrm{TEM}} + bla_{\mathrm{SHV}}$	1 (16.7)	0 (0)	1 (5.9)

FOX: Cefoxitin; CRO: Ceftriaxone; FEP: Cefepime; AMC: Amoxicillin/Clavulanic acid; TZP: Piperacillin/Tazobactam; MEM: Meropenem; n: sample size; ELVH: Embu Level V Hospital; KNH: Kenyatta National Hospital.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

The emergence and spread of antimicrobial-resistant strains of commensal and pathogenic bacteria has significantly hampered the global efforts to mitigate infectious illnesses (Hailemariam *et al.*, 2021; Jain *et al.*, 2021). The World Health Organisation (WHO) report shows there is insufficient data to guide policy formulation and recommendations on antimicrobial resistance (AMR), especially in Africa (WHO, 2022). The increase in AMR in Kenya as well as across East Africa has been aided by the lack of insufficient stewardship programs in hospitals, inadequate laboratory infrastructure, poor pharmacovigilance systems, and lack of updated treatment guidelines which are based on local antimicrobial susceptibility patterns at the national and facility levels (Abebe *et al*., 2019; Hailemariam *et al*., 2021; Ministry of Health, 2017).

Studies indicates that *Klebsiella pneumoniae* and *Escherichia coli* represent the most common occurring enterobacterial microorganisms causing various illnesses and have demonstrated resistance to common antibiotics, with some exhibiting multidrug-resistant (MDR) phenotypes thus limiting therapeutic options (Ny *et al.*, 2019; Sharahi *et al.*, 2021; Zanichelli *et al*., 2019). The predominance of ESBL production among *Enterobacteriaceae* ranges from below 1% to greater than 70% worldwide (Shakya *et al.*, 2017). Therefore, this research assessed the phenotypic and genetic determinants of antimicrobial resistance of ESBL-producing *E. coli* and *K. pneumoniae* from patients seeking treatment at Embu Level 5 hospital and Kenyatta National Hospital, Kenya.

The bacterial isolates from both facilities were identified through conventional biochemical techniques and molecular characterization through primers targeting species specific genes. All the 21 representative *E. coli* isolates (100%) from both facilities were positively identified as *E. coli* by sequencing and blasting after molecular analysis using the *gadA* gene. However, only 4 *K. pneumoniae* isolates (33.3%) from the Embu level 5 hospital were positively identified *as K. pneumoniae* after molecular analysis using the *rpoB* gene. At the KNH, all the 9 representative *K. pneumoniae* isolates (100%) showed similarity to *K. pneumoniae*.

These results show that the VITEK® 2 and MALDITOF MS systems used at the KNH demonstrated higher specificity and sensitivity compared to the IMVIC analysis used at the Embu level 5 hospital for biochemical analysis. The sample size collected at the ELVH was small compared to KNH. These differences could be due to the staffing and the availability of lab diagnostic resources at both microbiology labs. However, none of the labs performed PCR and sequencing as part of their routine surveillance. The molecular analysis was necessary to differentiate the bacterial isolates due to the greater reproducibility, specificity and sensitivity of that methodology(Książczyk *et al*., 2016).

The proportion of ESBL production amidst the *E. coli* isolates was elevated in both facilities with no significant difference (Table 4.3). These findings corroborate those reported previously in Kenya by other scholars that over fifty percent the clinical isolates of *E. coli* were ESBL producers(Maina *et al*., 2013; Magale *et al*., 2015). Additionally, surveys in Rwanda (Sutherland *et al*., 2019), Tanzania (Silago *et al*., 2021), Ethiopia (Teklu *et al*., 2019), Uganda(Baguma *et al*., 2017; Najjuka *et al*., 2016), Nigeria (Mofolorunsho *et al*., 2021b), Burkina Faso (Ouédraogo *et al*., 2016), Chad(Mahamat *et al*., 2019) and Nepal (Nepal *et al*., 2017; Koirala *et al*., 2021) revealed a similar pattern of ESBL production in *Escherichia coli* isolates. However, the ESBL production amidst *E. coli* clinical isolates was lower, as compared to this study, in Kenya(Kiiru *et al.*, 2012; Muriuki *et al.*, 2022), Uganda (Mbyemeire *et al.*, 2021; Iramiot *et al.*, 2018), Tanzania (Letara *et al.*, 2021), Canada(Denisuik *et al.*, 2019), Thailand(Sawatwong *et al.*, 2019), Brazil (Pereira *et al.*, 2019), Iran (Sharahi *et al.*, 2021), Nepal (Shilpakar *et al.*, 2021), the USA, Europe, Asia-Pacific as well as Latin America(Castanheira *et al.*, 2021).

The elevated ESBL production may be explained by the increased utilisation of third generation cephalosporins or fluoroquinolones, bed sharing, overcrowding in addition to previous or prolonged hospitalisation. Other risk factors include disparities in the infection prevention control policies or treatment guidelines within the hospital settings, availability of rapid lab diagnosis and the antimicrobial stewardship programs in place (Onduru *et al.,* 2021; Chokshi *et al.,* 2019; Sonda *et al.,* 2016; Castro-Sánchez *et al.*, 2016). The ESBL producing *E. coli* strains in phylogroups A and D contain virulence factors (toxins, fimbrial adhesins, iron capture systems) which have a tendency to develop resistance to oxyimino cephalosporins through AmpC β-lactamases as well as acquisition of CTX-M plasmids, respectively (Yu *et al.*, 2021). Biofilm formation by *E. coli* on hospital surfaces and indwelling medical devices may also be involved in the antimicrobial non-susceptibility towards extended spectrum cephalosporins (Jamal *et al.*, 2018)

The *K. pneumoniae* isolates had an elevated proportion in ESBL production at both facilities with no significant difference (Table 4.3). Previous studies displayed a similar pattern of ESBL production in Kenya at the Kilifi county Hospital (Henson *et al.*, 2017) and at the KNH (Magale *et al.*, 2015). Similarly, surveys in Uganda(Baguma *et al.*, 2017; Najjuka *et al.*, 2016), Tanzania(Silago *et al.*, 2021), Ethiopia (Teklu *et al.*, 2019) and Nepal(Koirala *et al.*, 2021; Nepal *et al.*, 2017) reported such high proportions. Lower ESBL production ranges were previously reported in Kenya at the Aga Khan University Hospital (Maina *et al.*, 2013) and KEMRI (Taitt *et al*., 2017). Likewise, studies done in Tanzania(Letara *et al*., 2021), Uganda (Mbyemeire *et al*., 2021), Ethiopia (Abayneh *et al*., 2018), Nigeria (Mofolorunsho *et al*., 2021b), Burkina Faso(Ouédraogo *et al*., 2016), Chad (Mahamat *et al*., 2019), Iran (Sharahi *et al*., 2021), Nepal(Shilpakar *et al*., 2021), Thailand (Sawatwong *et al*., 2019), Brazil(Pereira *et al*., 2019) and the USA(McDanel *et al*., 2017) observed low proportions.

This increased ESBL production may be associated with the extensive exposure of patients to oxyimino cephalosporins in clinical settings causing selective pressure against the antibiotics (Castanheira *et al*., 2021). *Klebsiella pneumoniae* has intrinsic resistance to ampicillin and ticarcillin because of the core gene bla_{SHV} in its chromosome causing production of the class A ESBL enzyme SHV (Wyres *et al*., 2020). Mutations could also lead to hydrolysis of ceftazidime and cefotaxime (Castanheira *et al*., 2021). The differences in proportions of ESBL production could be due to variations in analytical methods, collection techniques and sample size (Ejaz, 2022).

This research revealed that over half of ESBL-producing *E. coli* samples at both hospitals exhibited high resistance to ceftriaxone, levofloxacin and azithromycin (Table 4.4). Nonetheless, susceptibility to meropenem, amikacin and tigecycline was recorded with the urinary isolates being sensitive to nitrofurantoin. These findings corroborate those reported previously in Kenya at the KNH (Wangai *et al*., 2019; Magale *et al*., 2015) and two private hospitals in Nairobi (Lord *et al*., 2021; Maina *et al*., 2013). Other studies previously done in Rwanda (Sutherland *et al*., 2019), Chad (Mahamat *et al*., 2019) Nepal (Nepal *et al*., 2017; Shilpakar *et al*., 2021), Brazil (Pereira *et al*., 2019) and Canada (Denisuik *et al*., 2019) showed a similar antimicrobial resistance pattern. On the contrary, this study findings differ from those documented in Uganda (Iramiot *et al*., 2018), which showed low resistance of *E. coli* samples derived from outpatients to ceftriaxone and levofloxacin. Moreover, some studies showed a high resistance of *E. coli* samples to carbapenems in Nigeria (Mofolorunsho *et al*., 2021b) and Nepal (Koirala *et al*., 2021) which can be associated with the production of carbapenemases.

These differences may be attributed to patient factors, specific antimicrobial therapy regimens in various hospitals, the environment and specific selective pressures, which affect the microbial characteristics associated with diverse resistance patterns (Kiiru *et al.*, 2012; Muriuki *et al.*, 2022). *Escherichia coli* has the ability to produce ESBLs which hydrolyse oxyimino-cephalosporins like ceftriaxone with co-resistance to fluoroquinolones like levofloxacin through plasmid-mediated quinolone resistance. Intrinsic resistance to macrolides has also been reported in *E. coli* through erythromycin ribosomal methyltransferases (*erm*) and multidrug efflux pumps (Peterson and Kaur, 2018; Fyfe *et al*., 2016), hence the observed resistance to azithromycin.

