ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *ENTEROBACTERIACEAE* ISOLATED FROM URINE SAMPLES AT THE KENYATTA NATIONAL HOSPITAL MICROBIOLOGY LABORATORY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF MASTER OF SCIENCE IN TROPICAL AND INFECTIOUS DISEASES OF THE UNIVERSITY OF NAIROBI - INSTITUTE OF TROPICAL AND INFECTIOUS DISEASES (UNITID)

University of Nairobi 2013

DECLARATION

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

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W64/69495/2011

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DEDICATION

This work is dedicated to my family whose love and support never fails.

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS

AST   - Antimicrobial susceptibility testing

AMP   - Ampicillin

CLSI   - Clinical and Laboratory Standards Institute

CRE   - Carbapenem Resistant *Enterobacteriaceaea*

CTX   - Cefotaxime

ESBL   - Extended Spectrum Beta Lactamase

EUCAST   - European Committee on Antimicrobial Susceptibility Testing

GNB   - Gram Negative Bacteria

GNR   - Gram Negative Rod

IMP   - Imipenem

KNH   - Kenyatta National Hospital

KPC   - *Klebsiella Pneumoniae* Carbapenemases

MIC   - Minimum Inhibitory Concentration

MRP   - Meropenem

NDM   - New Delhi Metallo-β-lactamase

NTS   - Non-Typhoidal Salmonellae

OXA   - Oxacillinase group of β-lactamases

SAST   - Subcommittee on Antimicrobial Susceptibility Testing

SPSS   - Statistical Package for Social Sciences

SHV   - SulfHydrylVariable

TEM   - Temoniera

TSI   - Triple Sugar Iron

U.o.N   - University of Nairobi

VIM   - Verona Integron-encoded Metallo-β-lactamase

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ABSTRACT

Background: Antimicrobial resistance is an increasingly emerging problem worldwide and is a critical challenge for infectious diseases management around the world.Data from the National Healthcare Safety Network indicate that gram-negative bacteria are responsible for more than 30% of hospital-acquired infections and more than 40% of infections in patients in intensive care units. These infections are difficult to manage translating to a higher rate of morbidity and mortality as well as prolonged length of hospital and intensive care unit stay as well as increased expenses for the healthcare systems.

Methods: This was a hospital based cross sectional descriptive survey aimed at identifying antimicrobial susceptibility patterns of the *Enterobacteriaceae* isolated from urine sampled from both inpatients and outpatients in KNH microbiology laboratory in the period January 2012 to December 2012.Data were retrieved from the archives.A coded form was used to abstract the data. The study was approved by the KNH / U.o.N Ethics and Research Committee.

Results: In this study, among both sexes tested, incidence of *Enterobacteriaceae* infections was higher in females (56%) than males (44%). *Enterobacteriaceae* isolated were: *E.coli* (46%), *Klebsiella* spp. (19.5%), *Citrobacter* spp. (15.9%), *Proteus* spp. (7.1%), *Enterobacter* spp. (5.8%), *Acinetobacter* spp. (5.5%). Tested Carbapenems were found to have better activity against majority of the isolates. ESBL production was found to be high (>60%).

Conclusion: *E. coli* was the most common isolate. Tested carbapenems were more effective, than other drugs, against all the isolates. *Proteus* spp. were the prevalent ESBL producers. *Acinetobacter* spp. and *E. coli* showed higher and lower resistance respectively to carbapenems.

Keywords: Antimicrobial susceptibility patterns, *Enterobacteriaceae***.**

CHAPTER 1: BACKGROUND

Antimicrobial resistance is an increasingly emerging problem worldwide and is a critical challenge for infectious diseases management around the world (Masterton, 2000, Kollef et al., 2001). Infections caused by resistant strains have been associated with a higher rate of morbidity and mortality as well as prolonged hospital and intensive care unit stay and increased expenses for the healthcare systems (Kollef, 2003).