The present research showed elevated resistance of *K. pneumoniae* isolates to ceftriaxone, amoxicillin/clavulanic acid in addition to cefepime (Table 4.5). Additionally, most of the isolates had sensitivity towards amikacin, tigecycline as well as meropenem. The urinary isolates exhibited some resistance to nitrofurantoin. Comparable findings were reported in Kenya at a tertiary hospital in Eldoret (Apondi *et al*., 2016), a private hospital in Nairobi (Lord *et al*., 2021), Kilifi county hospital (Henson *et al*., 2017) and at the KNH (Wangai *et al*., 2019). Previous studies which agree with these findings were done in Thailand (Sawatwong *et al*., 2019), Ethiopia (Teklu *et al*., 2019) and Nepal (Shilpakar *et al*., 2021). However, some surveys found that *K. pneumoniae* isolates showed resistance towards carbapenems in Nigeria (Mofolorunsho *et al*., 2021b) and Nepal (Koirala *et al*., 2021).

The resistance observed to ceftriaxone and cefepime could be due to the high ESBL production noted in the *K. pneumoniae* isolates as these enzymes hydrolyse extended spectrum beta lactam antibiotics rendering them ineffective (Castanheira *et al.*, 2021; Paterson and Bonomo, 2005). The co-production of other beta lactamases like AmpC or inhibitor resistant β-lactamases which confer resistance against β-lactamase inhibitors, clavulanate and sulbactam, could explain the amoxicillin/clavulanic acid resistance (Castanheira *et al.*, 2021; Khalifa *et al.*, 2021; Rodriguez-Baño *et al.*, 2018). Mutational loss or alteration of porins in ESBL producing strains of *K. pneumoniae* can cause elevated resistance to fluoroquinolones as well as β lactam antibiotics (Ejaz, 2022; Khalifa *et al.*, 2021). The nitrofurantoin resistance observed in the urinary isolates perhaps is due to mutations disrupting product activation by nitro reductase and $\mathit{bla}_{\text{CTX-M-14}}$ mutations which catalyzes nitrofurantoin hydrolysis (Van Hugh Le and Rakonjac, 2021). Moreover, the high resistance frequencies in the studied bacterial isolates depicts the burden of antimicrobial resistance, which limits treatment options leading to life-threatening sequelae (Tadesse *et al.*, 2017; Leopold *et al.*, 2014).

Research shows that the multi-drug resistance of various clinical samples of the two pathogens significantly complicates antimicrobial chemotherapy by reducing treatment options (WHO, 2021). Both pathogens demonstrated less than 50 percent multi-drug resistance (MDR) to the tested antibiotics. In Kenya, a study on *K. pneumoniae* isolates exhibited similar proportions(Taitt *et al.*, 2017) while another in Spain (Arana *et al.*, 2016) showed similar low percentages in MDR ESBL producing *E. coli* and *K. pneumoniae* isolates. Other studies showed varying MDR proportions, in hospital settings among both isolates with over 50 percent observed against a considerable range of antibiotics in Kenya (Henson *et al.*, 2017; Apondi *et al.*, 2016), Portugal (Carvalho *et al.*, 2021), Canada (Karlowsky *et al.*, 2021) and Nepal (Koirala *et al.*, 2021).

There were varied resistance patterns exhibited by the studied isolates, despite their collection from similar settings. This finding may be attributed to the frequency of specific antibiotic administration, among other factors such as those reported previously by other scholars (San *et al.*, 2022; Zhang *et al.*, 2012). Perhaps, the lack of proper antimicrobial resistance surveillance and stewardship, compounded by the inadequate testing and documentation capacity, may be responsible for the high resistance rates (Elton *et al.*, 2020; Wang and Ogunseitan, 2022). Thus, identifying multidrug-resistance (MDR) patterns exhibited by pathogenic bacterial strains help guide the course of therapy (Seale *et al.*, 2017; WHO, 2016). The study findings revealed that all the ESBL-producing *K. pneumoniae* and *E. coli* isolates at ELVH exhibited resistance to one antibiotic under investigation. Notably, a few non-ESBL-producers of both strains obtained from the KNH demonstrated resistance to various antibiotics.

Thus, following the observed resistance patterns, this study investigated the phenotypic MDR patterns exhibited by the clinical isolates of both pathogens at ELVH and KNH. The findings revealed ceftriaxone-cefepime-azithromycin-levofloxacin and ceftriaxone-azithromycin-levofloxacin as the most prevalent resistant phenotypic patterns of *E. coli* isolates in ELVH and KNH, respectively. This differs from a survey done in Portugal (Carvalho *et al.*, 2021) which found most ESBL producing *E. coli* isolates exhibited amoxicillin/clavulanic acid-cefotaxime-ceftazidime-tetracycline-tobramycin-ciprofloxacin-gentamicin MDR phenotype. A surveillance study in Canada (Karlowsky *et al.*, 2021) found the most frequent MDR phenotype to be ceftriaxone-ciprofloxacin-sulfamethoxazole/trimethoprim among *E. coli* isolates. Various antibiotic resistance mechanisms could explain these resistance phenotypes caused by biochemical factors consisting of drug target modification, inactivation by group transfer (transferases), plus antibiotic inactivation, and genetic factors involving mutations along with horizontal gene transfer (Padmini *et al.*, 2017; Shaikh *et al.*, 2014).

Earlier research indicates that MDR phenotypes (resistance patterns) are determined by gene combinations or specific mutations and the environment where the microbes exist, which are highly variable (Meybodi *et al.*, 2021; Saravanan and Raveendaran, 2013). This may further explain the differences in MDR phenotypes of clinical isolates observed between the two hospitals. *Escherichia coli* strains possess virulence factors consisting of siderophores, fimbriae, adhesins, outer membrane proteins and toxins which promote their biofilm formation potential in addition to increased antimicrobial resistance (Yu *et al.*, 2021; Sarowska *et al.*, 2019).

Besides, ceftriaxone-amoxicillin/clavulanic acid-piperacillin/tazobactam-azithromycin-minocycline was the most frequent resistance pattern in *K. pneumoniae* isolates obtained from the ELVH, while FOX-CRO-FEP-AMC-TZP-AZM-LVX-MI and CRO-AMC-TZP-AZM-MI are the most frequent MDR phenotypes observed in *K. pneumoniae* isolates, which were acquired at KNH. Contrary findings were observed in Portugal(Carvalho *et al.*, 2021) where the carbapenems imipenem, meropenem and ertapenem formed part of the MDR phenotypes. In Canada (Karlowsky *et al.*, 2021) the most frequent phenotype was gentamicin-ceftriaxone-ciprofloxacin-sulfamethoxazole/trimethoprim. In particular, a non ESBL producing *K. pneumoniae* isolate at KNH exhibited a resistance phenotype consisting of cephalosporins, β lactam/β lactamase inhibitors, azithromycin, levofloxacin and tigecycline despite harbouring a combination of bla_{TEM} + bla_{SHV} + bla_{OXA}. This could indicate the presence of other antimicrobial resistance mechanisms like reduced membrane permeability, enzymatic drug degradation, drug target modification and active drug efflux (Padmini *et al.*, 2017; Shaikh *et al.*, 2014). *Klebsiella pneumoniae* has virulence factors comprising of the capsule, siderophores, fimbriae, porins, pumps, outer membrane proteins and allantion mechanisms which aid in biofilm formation, increase pathogenicity as well as evading antimicrobial agents (Paczosa and Mecsas, 2016).

The antimicrobial resistance, including MDR, exhibited by pathogenic enterobacteria, especially *K. pneumoniae* and *E. coli,* is primarily driven by a plasmid-mediated β-lactamase (Boonyasiri *et al.*, 2021; Dela *et al.*, 2022; Khalifa *et al.*, 2021). This enzyme hydrolyses and inactivates β-lactam antibiotics, including monobactams and cephalosporins, among a wide array of other antibiotics, rendering them ineffective. Molecular studies have revealed various ESBL variants in *K. pneumoniae* and *E. coli*, according to the gene or gene combinations they express (Aronin *et al.*, 2022; Na *et al.*, 2019; Pereira *et al.*, 2019).

The bla_{TEM} gene was the predominant gene observed in both isolates, then bla_{SHV}, bla_{OXA} and $bla_{\text{CTX-M-group-1}}$ from both hospitals. Surveys carried out in Uganda(Mbyemeire *et al*., 2021) and Iran(Sharahi *et al*., 2021) revealed a similar pattern whereby bla_{TEM} and bla_{SHV} genes were the most frequent. Surveys focusing on *K. pneumoniae* isolates done in Kenya(Henson *et al*., 2017) and Tanzania(Sonda *et al*., 2018) corroborate this study's findings. In addition, previous work analysing *E. coli* isolates in Kenya(Muriuki *et al*., 2022) displayed similar results. Studies which contradict these findings have been done in Burkina Faso(Ouédraogo *et al*., 2016), Chad (Mahamat *et al*., 2019), Brazil(Pereira *et al*., 2019) and Canada(Denisuik *et al*., 2019) whereby $bla_{\text{CTX-M}}$ was the most frequent gene . In Tanzania(Silago *et al*., 2021) a survey noted that over half the ESBL production in both pathogens had a blend of bla_{TEM}, $bla_{\text{CTX-M}}$ and bla_{SHV} present. Moreover, recent data indicates that the $bla_{\text{CTX-M}}$ is the commonest ESBL gene variant, and in some regions, it transcends the bla_{TEM} and bla_{SHV} ESBLs (Ejaz, 2022; Khalifa *et al.*, 2021). These differences may partly be attributable to geographic and environmental differences and specific gene factors, such as inherent mutations (Shawky *et al.*, 2021).

Studies shows that the detection of single or multiple ESBL genes drives MDR in *E. coli* and *K. pneumoniae* strains, among other clinically important bacteria (Maina *et al.*, 2017; Silago *et al.*, 2021). Accordingly, this study revealed the relationship between the ESBL genes and MDR phenotypes in clinical isolates of both pathogens obtained from ELVH and KNH. The findings showed diverse ESBL gene combinations in various MDR phenotypes of the *K. pneumoniae* and *E. coli* and samples, in accordance with previous research done in Kenya(Muriuki *et al.*, 2022), Tanzania (Silago *et al.*, 2021) and Rwanda(Sutherland *et al.*, 2019). This depicts the diverse and intricate mechanisms these microbes employ to evade antibacterial action, including the acquisition of resistance genes from other bacterial strains, and expressing these genes in various combinations, due to antimicrobial-induced selection (Heiden *et al.*, 2020; Sonda *et al.*, 2018).

Previous surveys indicate various ESBL-associated genes may have other functional roles apart from the plasmid-mediated β-lactamase hydrolysis of antibiotics (Ejaz, 2022). For instance, the $bla_{\text{CTX-M}}$ gene has been proven to decrease the sensitivity of *E. coli* and *K. pneumoniae* strains to carbapenems and cephalosporins (Sakkas *et al.*, 2019). Similar resistance patterns and genetic traits between isolates collected from the KNH and ELVH denote an active distribution of resistant pathogens. Perhaps, the proximity of Embu County to Nairobi County, where KNH is located, may have facilitated the transfer of resistant strains through persons during their mobility.

Besides, plasmids carrying the bla_{OXA} gene harbour other non-ESBL genes such as the bla_{CYM}, bla_{NDM}, and $bla_{\text{VIM,}}$ which confer enhanced metallo-β-lactamase activity, alteration of porin synthesis, and the acquisition of other drug-degrading enzymes (Rahman *et al.*, 2018). It is noteworthy that ESBL-associated genes are diverse and present varied characteristics (Adekanmbi *et al.*, 2020; Castanheira *et al.*, 2021). The various genetic combinations in the studied isolates may have been responsible for the elevated resistance toward particular antibiotics as seen in this present study. Moreover, a combination of these genes may modify the activity of multiple antibiotics leading to the MDR (Adekanmbi *et al.*, 2020; Rahman *et al.*, 2018), as witnessed in this study. The resistance detected in some non-ESBL producing clinical isolates of the studied microbes may be due to porin loss and the acquisition of other resistance mechanisms like mutation of antibiotic target proteins, amplification of multidrug efflux pumps and modification of the outer membrane permeability (Khalifa *et al.*, 2021).

Previous reports indicate that selective pressure due to antibiotic use may induce point mutations in various bacterial genes, especially in clinical settings (Adekanmbi *et al.*, 2020). These mutations modify protein products, leading to enhanced adaptability of the bacterial pathogens to antibiotics. As a result, these strains acquire extended resistance to various previously efficacious antibiotics of various classes, complicating their treatment. Moreover, similar resistance traits among *K. pneumoniae* and *E. coli* isolates might perhaps be attributed to horizontal gene transfer through plasmid carriers (Boonyasiri *et al.*, 2021; Saravanan and Raveendaran, 2013). However, gene interactions between the two strains should be investigated further. Additional molecular characterisation to distinguish particular mutations in resistance-associated genes, especially in the synthesis and action of ESBLs, may help us understand their dynamic functioning and develop more efficacious therapies (Suresh *et al.*, 2018; WHO, 2016).

Proper surveillance mechanisms are imperative considering the intricate nature of resistance to antimicrobial agents, especially among *E. coli* and *K. pneumoniae*, which are notorious sources of nosocomial infections (Isgren *et al.*, 2022; Karki *et al.*, 2021; San *et al.*, 2022; Sharahi *et al.*, 2021). Additionally, appropriate characterisation of resistance patterns and genetic traits in these enterobacteria may help redefine treatment regimens, averting further exacerbation as well as distribution of resistant strains (Berhe *et al.*, 2021).

As a result of the complex dynamics of antimicrobial resistance coupled with the high burden of MDR-related healthcare, especially in Kenya, close stewardship and regular surveillance programs in health facilities are necessary (Seale *et al.*, 2017). This may include policy formulation and implementation, development of testing, documentation, reporting infrastructure, and adequate staff training, among others (Elton *et al.*, 2020; Ministry of Health, 2017). Besides, the frequency and transmission of MDR strains may be partly mitigated through quarantine of patients with confirmed MDR phenotypes, strict disinfection of medical devices, handwashing and hygiene, and limiting the usage of specific antimicrobial drugs, especially those prone to resistance (Bairán *et al.*, 2020; Lee *et al.*, 2013).

The study had a few limitations including the small sample size of *K. pneumoniae* isolates at ELVH which was below the threshold of 30 required for AST investigation (CLSI, 2014). There was incomplete patient data entered in the hospital lab registers at the two microbiology laboratories. The strengths of the study were that phenotypic confirmation of ESBL production as well as molecular detection of the selected ESBL genes responsible for ESBL phenotypes implicated in beta lactam resistance.

5.2 Conclusions

In accordance with the research findings, the following inferences were made according to the formulated objectives.

i. Automated biochemical methods like VITEK® 2 and molecular techniques for identification of *E. coli* and *K. pneumoniae* were more specific as well as selective compared to the conventional phenotypic techniques.

ii. *Escherichia coli and K. pneumonia* isolated from patients seeking treatment at Embu Level V Hospital and KNH were ESBL-producers displaying MDR phenotypes to beta-lactams, levofloxacin, azithromycin and amoxicillin/clavulanic acid. The isolates were sensitive to meropenem, amikacin and tigecycline.

iii. bla_{TEM} gene was prevalent in *K. pneumoniae* and *E. coli* exhibiting extended spectrum β-lactam resistance phenotypes, while the isolates harbouring $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}2}}$ genotype were less prevalent.

5.3 Recommendations

i. Routine surveillance using automated methods is needed in hospital settings for effective real-time diagnosis of pathogenic bacterial isolates to improve therapeutic outcomes.

ii. Antimicrobial stewardship programs should be established to curb the dissemination of MDR bacterial pathogens through updated treatment guidelines and infection prevention control protocols.

iii. More extensive large-scale studies on the genetic determinants present in MDR isolates is recommended to establish AMR patterns at county and national level.

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APPENDICES

Appendix 1: Selected *Escherichia coli* isolates with percentage identities and accession numbers

Isolate ID	Target gene	Accession no.	Homologue	% Identity
ECE3	*gadA*	OL743740	*Escherichia coli*	99%
ECE33	*gadA*	OL743741	*Escherichia coli*	99%
ECK16	*gadA*	OL743742	*Escherichia coli*	99%
ECK49	*gadA*	OL743743	*Escherichia coli*	99%
ECK30	*gadA*	OL743744	*Escherichia coli*	99%
ECK23	*gadA*	OL743745	*Escherichia coli*	99%
ECK96	*gadA*	OL743746	*Escherichia coli*	99%
ECE1	*gadA*	OL743747	*Escherichia coli*	99%
ECK9	*gadA*	OL743748	*Escherichia coli*	99%
ECK95	*gadA*	OL743749	*Escherichia coli*	99%
ECK60	*gadA*	OL743750	*Escherichia coli*	99%
ECE9	*gadA*	OL743751	*Escherichia coli*	99%
ECE6	*gadA*	OL743752	*Escherichia coli*	99%
ECK53	*gadA*	OL743753	*Escherichia coli*	99%
ECK44	*gadA*	OL743754	*Escherichia coli*	99%
ECE36	*gadA*	OL743755	*Escherichia coli*	99%
ECK20	*gadA*	OL743756	*Escherichia coli*	99%

Appendix 2: Selected *K. pneumoniae* isolates with percentage identities and accession numbers

Isolate ID	Target gene	Accession no.	Homologue	% Identity
KPE5	*rpoB*	OL743757	*K. pneumoniae*	99%
KPE3	*rpoB*	OL743758	*K. pneumoniae*	99%
KPK53	*rpoB*	OL743759	*K. pneumoniae*	99%
KPK55	*rpoB*	OL743760	*K. pneumoniae*	99%
KPE9	*rpoB*	OL743761	*K. pneumoniae*	99%
KPK85	*rpoB*	OL743762	*K. pneumoniae*	99%
KPK81	*rpoB*	OL743763	*K. pneumoniae*	99%
KPK51	*rpoB*	OL743764	*K. pneumoniae*	99%
KPK83	*rpoB*	OL743765	*K. pneumoniae*	99%
KPE6	*rpoB*	OM310867	*K. pneumoniae*	99%
KPE12	*rpoB*	OM310868	*E. coli*	99%
KPE2	*rpoB*	OM310869	*E. coli*	99%
KPE4	*rpoB*	OM310870	*E. coli*	99%

Appendix 3: ESBL genes percentage identities and accession numbers

Isolate ID	Target gene	Accession no	Homologue	% Identity
KPE2	*bla*TEM	OM310856	*Klebsiella pneumoniae*	99%
KPE1	*bla*TEM	OM310857	*Klebsiella pneumoniae*	99%
KPE4	*bla*TEM	OM310858	*Klebsiella pneumoniae*	99%
ECK30	*bla*TEM	OM310859	*Escherichia coli*	99%
KPE5	*bla*TEM	OM310860	*Klebsiella pneumoniae*	99%
KPE6	*bla*TEM	OM310861	*Klebsiella pneumoniae*	99%
KPK83	*bla*TEM	OM310862	*Klebsiella pneumoniae*	99%
KPK85	*bla*TEM	OM310863	*Klebsiella pneumoniae*	99%
ECK44	*bla*TEM	OM310864	*Escherichia coli*	99%
KPE 12	*bla*TEM	OM310865	*Klebsiella pneumoniae*	99%
KPK53	*bla*TEM	OM310866	*Klebsiella pneumoniae*	99%
KPE5	*bla*SHV	OM310830	*Klebsiella pneumoniae*	99%
KPK53	*bla*SHV	OM310831	*Klebsiella pneumoniae*	99%
ECE3	*bla*SHV	OM310832	*Escherichia coli*	99%
KPK83	*bla*SHV	OM310833	*Klebsiella pneumoniae*	99%
KPE12	*bla*SHV	OM310834	*Klebsiella pneumoniae*	99%
KPK53	*bla*SHV	OM310835	*Klebsiella pneumoniae*	99%
KPK85	*bla*SHV	OM310836	*Klebsiella pneumoniae*	99%
ECK95	*bla*SHV	OM310837	*Escherichia coli*	99%