Antibiotics are among the most commonly prescribed drugs in hospitals (Ravi et al., 2000). Studies in Africa have shown that about 90.1% individuals seek care outside the home and 36.2% of these receive antibiotics. Of all those who received antibiotics, 31.7 % did not receive a prescription from a doctor and about 26.4% obtained antibiotics from an informal dispenser (Vialle et al., 2012). Since the discovery of antimicrobial agents, micro-organisms have developed virtually unlimited resistance to them through mechanisms such as mutations and increased enzyme production. Hospitals, and particularly intensive care units, are an important breeding ground for the development of antibiotic resistant bacteria.

Data from the National Healthcare Safety Network indicate that gram-negative bacteria are responsible for more than 30% of hospital-acquired infections and more than 40% of infections in patients in intensive care units (Paleg et al., 2010, Kallen et al., 2010). Hospital-acquired infections caused by gram-negative bacteria are difficult to manage, due to the increasingly varied resistance mechanisms that these bacteria can develop (Tumbarello et al., 2007, Giske et al., 2008).

Urinary tract infections are common in human beings and are the leading cause of Gram-negative bacteremia in patients of all ages and are associated with a high risk of morbidity and mortality and account for significant health care costs (Padmapriya et al., 2012). Studies have

demonstrated that the geographical variability of pathogen occurrence in cases of UTI among inpatients and outpatients populations is limited by the predominance of Gram-negative species usually *Enterobacteriaceae* and particularly *E. coli* and *Enterobacter* spp. in various regions of the world (Twaij, 2000). It is necessary to identify the causative agent and spectrum of its antimicrobial susceptibilities in order to treat UTI. Due to the increasing drug resistance in UTIs, there is need for regular monitoring of the antibiotic susceptibility of uropathogens in a particular area. The distribution of antimicrobial susceptibility data of UTI-causing microorganisms changes from time to time and from place to place (Clinical and Laboratory Standards Institute, 2012).

Therefore, this study aims to determine the susceptibility patterns of the *Enterobacteriaceae* isolated from records of urine collected from inpatients and outpatients at the Kenyatta National Hospital medical microbiology laboratory.

The information obtained from this study will be of great value in implementing policies on rational antimicrobial usage. The evidence could also be used in guiding antimicrobial stewardship programs in a bid to halt the expansion of microorganism resistance and will also be useful in formation of antibiotic formularies at the hospital for empiric treatment of *Enterobacteriaceae* infections.

CHAPTER 2: LITERATURE REVIEW

2.1. INTRODUCTION

2.1.1.*Enterobacteriaceae*

Enterobacteriaceae are gram negative rod bacteria. They include: *Escherichia coli, Klebsiella, Salmonella, Shigella, Proteus, Citrobacter, Enterobacter, Morganella, Plesiomonas, Serratia, Yersinia, Arizona, Providencia, Erwinia* and *Edwardsiella species* among others. Those that have been shown to affect the gastrointestinal tract include certain strains of *E. coli* and *Salmonella*, all four species of *Shigella*, and *Yersinia enterocolitica.* Examples of genera that cause opportunistic infections (including septicemia, pneumonia, meningitis and urinary tract infections) are: *Citrobacter*, *Enterobacter*, *Escherichia, Hafnia*, *Morganella*, *Providencia* and *Serratia*. Some of the organisms additionally cause community-acquired diseases in otherwise healthy people. *Klebsiella pneumoniae* is often involved in respiratory infections. The organism has a prominent capsule aiding pathogenicity. The commonest community acquired urinary tract infection is caused by *E. coli*. The vast majority of urinary tract infections are ascending, often from fecal contamination. *Proteus* species are another common cause of urinary tract infection; the organism produces a urease that degrades urea thereby producing alkaline urine. Selection of antibiotic therapy is complex due to the diversity of these organisms.