Appendix 4: ESBL genes percentage identities and accession numbers

Isolate ID	Target gene	Accession no	Homologue	% Identity
ECE6	*bla*OXA	OM310838	*Escherichia coli*	99%
ECE36	*bla*OXA	OM310839	*Escherichia coli*	99%
ECE9	*bla*OXA	OM310840	*Escherichia coli*	99%
ECK16	*bla*OXA	OM310841	*Escherichia coli*	99%
KPK51	*bla*OXA	OM310842	*Klebsiella pneumoniae*	99%
KPK53	*bla*OXA	OM310843	*Klebsiella pneumoniae*	99%
ECK9	*bla*OXA	OM310844	*Escherichia coli*	99%
KPK83	*bla*OXA	OM310845	*Klebsiella pneumoniae*	99%
ECE1	*bla*OXA	OM310846	*Escherichia coli*	99%
KPK85	*bla*OXA	OM310847	*Klebsiella pneumoniae*	99%
KPK81	*bla*OXA	OM310848	*Klebsiella pneumoniae*	99%
ECK23	*bla*CTXMgp1	OM310849	*Escherichia coli*	99%
ECE20	*bla*CTXMgp1	OM310850	*Escherichia coli*	99%
KPK60	*bla*CTXMgp2	OM310851	*Klebsiella pneumoniae*	99%
ECK96	*bla*CTXMgp9	OM310852	*Escherichia coli*	99%
ECK60	*bla*CTXMgp9	OM310853	*Escherichia coli*	99%
ECK34	*bla*CTXMgp9	OM310854	*Escherichia coli*	99%
ECE33	*bla*CTXMgp9	OM310855	*Escherichia coli*	99%

Appendix 5: Antibiogram for *Klebsiella pneumoniae* isolates

Antibiogram for *K. Pneumoniae* isolates from KNH Cont'

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MIN	TGC	FM
KPK 01	22S	23S	31S	26S	15I	27S	28S	22S	28S	13I	18S	
KPK 02	20S	21S	11R	18R	12R	23S	27S	19S	25S	19S	19S	
KPK 03	25S	20S	8R	17R	15I	23S	25S	21S	26S	15I	16R	17S
KPK 04	24S	17I	10R	16R	15I	20I	27S	6R	6R	13I	15R	14R
KPK 05	20S	21S	10R	19SDD	14I	22S	26S	23S	27S	17S	20S	
KPK 06	20S	22S	8R	16R	13R	21S	27S	24S	27S	13I	20S	
KPK 07	22S	20S	11R	15R	11R	22S	26S	25S	27S	18S	20S	
KPK 08	19S	21S	11R	17R	12R	22S	26S	25S	28S	18S	20S	
KPK 09	20S	21S	12R	17R	12R	22S	26S	26S	28S	19S	22S	
KPK 10	21S	20S	8R	15R	8R	19I	25S	23S	19I	15I	18S	
KPK 11	19S	21S	10R	18R	12R	20I	29S	25S	27S	19S	21S	
KPK 12	20S	20S	8R	16R	12R	21S	27S	23S	26S	18S	20S	
KPK 13	20S	20S	12R	17R	12R	22S	28S	25S	26S	17S	21S	
KPK 14	20S	21S	13R	18R	11R	24S	27S	25S	27S	19S	20S	
KPK 15	22S	25S	12R	25S	19S	26S	30S	6R	20I	18S	22S	
KPK 16	26S	20S	12R	17R	18S	24S	30S	22S	20I	17S	19S	
KPK 17	24S	19S	11R	13R	14I	22S	27S	23S	26S	17S	20S	
KPK 18	19S	21S	11R	17R	13R	22S	27S	23S	26S	19S	21S	
KPK 19	18S	21S	10R	24SDD	12R	21S	25S	25S	26S	18S	21S	
KPK 20	26S	18S	6R	15R	15I	23S	24S	24S	20I	14I	18S	14R
KPK 21	21S	21S	10R	16R	18S	25S	26S	6R	18I	16S	18S	15I
KPK 22	18S	23S	6R	19R	12R	19I	25S	21S	28S	20S	22S	
KPK 23	22S	21S	11R	21SDD	12R	23S	24S	8R	20I	20S	24S	
KPK 24	21S	21S	10R	14R	10R	20I	25S	24S	22S	16S	19S	16I

Antibiogram for *K. Pneumoniae* isolates from KNH Cont'

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MIN	TGC	FM
KPK 25	24S	21S	6R	20SDD	7R	15R	25S	6R	21S	11R	20S	
KPK 26	19S	23S	6R	17R	6R	20I	25S	23S	28S	19S	21S	
KPK 27	21S	23S	10R	17R	13R	24S	30S	25S	28S	19S	21S	
KPK 28	23S	21S	10R	19SDD	17I	22S	24S	24S	27S	18S	20S	
KPK 29	16I	30S	27S	28S	8R	15R	23S	21S	7R	16S	20S	15I
KPK 30	21S	18S	13R	22SDD	18S	20I	26S	26S	6R	14I	19S	
KPK 31	19S	21S	11R	20SDD	13R	21S	28S	26S	26S	20S	23S	
KPK 32	19S	23S	11R	20SDD	13R	22S	29S	26S	28S	19S	23S	
KPK 33	18S	24S	10R	20SDD	13R	20I	28S	26S	30S	21S	22S	
KPK 34	23S	20S	30S	31S	22S	23S	26S	24S	29S	16S	21S	
KPK 35	24S	21S	12R	23SDD	13R	21S	28S	24S	26S	18S	22S	
KPK 36	22S	22S	8R	19SDD	13R	20I	26S	22S	21S	10R	21S	
KPK 37	17S	23S	8R	18R	12R	22S	28S	24S	29S	20S	22S	
KPK 38	18S	22S	8R	20SDD	12R	20I	28S	25S	29S	21S	22S	
KPK 39	18S	20S	12R	21SDD	13R	21S	30S	26S	30S	19S	24S	
KPK 40	23S	19S	8R	16R	10R	18I	25S	25S	6R	8R	20S	
KPK 41	20S	26S	8R	14R	13R	20I	25S	24S	30S	21S	20S	
KPK 42	25S	21S	6R	16R	6R	20I	29S	22S	27S	16S	20S	
KPK 43	25S	22S	30S	33S	23S	23S	26S	22S	28S	19S	21S	
KPK 44	24S	24S	12R	22SDD	16I	20I	26S	25S	26S	10R	21S	
KPK 45	23S	22S	28S	33S	23S	24S	25S	25S	30S	12R	22S	
KPK 46	19S	23S	10R	18R	14I	21S	26S	23S	27S	18S	20S	
KPK 47	19S	20S	8R	18R	13R	17R	28S	24S	30S	18S	20S	
KPK 48	22S	21S	28S	30S	21S	24S	25S	22S	29S	18S	20S	

Antibiogram for *K. Pneumoniae* isolates from KNH Cont'

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MIN	TGC	FM
KPK 49	20S	20S	11R	16R	12R	21S	27S	23S	28S	19S	21S	
KPK 50	20S	22S	8R	20SDD	13R	21S	26S	10R	23S	20S	22S	
KPK 51	24S	21S	10R	17R	19S	23S	26S	6R	22S	16S	20S	15I
KPK 52	23S	20S	8R	17R	7R	17R	23S	21S	27S	16S	19S	
KPK 53	26S	13R	27S	30S	6R	16R	20I	12R	6R	13I	18S	8R
KPK 54	21S	20S	10R	6R	10R	25S	29S	22S	6R	18S	22S	
KPK 55	6R	19S	6R	6R	6R	6R	6R	6R	8R	14I	15R	
KPK 56	23S	6R	10R	18R	11R	22S	28S	20S	6R	13I	20S	18S
KPK 57	23S	6R	6R	6R	6R	12R	26S	10R	6R	7R	22S	
KPK 58	25S	23S	6R	19SDD	10R	24S	27S	7R	21S	20S	21S	
KPK 59	26S	21S	12R	20SDD	10R	17R	26S	7R	21S	11R	20S	
KPK 60	23S	16I	6R	17R	16I	24S	30S	6R	8R	14I	19S	
KPK 61	19S	20S	8R	17R	13R	20I	27S	23S	26S	18S	22S	
KPK 62	20S	19S	10R	19SDD	10R	22S	30S	23S	27S	19S	21S	
KPK 63	18S	20S	11R	18R	12R	21S	30S	23S	27S	18S	22S	
KPK 64	18S	24S	8R	18R	12R	20I	29S	25S	28S	18S	20S	
KPK 65	20S	24S	8R	20SDD	12R	22S	28S	24S	27S	17S	21S	
KPK 66	22S	20S	6R	15R	14I	20I	24S	22S	20I	15I	19S	
KPK 67	26S	23S	10R	6R	16I	22S	27S	23S	27S	17S	24S	
KPK 68	15I	19S	10R	18R	13R	18I	20I	7R	18I	11R	19S	
KPK 69	21S	18S	11R	16R	11R	20I	29S	20S	30S	18S	21S	
KPK 70	18S	22S	11R	17R	12R	21S	27S	25S	26S	18S	20S	
KPK 71	23S	18S	6R	15R	12R	18I	21I	7R	19I	18S	24S	
KPK 72	18S	21S	6R	18R	10R	18I	28S	7R	26S	22S	22S	
KPK 73	25S	19S	8R	17R	18S	25S	24S	7R	20I	15I	20S	

Antibiogram for *K. Pneumoniae* isolates from KNH Cont'

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MIN	TGC	FM
KPK 74	21S	19S	12R	18R	10R	22S	26S	25S	25S	18S	22S	
KPK 75	23S	26S	30S	17R	12R	25S	27S	23S	30S	23S	20S	
KPK 76	18S	19S	6R	18R	12R	20I	26S	22S	28S	18S	21S	
KPK 77	20S	22S	8R	18R	12R	19I	25S	22S	22S	19S	21S	
KPK 78	18S	21S	6R	17R	12R	20I	26S	25S	26S	18S	23S	
KPK 79	22S	18S	10R	21SDD	13R	18I	25S	18S	26S	16S	21S	
KPK 80	18S	21S	10R	20SDD	14I	20I	25S	21S	25S	18S	23S	
KPK 81	18S	22S	8R	18R	12R	20I	27S	22S	28S	19S	20S	
KPK 82	18S	23S	8R	19SDD	13R	19I	25S	22S	28S	18S	22S	
KPK 83	17S	24S	8R	17R	13R	18I	24S	23S	29S	20S	20S	
KPK 84	18S	24S	10R	16R	11R	20I	25S	24S	31S	19S	21S	
KPK 85	18S	24S	6R	16R	10R	18I	23S	22S	29S	20S	22S	
KPK 86	18S	23S	8R	17R	12R	19I	25S	24S	28S	20S	23S	
KPK 87	21S	6R	6R	6R	6R	15R	26S	6R	6R	11R	20S	15I
KPK 88	17S	22S	8R	15R	12R	18I	24S	6R	25S	18S	19S	
KPK 89	24S	21S	10R	6R	15I	19I	23S	25S	22S	16S	20S	
KPK 90	16I	23S	6R	6R	10R	18I	24S	24S	28S	17S	23S	
KPK 91	21S	14R	8R	14R	15I	18I	24S	14S	18I	12R	16R	
KPK 92	17S	24S	8R	13R	12R	19I	25S	23S	29S	20S	21S	
KPK 93	18S	22S	6R	16R	10R	18I	23S	7R	21S	18S	22S	
KPK 94	16I	20S	6R	6R	11R	18I	22I	22S	29S	18S	22S	
KPK 95	24S	17I	22S	25S	15I	18I	26S	25S	6R	8R	20S	15I
KPK 96	25S	23S	28S	29S	21S	23S	26S	23S	26S	19S	20S	
KPK 97	15I	21S	6R	19SDD	13R	18I	23S	22S	25S	25S	23S	
KPK 98	21S	20S	12R	21SDD	13R	15R	27S	21S	26S	18S	21S	

Antibiogram for *K. Pneumoniae* isolates from KNH Cont'

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MIN	TGC	FM
KPK 99	16I	22S	6R	19SDD	14I	18I	22I	25S	28S	18S	22S	
KPK 100	23S	21S	6R	15R	18S	17R	23S	21S	27S	18S	23S	
KPK 101	22S	21S	10R	20SDD	14I	19I	24S	20S	28S	19S	22S	
KPK 102	20S	21S	6R	18R	6R	19I	23S	6R	20I	19S	21S	
KPK 103	20S	6R	10R	18R	10R	21S	24S	20S	20I	17S	20S	
KPK 104	21S	6R	10R	15R	10R	22S	28S	20S	6R	19S	20S	
KPK 105	17S	21S	10R	17R	10R	18I	21I	21S	26S	18S	21S	
KPK 106	15I	23S	6R	16R	13R	17R	20I	25S	29S	19S	19S	
KPK 107	16I	20S	8R	18R	12R	18I	24S	22S	27S	18S	21S	
KPK 108	17S	21S	10R	19SDD	13R	21S	25S	23S	28S	20S	23S	
KPK 109	21S	20S	13R	19SDD	12R	19I	23S	7R	21S	16S	20S	
KPK 110	23S	6R	6R	6R	6R	6R	6R	6R	6R	17S	26S	
KPK 111	21S	20S	24S	29S	22S	20I	23S	21S	25S	17S	22S	16I
KPK 112	16I	21S	6R	23SDD	11R	16R	22I	24S	27S	20S	21S	
KPK 113	18S	22S	6R	27S	16I	17R	24S	15S	28S	15I	21S	17S
KPK 114	16I	20	6R	17R	12R	18I	23S	22S	26S	19S	21S	
KPK 115	17S	21S	6R	14R	11R	21S	20I	24S	26S	18S	23S	
STD A	23S	15R	6R	23SDD	19S	26S	28S	17S	8R	8R	22S	
STD B	20S	15R	8R	8R	20S	26S	25S	15S	10R	13R	23S	12R

KEY: AN- amikacin, FOX- cefoxitin, CRO-ceftriaxone, FEP- cefepime, AMC- amoxicillin/clavulanic acid, TZP- piperacillintazobactam, MEM- meropenem, AZM- azithromycin, LVX- levofloxacin, MIN- minocycline, TGC- tigecycline, FM- nitrofurantoin.

Antibiogram for *K. Pneumoniae* isolates from EMBU LEVEL 5 HOSPITAL

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MIN	TGC	FM
KPE01	21S	6R	6R	24SDD	6R	25S	30S	8R	27S	10R	8R	6R
KPE02	20S	24S	6R	21SDD	18S	24S	26S	13S	30S	18S	21S	
KPE03	22S	23S	25S	30S	20S	22S	21I	20S	28S	18S	18S	6R
KPE04	20S	22S	25S	28S	16I	24S	26S	23S	30S	19S	20S	14R
KPE05	22S	23S	6R	6R	13R	15R	25S	6R	28S	21S	21S	18S
KPE06	21S	24S	6R	6R	13R	19I	22I	7R	30S	19S	22S	16I
KPE07	23S	6R	6R	22SDD	6R	23S	30S	15S	24S	10R	6R	6R
KPE08	25S	6R	16R	23SDD	6R	22S	30S	18S	30S	8R	10R	6R
KPE09	23S	23S	16R	29S	20S	22S	20I	15S	30S	20S	19S	8R
KPE10	20S	6R	15R	21SDD	7R	20I	18R	20S	25S	16S	15R	6R
KPE11	24S	23S	6R	22SDD	12R	22S	26S	7R	6R	19S	20S	
KPE12	21S	20S	6R	26S	18S	23S	26S	7R	6R	19S	23S	
STD B	22S	16R	6R	8R	17S	26S	26S	10R	23S	12R	21S	12R

KEY: AN- amikacin, FOX- cefoxitin, CRO-ceftriaxone, FEP- cefepime, AMC- amoxicillin/clavulanic acid, TZP- piperacillin/tazobactam, MEM- meropenem, AZM- azithromycin, LVX- levofloxacin, MIN- minocycline, TGC- tigecycline, FM- nitrofurantoin.

S- sensitive, I- intermediate, SDD-susceptible dose dependent, R- resistance.

Appendix 6: Antibiogram for *Escherichia coli* isolates

Antibiogram for *Escherichia coli* isolates from KNH

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MI	TGC	FM
ECK 01	21S	25S	26S	29S	16I	21S	29S	25S	16R	16S	21S	17S
ECK 02	20S	26S	11R	27S	21S	25S	30S	26S	21S	19S	23S	
ECK 03	22S	21S	28S	35S	29S	23S	29S	21S	35S	10R	23S	
ECK 04	25S	25S	29S	30S	17I	30S	25S	23S	30S	6R	19S	15I
ECK 05	22S	6R	28S	7R	6R	21S	31S	10R	6R	10R	21S	
ECK 06	23S	26S	6R	31S	19S	13R	29S	12R	10R	19S	23S	22S
ECK 07	21S	26S	13R	23SDD	17I	25S	29S	11R	7R	17S	20S	
ECK 08	26S	25S	12R	20SDD	19S	25S	30S	13S	26S	16S	26S	
ECK 09	18S	7R	6R	7R	13R	16R	25S	14S	6R	11R	22S	
ECK 10	21S	20S	6R	15R	13R	21S	26S	6R	6R	14I	27S	
ECK 11	20S	6R	6R	6R	6R	16R	26S	6R	6R	10R	22S	14I
ECK 12	21S	24S	29S	31S	19S	25S	30S	26S	23S	15I	22S	
ECK 13	21S	6R	6R	12R	12R	17R	26S	14S	6R	10R	21S	18S
ECK 14	20S	26S	6R	31S	20S	17R	26S	25S	28S	17S	21S	17S
ECK 15	21S	24S	31S	29S	18S	27S	29S	22S	26S	16S	21S	
ECK 16	19S	26S	9R	19SDD	14I	20I	30S	15S	10R	13I	26S	
ECK 17	21S	6R	6R	10R	6R	14R	24S	10R	6R	10R	25S	
ECK 20	21S	24S	6R	21SDD	17I	26S	29S	27S	28S	19S	24S	
ECK 21	21S	27S	12R	31S	18S	22S	24S	16S	26S	21S	22S	
ECK 22	20S	6R	11R	10R	10R	26S	27S	13S	6R	10R	23S	
ECK 23	21S	29S	6R	19SDD	21S	22S	26S	12R	30S	18S	25S	
ECK 24	21S	23S	17R	25S	21S	26S	27S	14S	26S	18S	23S	
ECK 25	22S	21S	8R	20SDD	18S	22S	28S	28S	29S	15I	25S	
ECK 26	19S	26S	11R	28S	21S	23S	27S	15S	10R	26S	30S	

Antibiogram for *Escherichia coli* isolates from KNH Cont’