2.2.ANTIBIOTIC RESISTANCE IN *ENTEROBACTERIACEAE*

The emergence of antimicrobial resistance is primarily due to excessive and often unnecessary use of antibiotics in humans and animals. A study done in low and middle income countries showed a considerable increasing resistance in *Enterobacteriaceae* (Ashley et al., 2011). The

data revealed that affordable first line agents such as ampicillin and gentamicin are unlikely to be clinically efficacious in a substantial proportion of infections. This results in increasing reliance on the third generation cephalosporins for empirical treatment of serious infections. However, the spread of extended-spectrum beta-lactamase producing strains into the community (Ashley et al., 2011), probably accelerated by this increased consumption, is eroding the usefulness of these drugs. Alternative agents for treating multi-resistant coliform infections, such as the carbapenems, are unaffordable for treatment of community- acquired infections in low-income countries. In Africa, where Non-Typhoidal Salmonella (NTS) are of greater importance, there have been no clinical trials of fluoroquinolones. As quinolone-resistant *salmonellae* infections become more common, an alternative oral antimicrobial is required for settings where parenteral ceftriaxone is not a treatment option. Azithromycin is clearly an excellent drug for these infections, but laboratory data to support clinical trial data are lacking (Ashley et al., 2011).

2.3. BETA-LACTAMASES

Beta-lactamases (β-lactamases) are enzymes produced by some bacteria which provide resistance to beta-lactam antibiotics such as penicillins, cephamycins, and carbapenems (ertapenem). Carbapenems are however relatively resistant to beta-lactamases. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure. All these antibiotics have a common element in their molecular structure: a four-atom ring known as a beta-lactam. Through hydrolysis, the lactamase enzyme breaks the β-lactam ring open thus deactivating the molecule's antibacterial properties.

2.3.1. Classification of beta-lactamases

A. Functional classification for beta lactamases (Bush et al., 1995)

Group 1 are cephalosporinases not inhibited by Clavulanic acid, belonging to the molecular class C.

Group 2 are penicillinases, cephalosporinases, or both inhibited by Clavulanic acid, corresponding to the molecular classes A and D reflecting the original TEM and SHV genes. They are divided into two subclasses, 2a and 2b:

> **Group 2a** (penicillinase, Molecular Class A): The *2a subgroup* contains just penicillinases.

> **Group 2b** (broad-spectrum, Molecular Class A): These are capable of inactivating penicillins and cephalosporins at the same rate. Furthermore, new subgroups were segregated from subgroup 2b:

Group 2be (extended-spectrum, Molecular Class A) these are capable of inactivating third-generation cephalosporins (ceftazidime, cefotaxime, and cefpodoxime) as well as monobactams (aztreonam).

Group 2br (inhibitor-resistant, Molecular Class A): They are also called inhibitor-resistant TEM-derivative enzymes; nevertheless, they are commonly still susceptible to tazobactam, except where an amino acid replacement exists at position met69.

Group 2c (carbenicillinase, Molecular Class A): These inactivate carbenicillin more than benzylpenicillin, with some effect on cloxacillin.

Group 2d (cloxacilinase, Molecular Class D or A): These inactivate cloxacillin more than benzylpenicillin, with some activity against carbenicillin; these enzymes are poorly inhibited by clavulanic acid, and some of them are ESBLs

Group 2e (cephalosporinase, Molecular Class A): These are cephalosporinases that can also hydrolyse monobactams, and they are inhibited by clavulanic acid

Group 2f (carbapenamase, Molecular Class A): These are serine-based carbapenemases

Group 3 (metalloenzyme, Molecular Class B): Metallo B-lactamases are able to hydrolyse penicillins, cephalosporins, and carbapenems. Thus, carbapenems are inhibited by both group 2f (serine-based mechanism) and group 3 (zinc-based mechanism)

Group 4 (penicillinase, No Molecular Class): These are penicillinases that are not inhibited by clavulanic acid, and they do not yet have a corresponding molecular class.