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MI	TGC	FM
ECK 27	24S	26S	12R	20SDD	18S	25S	30S	13R	8R	23S	25S	
ECK 28	23S	19S	9R	16R	14I	21S	28S	27S	25S	15I	26S	18S
ECK 29	24S	25S	9R	17R	16I	23S	28S	26S	25S	19S	21S	
ECK 30	21S	29S	10R	10R	18S	25S	28S	26S	8R	18S	23S	13R
ECK 31	20S	18S	27S	10R	14I	25S	31S	13S	7R	16S	21S	
ECK 32	21S	19S	6R	10R	12R	19I	25S	14S	7R	16S	21S	
ECK 33	22S	24S	6R	18R	12R	6R	6R	6R	7R	16S	22S	
ECK 34	22S	10R	12R	16R	6R	26S	29S	6R	29S	15I	20S	
ECK 35	19S	25S	6R	17R	13R	19I	26S	6R	7R	16S	22S	
ECK 36	18S	22S	6R	19SDD	14I	21S	27S	17S	10R	17S	23S	
ECK 37	27S	26S	12R	22SDD	20S	26S	28S	15S	10R	18S	24S	
ECK 38	21S	26S	26S	30S	19S	26S	27S	25S	26S	18S	22S	17S
ECK 39	21S	23S	12R	22SDD	16I	25S	27S	15S	8R	19S	24S	
ECK 40	21S	25S	17R	30S	18S	26S	30S	15S	10R	17S	19S	
ECK 41	24S	26S	30S	33S	21S	26S	31S	29S	23S	18S	23S	
ECK 42	23S	26S	13R	26S	14I	21S	28S	12R	7R	18S	24S	
ECK 43	21S	21S	12R	19SDD	16I	25S	30S	26S	6R	13I	21S	
ECK 44	17S	24S	17R	22SDD	17I	25S	28S	13S	8R	19S	21S	
ECK 45	21S	26S	16R	24SDD	20S	26S	31S	14S	7R	18S	24S	
ECK 46	21S	21S	12R	17R	18S	25S	27S	6R	6R	10R	22S	
ECK 47	24S	29S	14R	26S	21S	24S	31S	6R	12R	15I	24S	
ECK 48	21S	20S	11R	19SDD	18S	23S	25S	6R	6R	10R	25S	
ECK 49	21S	7R	6R	11R	6R	13R	25S	12R	6R	11R	21S	
ECK 50	21S	21S	13R	20SDD	16I	26S	29S	11R	6R	13I	21S	25S
ECK 51	17S	20S	8R	21SDD	13R	19I	23S	23S	27S	17S	19S	

Antibiogram for *Escherichia coli* isolates from KNH Cont'

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MI	TGC	FM
ECK 52	16I	21S	6R	19SDD	12R	22S	27S	14S	6R	19S	22S	23S
ECK 53	20S	22S	26S	28S	14I	21S	31S	21S	18I	16S	21S	13R
ECK 54	22S	25S	30S	32S	13R	22S	30S	25S	30S	13I	24S	
ECK 55	19S	24S	31S	33S	23S	25S	28S	26S	28S	21S	21S	
ECK 56	20S	20S	10R	19SDD	18S	22S	25S	8R	6R	11R	23S	
ECK 57	24S	23S	26S	33S	18S	24S	29S	24S	6R	14I	25S	
ECK 58	23S	26S	13R	19SDD	20S	25S	31S	8R	24S	13I	24S	
ECK 59	22S	22S	6R	20SDD	19S	25S	24S	6R	22S	15I	24S	
ECK 60	18S	6R	6R	6R	6R	16R	25S	6R	6R	10R	24S	22S
ECK 61	21S	26S	6R	20SDD	19S	27S	29S	21S	31S	17S	20S	17S
ECK 62	24S	26S	29S	34S	19S	18I	28S	26S	31S	13I	26S	
ECK 63	20S	27S	29S	34S	22S	24S	28S	8R	23S	10R	23S	20S
ECK 64	23S	25S	31S	39S	21S	30S	33S	21S	26S	15I	29S	24S
ECK 65	21S	7R	6R	6R	6R	16R	27S	8R	6R	10R	25S	23S
ECK 66	22S	23S	6R	23SDD	20S	24S	30S	6R	18I	22S	23S	
ECK 67	20S	6R	6R	6R	6R	17R	24S	6R	6R	14I	23S	13R
ECK 68	21S	20S	14R	24SDD	19S	24S	26S	6R	6R	13I	20S	
ECK 69	23S	28S	32S	34S	21S	31S	31S	27S	30S	16S	26S	21S
ECK 70	21S	20S	11R	22SDD	20S	21S	29S	8R	6R	11R	23S	
ECK 71	25S	26S	6R	14R	18S	23S	30S	13S	35S	23S	26S	
ECK 72	24S	6R	6R	6R	6R	6R	8R	8R	6R	18S	22S	
ECK 73	26S	21S	6R	16R	16I	23S	30S	26S	6R	12R	24S	
ECK 74	23S	7R	6R	7R	6R	6R	8R	6R	7R	19S	23S	20S
ECK 75	23S	6R	6R	6R	6R	6R	8R	7R	6R	19S	21S	
ECK 76	23S	26S	10R	24SDD	18S	25S	29S	6R	26S	21S	23S	

Antibiogram for *Escherichia coli* isolates from KNH Cont’

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MI	TGC	FM
ECK 77	26S	29S	31S	38S	21S	31S	29S	29S	27S	17S	27S	21S
ECK 78	25S	30S	36S	19SDD	22S	31S	33S	6R	36S	18S	29S	23S
ECK 79	19S	24S	6R	21SDD	15I	24S	31S	6R	6R	12R	25S	
ECK 80	23S	29S	29S	36S	23S	30S	31S	14S	36S	23S	29S	
ECK 81	23S	20S	13R	24SDD	17I	25S	30S	6R	6R	11R	25S	20S
ECK 82	21S	25S	32S	33S	19S	25S	31S	21S	6R	7R	23S	15I
ECK 83	25S	31S	31S	36S	16I	29S	31S	24S	36S	25S	26S	21S
ECK 84	24S	26S	31S	31S	15I	26S	30S	22S	27S	16S	21S	18S
ECK 85	19S	6R	6R	6R	6R	6R	6R	6R	8R	23S	26S	19S
ECK 86	18S	24S	11R	22SDD	11R	21S	30S	10R	8R	14I	21S	
ECK 87	19S	18S	6R	17R	6R	21S	25S	6R	6R	13I	21S	
ECK 88	24S	29S	30S	36S	20S	26S	28S	29S	8R	26S	26S	20S
ECK 89	19S	15I	6R	10R	6R	13R	26S	6R	6R	14I	20S	14R
ECK 90	22S	23S	31S	31S	10R	24S	31S	26S	36S	25S	21S	
ECK 91	16I	24S	6R	21SDD	6R	21S	30S	6R	6R	13I	24S	
ECK 92	26S	27S	31S	35S	13R	24S	31S	6R	32S	16S	24S	
ECK 93	23S	26S	6R	22SDD	10R	16R	27S	28S	24S	21S	21S	
ECK 94	24S	25S	6R	20SDD	7R	24S	32S	6R	23S	16S	22S	18S
ECK 95	16I	23S	11R	24SDD	10R	20I	31S	6R	6R	13I	24S	
ECK 96	26S	24S	6R	19SDD	8R	19I	24S	6R	21S	19S	20S	16I
ECK 97	23S	26S	6R	18R	17I	26S	29S	10R	27S	15I	24S	
ECK 98	20S	19S	6R	16R	14I	23S	30S	7R	7R	16S	25S	
ECK 99	26S	6R	6R	6R	6R	6R	6R	6R	6R	16S	22S	21S
ECK 100	21S	27S	6R	26S	22S	29S	30S	12R	8R	21S	24S	
ECK 101	19S	23S	8R	21SDD	11R	22S	29S	26S	28S	21S	24S	

Antibiogram for *Escherichia coli* isolates from KNH Cont’

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MI	TGC	FM
ECK 102	24S	6R	6R	13R	6R	10R	19R	8R	13R	20S	18S	
ECK 103	22S	6R	6R	6R	6R	6R	8R	13S	6R	13I	27S	
ECK 104	23S	26S	16R	27S	25S	28S	30S	17S	26S	21S	24S	
ECK 105	24S	26S	6R	15R	15I	26S	33S	16S	7R	24S	26S	
ECK 106	26S	29S	8R	23SDD	22S	31S	35S	6R	7R	31S	30S	
STD A	24S	25S	30S	30S	18S	24S	27S	23S	30S	18S	22S	20S
STD B	25S	29S	28S	35S	20S	26S	30S	30S	32S	20S	26S	22S

KEY: AN- amikacin, FOX- cefoxitin, CRO-ceftriaxone, FEP- cefepime, AMC- amoxicillin/clavulanic acid, TZP- piperacillin/tazobactam, MEM- meropenem, AZM- azithromycin, LVX- levofloxacin, MIN- minocycline, TGC- tigecycline, FM- nitrofurantoin.

S- sensitive, I- intermediate, SDD-susceptible dose dependent, R- resistance.

Antibiogram for *Escherichia coli* isolates from Embu Level 5 Hospital

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MI	TGC	FM
EC01	22S	18S	22R	21SDD	16I	14R	21I	6R	26S	18S	17R	17S
EC02	21S	22S	26S	26S	19S	23S	25S	20S	27S	19S	18S	
EC03	26S	23S	26S	26S	18S	22S	26S	16S	10R	15I	24S	
EC04	17S	19S	30S	24SDD	17I	26S	25S	14S	26S	18S	18S	
EC05	6R	6R	6R	10R	21S	24S	15R	6R	10R	12R	12R	17S
EC06	15I	22S	6R	6R	11R	17R	25S	10R	7R	17S	19S	15I
EC07	24S	23S	27S	27R	18S	18I	29S	22S	13R	21S	21S	16I
EC08	25S	26S	32S	32S	22S	27S	31S	21S	24S	15I	22S	
EC09	21S	21S	6R	6R	13R	20I	27S	14S	6R	20S	20S	17S
EC10	20S	23S	27S	29S	19S	22S	29S	25S	28S	19S	19S	
EC11	22S	26S	29S	31S	20S	25S	28S	6R	31S	21S	23S	23S
EC12	23S	25S	10R	18R	21S	26S	29S	8R	7R	11R	21S	15I
EC13	25S	6R	27S	31S	15I	26S	28S	15S	24S	21S	21S	
EC14	12R	6R	18R	18R	14I	25S	18R	6R	23S	13I	17R	
EC15	21S	24S	26S	31S	23S	27S	28S	13S	26S	23S	23S	
EC16	22S	23S	30S	31S	20S	26S	29S	24S	30S	22S	21S	15I
EC17	24S	25S	31S	32S	21S	25S	30S	25S	31S	23S	24S	15I
EC18	9R	6R	15R	15R	26S	27S	16R	6R	24S	15I	21S	20S
EC19	21S	24S	30S	31S	21S	25S	30S	24S	30S	21S	22S	19S
EC20	21S	6R	6R	6R	6R	11R	31S	6R	6R	13I	25S	12R
EC21	20S	21S	8R	16R	15I	21S	31S	10R	6R	20S	22S	18S
EC22	20S	22S	8R	16R	14I	20I	29S	11R	6R	21S	24S	17S
EC23	25S	24S	6R	20SDD	20S	26S	28S	19S	30S	20S	25S	17S
EC24	22S	23S	15R	31S	17I	21S	30S	19S	26S	14I	21S	17S
EC25	11R	28S	13R	15R	19S	17R	16R	20S	27S	21S	23S	17S
EC26	21S	25S	31S	29S	20S	22S	28S	19S	29S	20S	21S	18S

Antibiogram for *Escherichia coli* isolates from Embu Level 5 Hospital Cont'

SAMPLES	AN	FOX	CRO	FEP	AMC	TZP	MEM	AZM	LVX	MI	TGC	FM
EC27	23S	24S	27S	30S	17I	23S	27S	15S	10R	24S	23S	23S
EC28	15I	25S	24S	31S	18S	21S	26S	21S	7R	27S	22S	23S
EC29	23S	26S	31S	31S	20S	22S	27S	20S	29S	20S	24S	18S
EC30	26S	25S	26S	30S	18S	23S	31S	28S	29S	22S	22S	16I
EC31	19S	27S	6R	13R	19S	20I	29S	16S	33S	26S	25S	20S
EC33	21S	26S	6R	17R	19S	21S	28S	10R	7R	21S	24S	19S
EC34	24S	27S	7R	15R	20S	21S	29S	10R	8R	22S	25S	20S
EC36	25S	26S	31S	31S	19S	26S	30S	27S	26S	22S	23S	19S
STD A	24S	25S	30S	30S	18S	24S	27S	23S	30S	18S	22S	20S
STD B	25S	29S	28S	35S	20S	26S	30S	30S	32S	20S	26S	22S

KEY: AN- amikacin, FOX- cefoxitin, CRO-ceftriaxone, FEP- cefepime, AMC- amoxicillin/clavulanic acid, TZP- piperacillin/tazobactam, MEM- meropenem, AZM- azithromycin, LVX- levofloxacin, MIN- minocycline, TGC- tigecycline, FM- nitrofurantoin.

S- sensitive, I- intermediate, SDD-susceptible dose dependent, R- resistance.

Appendix 7: ESBL Phenotypic Confirmatory Disk Diffusion Test for *K. pneumoniae* isolates

ESBL confirmatory test for *K. pneumoniae* isolates from KNH				
SAMPLES	**CEFTAZIDIME**	**CEFTAZIDIME/CLAVULANATE**	**CEFOTAXIME**	**CEFOTAXIME/CLAVULANATE**
KPK 01	22R	29R	21R	29R
KPK 02	23S	27S	15R	29R
KPK 03	12R	25R	7R	23R
KPK 04	11R	22R	9R	22R
KPK 05	16R	24R	7R	23R
KPK 06	17R	25R	10R	26R
KPK 07	16R	27R	7R	24R
KPK 08	17R	26R	9R	24R
KPK 09	16R	26R	9R	24R
KPK 10	13R	25R	8R	23R
KPK 11	18R	25R	8R	26R
KPK 12	16R	25R	6R	25R
KPK 13	17R	25R	7R	25R
KPK 14	16R	25R	8R	26R
KPK 15	21R	25R	6R	26R
KPK 16	16R	25R	10R	26R
KPK 17	15R	25R	10R	25R
KPK 18	18R	24R	11R	24R
KPK 19	18R	26R	10R	26R
KPK 20	12R	23R	7R	23R
KPK 21	17R	26R	8R	26R
KPK 22	17R	26R	9R	26R
KPK 23	17R	28R	11R	27R
KPK 24	14R	24R	9R	23R

ESBL confirmatory test for *K. pneumoniae* isolates from KNH Cont'

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
KPK 25	17R	24R	7R	23R
KPK 26	19R	25R	6R	25R
KPK 27	16R	24R	10R	25R
KPK 28	15R	25R	12R	25R
KPK 29	23S	24S	27S	28S
KPK 30	18R	24R	10R	25R
KPK 31	17R	26R	11R	27R
KPK 32	16R	25R	9R	26R
KPK 33	15R	25R	9R	26R
KPK 34	26S	27S	31S	32S
KPK 35	14R	26R	12R	25R
KPK 36	16R	25R	11R	26R
KPK 37	18R	28R	13R	27R
KPK 38	17R	26R	9R	25R
KPK 39	18R	24R	9R	26R
KPK 40	11R	23R	6R	24R
KPK 41	16R	24R	9R	25R
KPK 42	11R	24R	6R	24R
KPK 43	26S	28S	28S	31S
KPK 44	15R	25R	11R	28R
KPK 45	24S	30R	22R	29R
KPK 46	16R	25R	11R	26R
KPK 47	15R	25R	7R	25R
KPK 48	28S	30S	30S	30S

ESBL confirmatory test for *K. pneumoniae* isolates from KNH Cont'

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
KPK 49	16R	26R	6R	27R
KPK 50	17R	30R	9R	30R
KPK 51	17R	28R	11R	26R
KPK 52	12R	25R	8R	25R
KPK 53	25S	25S	24R	26R
KPK 54	16R	26R	11R	25R
KPK 55	10R	14R	9R	15R
KPK 56	11R	24R	9R	25R
KPK 57	6R	8R	6R	6R
KPK 58	12R	18R	8R	15R
KPK 59	16R	23R	11R	22R
KPK 60	18R	26R	8R	25R
KPK 61	16R	25R	8R	24R
KPK 62	16R	24R	11R	24R
KPK 63	17R	25R	11R	25R
KPK 64	19R	28R	14R	29R
KPK 65	17R	27R	13R	26R
KPK 66	13R	25R	9R	24R
KPK 67	13R	25R	8R	25R
KPK 68	11R	23R	12R	25R
KPK 69	15R	25R	10R	27R
KPK 70	17R	24R	11R	25R
KPK 71	16R	24R	10R	26R
KPK 72	18R	28R	8R	26R

ESBL confirmatory test for *K. pneumoniae* isolates from KNH Cont'

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
KPK 73	18R	25R	14R	26R
KPK 74	18R	26R	12R	26R
KPK 75	28S	29S	30S	30S
KPK 76	19R	24R	11R	26R
KPK 77	17R	28R	13R	27R
KPK 78	19R	27R	11R	27R
KPK 79	18R	27R	11R	25R
KPK 80	17R	27R	12R	26R
KPK 81	17R	28R	8R	27R
KPK 82	18R	26R	7R	25R
KPK 83	17R	27R	11R	27R
KPK 84	18R	30R	11R	30R
KPK 85	15R	25R	8R	28R
KPK 86	18R	28R	10R	29R
KPK 87	29S	30S	29S	30S
KPK 88	15R	28R	9R	28R
KPK 89	12R	25R	7R	26R
KPK 90	16R	25R	9R	25R
KPK 91	12R	16R	9R	24R
KPK 92	17R	24R	10R	24R
KPK 93	19R	26R	12R	28R
KPK 94	20R	29R	12R	28R
KPK 95	29S	29S	30S	30S
KPK 96	28S	30S	29S	30S

ESBL confirmatory test for *K. pneumoniae* isolates from KNH Cont'

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
KPK 97	18R	25R	11R	27R
KPK 98	16R	26R	11R	26R
KPK 99	17R	26R	11R	26R
KPK 100	15R	27R	10R	25R
KPK 101	17R	24R	10R	24R
KPK 102	17R	24R	10R	23R
KPK 103	20R	27R	14R	25R
KPK 104	17R	27R	10R	27R
KPK 105	15R	24R	8R	26R
KPK 106	16R	24R	6R	26R
KPK 107	12R	16R	9R	17R
KPK 108	12R	16R	7R	17R
KPK 109	12R	16R	10R	17R
KPK 110	6R	6R	6R	6R
KPK 111	25S	26S	29S	29S
KPK 112	11R	16R	6R	17R
KPK 113	16R	22R	7R	26R
KPK 114	18R	25R	7R	25R
KPK 115	19R	25R	10R	28R
STD A	23R	30R	6R	28R
STD B	6R	10R	6R	10R

ESBL confirmatory test for *K. pneumoniae* isolates from Embu level 5 Hospital

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
KPE 01	24S	25S	11R	21R
KPE 02	20R	26R	6R	26R
KPE 03	25S	28S	26R	30R
KPE 04	24S	28S	26R	28R
KPE 05	13R	24R	6R	23R
KPE 06	10R	23R	6R	23R
KPE 07	21R	22R	6R	16R
KPE 08	21R	21R	18R	20R
KPE 09	22R	24R	19R	22R
KPE 10	20R	20R	15R	16R
KPE 11	20R	22R	6R	20R
KPE 12	22R	24R	7R	25R
STD A	6R	8R	6R	15R
STD B	6R	10R	6R	10R

KEY: ESBL PRODUCTION
CEFTAZIDIME zone ≤ 22 mm, CEFOTAXIME zone ≤ 27mm
RESITANCE ≥ 5mm increase in zone diameter for either combination with clavulanate vs the agent when tested alone.

S- sensitive, I- intermediate, SDD-susceptible dose dependent, R- resistance.