B. Molecular classification of beta-lactamases

The molecular classification of β-lactamases is based on the nucleotide and amino acid sequences in these enzymes. To date, four classes are recognised (A-D), correlating with the functional classification. Classes A, C, and D act by a serine-based mechanism, whereas class B or metallo-β-lactamases need zinc for their action.

Extended-spectrum beta-lactamase (ESBL)

Extended-spectrum beta-lactamases (ESBLs) are beta-lactamases that hydrolyze penicillins, the monobactam aztreonam, and cephalosporins (including expanded-spectrum cephalosporins, such as cefotaxime, ceftriaxone, ceftizoxime, and ceftazidime) (Dubois et al., 2002). Thus ESBLs confer resistance to these antibiotics and related oxyimino-beta lactams. In typical circumstances, they derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β-lactamases. This extends the spectrum of β-lactam antibiotics susceptible to hydrolysis by these enzymes. An increasing number of ESBLs not of TEM or SHV lineage have recently been described (Emery et al., 1997). Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides). Therefore, antibiotic options in the treatment of ESBL-producing organisms are limited. Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, yet carbapenem-resistant isolates have recently been reported. ESBL-producing organisms may appear susceptible to some extended-spectrum

cephalosporins. However, treatment with such antibiotics has been associated with high failure rates (Gazin et al., 2012).

Types of ESBLs

TEM beta-lactamases (class A): TEM-1 is the most commonly encountered beta-lactamase in Gram-negative bacteria. Up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1 (Cooksey et al., 1990). They are also responsible for the ampicillin and penicillin resistance that is seen in *Haemophilus influenzae and Neisseria gonorrhoeae* in increasing numbers (Bradford, 2001). Although TEM-type beta-lactamases are most often found in *E. Coli* and *K. pneumoniae*, they have also been found in other species of Gram-negative bacteria with increasing frequency. Based upon different combinations of changes, currently 140 TEM-type enzymes have been described. Among them, TEM-10, TEM-12, and TEM-26 are among the most common in the United States (Bradford, 2001; Jacoby et al., 2005)

SHV beta-lactamases (class A): SHV-1 shares 68% of its amino acids with TEM-1 and has a similar overall structure. The SHV-1 beta-lactamase is most commonly found in *K.pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance in this species. More than 60 SHV varieties are known. They are the predominant ESBL type in Europe and the United States and are found worldwide. SHV-5 and SHV-12 are among the most common (Paterson et al., 2003).

CTX-M beta-lactamases (class A): These enzymes were named cefotaxime-modifying beta-lactamases for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates (ceftazidime, ceftriaxone, or cefepime). Rather than arising by mutation, they

represent examples of plasmid acquisition of beta-lactamase genes normally found on the chromosome of *Kluyvera* species, a group of rarely - pathogenic commensal organisms. They have mainly been found in strains of *Salmonella enteric* serovar *typhimurium* and *E. coli*, but have also been described in other species of *Enterobacteriaceae* and are the predominant ESBL type in parts of South America while CTX-M-14, CTX-M-3, CTX-M-2 and CTX-M-15 are the most widespread (Bradford, 2001; Jacoby et al., 2005).

***O*xacillinase (OXA) beta-lactamases (class D):** The OXA beta-lactamases are less common and are plasmid-mediated beta-lactamase varieties that could hydrolyze oxacillin and related anti-staphylococcal penicillins. The OXA-type beta-lactamases confer resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid. The OXA-type ESBLs have been found mainly in *P. aeruginosa* (Bradford, 2001; Jacoby et al., 2005)

Amp Ctype β-lactamases (Class C): Amp C type β-lactamases are also commonly isolated from extended-spectrum cephalosporin-resistant Gram-negative bacteria. Amp C β-lactamases (also termed class C or group 1) are typically encoded on the chromosome of many Gram-negative bacteria including *Citrobacter*, *Serratia* and *Enterobacter* species where its expression is usually inducible; it may also occur on *E.coli* but in these it is not usually inducible, although it can be hyper expressed. Amp C type β-lactamases may also be carried on plasmids (Philippon et al., 2002). Amp C β-lactamases, in contrast to ESBLs, hydrolyse broad and extended-spectrum cephalosporins (cephamycins as well as to oxyimino-β-lactams) but are not inhibited by β-lactamase inhibitors such as clavulanic acid.

Carbapenemases

Carbapenemases are a diverse group of β-lactamases that are active not only against the oxyimino-cephalosporins and cephamycins but also against the carbapenems. Carbapenemases, enzymes that hydrolyze carbapenem class antibiotics (ertapenem, imipenem, meropenem, and doripenem) usually hydrolyze all other currently available β-lactams, with the exception of aztreonam for some metallo beta lactamases. The genome encoding for the production of these enzymes may be located on plasmids for instance *K. pneumonia* carbapenemases, a feature that makes them of particular concern from an infection control perspective.

There are 3 classes of carbapenemases: serine class A, class B enzymes known as the metallo-β-lactamases [such as Verona integron-encoded metallo-beta-lactamase (VIM), IMP, and New Delhi Metallo-beta-lactamase (NDM)], and the class D OXA enzymes (Queenan et al., 2007). Carbapenemases have been identified in a wide range of gram-negative genera. The KPC enzyme, the most frequently identified class A carbapenemase in the United States, is most often found in the *Enterobacteriaceae* but has also been detected in *P aeruginosa* (Villegas et al., 2007). Metallo beta lactamases are most frequently seen in *Acinetobacter* spp. and *P.aeruginosa*, but recently NDM has become widespread in some regions of the world among species of *Enterobacteriaceae*, particularly *K pneumonia* (Villegas et al., 2007). OXA carbapenemases are frequently found in *Acinetobacter* species but have also been reported among other isolates of *Enterobacteriaceae*.

2.4. ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

This is indicated for pathogens contributing to an infectious process that warrants antimicrobial therapy if susceptibility to antimicrobials cannot be predicted reliably based on knowledge of their identity. Antimicrobial susceptibility testing (AST) is used routinely by diagnostic microbiology laboratories to direct therapy. The gold standard for susceptibility testing is determination of the minimum inhibitory concentration (MIC), i.e. the lowest concentration of antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation (Jenkins and Schuetz, 2012).

The range of antibiotic concentrations used for determining MICs is universally accepted to be in doubling dilution steps up or down from 1 mg/l. Methods for determining the MIC include the broth micro dilution method, where wells contain broth with different dilutions of antibiotics added, and agar dilution techniques that use agar into which antimicrobial agents have been incorporated at different concentrations. The E-test is a modified agar diffusion method in which an agar plate is inoculated with a bacterial isolate and a rectangular strip impregnated with antibiotic is overlaid; the drug diffuses out into the agar, producing an exponential gradient of drug concentrations. The MIC corresponding to the zone of inhibition is read off a scale on the strip (Jenkins and Schuetz, 2012).

In practice, estimating precise MICs for various drugs against individual isolates is labour-intensive and time-consuming, so the most common method employed by most diagnostic laboratories is a simpler agar diffusion test (Kirby–Bauer method), in which the organism under investigation is inoculated onto an agar plate and exposed to a diffusion gradient of antibiotic from an impregnated disc of filter paper placed on the agar surface. The circular area of growth

inhibition (zone of inhibition size) reflects the antibiotic activity. This method provides a simple and cheap 'breakpoint technique', using zone of inhibition cut-offs to classify bacterial isolates as either: susceptible, intermediate, or resistant. There is not one universally accepted system for AST, meaning different countries / laboratories use different breakpoints to define susceptibility of different bacteria (CLSI, 2005).