Appendix 8: ESBL Phenotypic Confirmatory Disk Diffusion Test for *Escherichia coli* isolates

ESBL confirmatory test for *Escherichia coli* isolates from KNH				
SAMPLES	**CEFTAZIDIME**	**CEFTAZIDIME/CLAVULANATE**	**CEFOTAXIME**	**CEFOTAXIME/CLAVULANATE**
ECK 01	23S	24S	25R	25R
ECK 02	21R	25R	14R	23R
ECK 03	29S	30S	29S	30S
ECK 04	25S	28S	24R	27R
ECK 05	25S	28S	26R	26R
ECK 06	6R	8R	6R	7R
ECK 07	21R	26R	11R	26R
ECK 08	19R	27R	13R	26R
ECK 09	6R	17R	6R	17R
ECK 10	14R	23R	11R	23R
ECK 11	6R	7R	6R	6R
ECK 12	25S	27S	27R	28R
ECK 13	10R	22R	6R	20R
ECK 14	18R	25R	15R	22R
ECK 15	26S	26S	28S	27S
ECK 16	19R	25R	11R	25R
ECK 17	7R	11R	6R	8R
ECK 20	18R	27R	9R	25R
ECK 21	22R	28R	16R	28R
ECK 22	16R	25R	11R	23R
ECK 23	16R	28R	8R	25R
ECK 24	21R	28R	17R	27R

ESBL confirmatory test for *Escherichia coli* isolates from KNH Cont'

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
ECK 25	17R	27R	9R	27R
ECK 26	20R	27R	9R	27R
ECK 27	21R	26R	11R	28R
ECK 28	21R	26R	10R	23R
ECK 29	16R	26R	7R	23R
ECK 30	17R	25R	8R	26R
ECK 31	27S	29S	26R	30R
ECK 32	7R	20R	6R	19R
ECK 33	6R	8R	10R	11R
ECK 34	20R	26R	14R	26R
ECK 35	16R	24R	6R	24R
ECK 36	16R	23R	9R	26R
ECK 37	24S	26S	20R	28R
ECK 38	27S	28S	28S	29S
ECK 39	21R	24R	13R	26R
ECK 40	27S	29S	25R	30R
ECK 41	27S	30S	28S	30S
ECK 42	20R	30R	14R	27R
ECK 43	20R	27R	12R	25R
ECK 44	13R	21R	15R	23R
ECK 45	14R	21R	13R	23R
ECK 46	17R	26R	9R	27R
ECK 47	20R	27R	14R	29R
ECK 48	15R	26R	12R	26R

ESBL confirmatory test for *Escherichia coli* isolates from KNH Cont'

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
ECK 49	6R	8R	6R	6R
ECK 50	20R	27R	10R	29R
ECK 51	18R	27R	13R	29R
ECK 52	17R	29R	12R	28R
ECK 53	26S	27S	30S	31S
ECK 54	27S	28S	30S	31S
ECK 55	26S	28S	29S	31S
ECK 56	17R	25R	14R	26R
ECK 57	26S	27S	29S	29S
ECK 58	20R	27R	12R	29R
ECK 59	21R	28R	13R	27R
ECK 60	6R	19R	6R	18R
ECK 61	20R	26R	13R	29R
ECK 62	28S	29S	31S	30S
ECK 63	25S	26S	29S	29S
ECK 64	31S	31S	32S	33S
ECK 65	6R	21R	6R	18R
ECK 66	23S	27S	11R	28R
ECK 67	6R	6R	6R	6R
ECK 68	14R	19R	15R	21R
ECK 69	29S	31S	28S	31S
ECK 70	18R	27R	13R	27R
ECK 71	15R	30R	6R	25R
ECK 72	6R	6R	6R	6R

ESBL confirmatory test for *Escherichia coli* isolates from KNH Cont’

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
ECK 73	15R	25R	9R	25R
ECK 74	6R	6R	6R	6R
ECK 75	6R	6R	6R	6R
ECK 76	20R	26R	12R	27R
ECK 77	30S	30S	30S	30S
ECK 78	30S	30S	30S	30S
ECK 79	25S	30S	12R	28R
ECK 80	30S	30S	30S	30S
ECK 81	19R	27R	14R	26R
ECK 82	29S	30S	31S	31S
ECK 83	30S	30S	30S	30S
ECK 84	29S	30S	30S	31S
ECK 85	6R	6R	6R	6R
ECK 86	20R	26R	13R	27R
ECK 87	18R	26R	10R	27R
ECK 88	29S	30S	30S	30S
ECK 89	8R	23R	6R	22R
ECK 90	30S	30S	30S	31S
ECK 91	26S	32R	10R	30R
ECK 92	28S	30S	30S	31S
ECK 93	18R	24R	11R	24R
ECK 94	16R	30R	12R	29R
ECK 95	25S	29S	13R	27R
ECK 96	14R	23R	10R	26R

ESBL confirmatory test for *Escherichia coli* isolates from KNH Cont'

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
ECK 97	18R	29R	8R	27R
ECK 98	17R	26R	7R	27R
ECK 99	6R	6R	6R	6R
ECK 100	24S	29S	17R	30R
ECK 101	18R	28R	13R	28R
ECK 102	6R	11R	6R	7R
ECK 103	6R	10R	6R	8R
ECK 104	22R	30R	19R	31R
ECK 105	19R	29R	9R	28R
ECK 106	20R	34R	9R	31R
STD A	30S	30S	30S	31S
STD B	30S	30S	30S	30S

ESBL confirmatory test for *Escherichia coli* isolates from Embu level 5 hospital

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
EC01	20R	23R	21R	22R
EC02	19R	24R	25R	25R
EC03	17R	22R	24R	21R
EC04	19R	21R	21R	21R
EC05	6R	6R	6R	6R
EC06	10R	21R	6R	21R
EC07	22R	23R	25R	26R
EC08	26S	29S	31S	27S
EC09	13R	23R	6R	24R
EC10	23S	26S	24R	26R
EC11	28S	30S	31S	31S
EC12	21R	29R	6R	26R
EC13	26S	28S	26R	28R
EC14	11R	13R	14R	16R
EC15	22R	26R	26R	26R
EC16	23S	26S	26R	26R
EC17	26S	26S	25R	28R

ESBL confirmatory test for *Escherichia coli* isolates from Embu level 5 hospital Cont'

SAMPLES	CEFTAZIDIME	CEFTAZIDIME/CLAVULANATE	CEFOTAXIME	CEFOTAXIME/CLAVULANATE
EC18	6R	6R	6R	6R
EC19	25S	26S	25R	26R
EC20	7R	18R	6R	20R
EC21	14R	26R	6R	26R
EC22	11R	24R	6R	26R
EC23	18R	23R	7R	26R
EC24	23S	26S	17R	26R
EC25	28S	30S	16R	29R
EC26	21R	23R	23R	25R
EC27	21R	25R	25R	26R
EC28	21R	26R	23R	25R
EC29	22R	26R	26R	27R
EC30	23S	25S	24R	25R
EC31	16R	26R	10R	24R
EC33	14R	25R	10R	26R
EC34	18R	26R	7R	24R
EC36	26S	29S	28S	30S
STD A	30S	30S	30S	31S
STD B	30S	30S	30S	30S

KEY: ESBL PRODUCTION
CEFTAZIDIME zone ≤ 22 mm, CEFOTAXIME zone ≤ 27mm
RESITANCE ≥ 5mm increase in zone diameter for either combination with clavulanate vs the agent when tested alone

S- sensitive, I- intermediate, SDD-susceptible dose dependent, R- resistance.

Dr Gabriel Aboge

21/11/2023

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE IN CLINICAL ISOLATES AT EMBU LEVEL FIVE HOSPITAL AND KENYATTA NATIONA

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off Waiyaki Way, Upper Kabete,
P. O. Box 30623, 00100 Nairobi, KENYA
Land line: 020 4007000, 020 2241349, 020 3310571, 020 8001077
Mobile: 0713 788 787 / 0735 404 245
E-mail: dg@nacosti.go.ke / registry@nacosti.go.ke
Website: www.nacosti.go.ke

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
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KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

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15th December 2020

Sylvia Mwelu Maveke
Reg. No.J56/11242/2018
Dept. of Public Health, Pharmacology and Toxicology
Faculty of Veterinary Medicine
College of Agriculture and Veterinary Sciences
University of Nairobi

Dear Sylvia

Re: Approval of Annual Renewal – Phenotypic and molecular characterization of extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumonia* in clinical isolates in Embu Level Five Hospital, Kenya (P866/10/2019)

Refer to your communication of received on 3rd November 2020.

This is to acknowledge receipt of the study progress report and hereby grant annual extension of approval for ethical research protocol P866/10/2019.

The approval dates are 9th January 2021 –8th January 2022.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH- UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH- UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH-UON ERC

c.c. The Principal, College of Health Sciences, UoN
The Senior Director CS, KNH
The Chairperson, KNH-UoN ERC

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
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KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref. No.KNH/ERC/Mod&SAE/369

16th December 2020

Sylvia Mwelu Maveke
Reg. J56/11242/2018
Dept. of Public Health, Pharmacology and Toxicology
Faculty of Veterinary Medicine
College of Agriculture and Veterinary Sciences
University of Nairobi

Dear Sylvia

Re: Approval of modifications – study titled 'Phenotypic and molecular characterization of extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumonia* in clinical isolates in Embu Level Five Hospital, Kenya' (P866/10/2019)

Your communication dated 3rd November 2020 refers.

Upon review, the KNH-UoN ERC has **approved** inclusion of Kenyatta National Hospital Microbiology Laboratory as an additional study site in order to achieve the approved sample size within the required timelines. The reasons are justified and the amendment will enrich the research outcomes.

These changes are incorporated in the revised proposal and are therefore acceptable.

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH-UON ERC

c.c. The Principal, College of Health Sciences, UoN
The Senior Director CS, KNH
The Chairperson, KNH-UoN ERC
The Chair, Dept. of Public Health, Pharmacology and Toxicology, UoN
Supervisors: Dr. Gabriel O.Aboge(UoN), Dr. Laetitia W. Kanja(UoN)