2.4.1. Detection of ESBL production among *Enterobacteriaceae*

Beta-lactamase enzymatic activity can be detected using nitrocefin, a chromogenic cephalosporin substrate which changes color from yellow to red upon beta-lactamase mediated hydrolysis (O'Collaghan et al 1972). The CLSI guidelines specify screening criteria and confirmatory testing approaches for detection of ESBL production by *Escherichia coli*, *K pneumoniae*, and *Proteus mirabilis* (CLSI, 2012). For organisms such as *Enterobacter* spp. and *Serratia* spp. Which produce Amp C-type enzymes, ESBL screening should not be performed because false-negative results can occur. These screening and confirmatory tests were necessary because standard disk diffusion and MIC tests did not uniformly identify isolates producing ESBLs. Because ESBLs are usually inhibited by clavulanic acid, the CLSI made use of this property in developing the tests recommended to clinical laboratories for their detection and recommendations that isolates producing ESBLs be reported as resistant to all penicillins, cephalosporins, and aztreonam.

On establishment of new, lower interpretive criteria for many of these compounds largely based on pharmacokinetic and pharmacodynamic principles and limited clinical data, the CLSI revised their recommendations for reporting (CLSI, 2012). When the new breakpoints are adopted by clinical laboratories, the CLSI recommends that results for specific cephalosporins and aztreonam be reported and interpreted as they are tested and that the ESBL screening and

confirmatory tests need only be performed for epidemiological and infection control purposes. By comparison, although the European Committee on Antimicrobial Susceptibility Testing's (EUCAST) breakpoints for the cephalosporins are similar to those now recommended by the CLSI, the former (EUCAST) recommends that laboratories continue to screen and confirm ESBL production due to the limited supporting clinical data and that cephalosporin reports be changed from susceptible to intermediate or intermediate to resistant if an isolate tests positive for ESBL production.

2.4.2. Detection of Carbapenemase activity among *Enterobacteriaceae*

Subcommittee on Antimicrobial Susceptibility Testing (SAST) recommended the modified Hodge test for the detection of carbapenemase activity in *Enterobactericeae* (CLSI, 2012). Advantages of this assay include its ease of performance, the ability to test several isolates on a single plate, and the detection of different classes of carbapenemases with one test (Anderson et al., 2007). The primary disadvantages are subjectivity in reading the results, its inability to differentiate the various carbapenemases (potentially useful from an epidemiological perspective), and the false-positive results that can occur with some organisms producing Amp C or ESBL enzymes.

The modified Hodge test was originally recommended for the detection of carbapenemases in bacteria for which carbapenem MICs were elevated but still fell within the susceptible range. When isolates tested positive, the SAST recommended that they be designated carbapenemase producing strains in the patient report with a warning indicating that the therapeutic outcomes of patients infected with such organisms and treated with the relevant carbapenem were unknown, particularly when alternative dosing regimens were used (e.g., continuous or prolonged infusion). In 2010, though, the SAST lowered the carbapenem breakpoints to capture most

carbapenemase-producing strains, which would now test either as resistant or intermediate to these compounds (CLSI, 2012). Implementation of the revised breakpoints eliminates the need for laboratories to routinely perform the modified Hodge test, although such testing may in some cases still be of value from an epidemiological or infection control perspective. A number of phenotypic tests allow detection and differentiation of class A (inhibition by boronic acid) and class C inhibition by chelating agents such as ethylenediaminetetraacetic acid (EDTA) carbapenemases, but these tests fail to detect class D (OXA) and are primarily used for strain characterization rather than for clinical purposes.

CHAPTER 3: RESEARCH DEFINITION

3.0. JUSTIFICATION

Antibiotics are among the most commonly prescribed drugs in hospitals (Ravi et al., 2000). Studies in Africa have shown that about 90.1% individuals seek care outside the home, 36.2 % of whom receive antibiotics. Of all those who receive antibiotics, 31.7 % do not receive a prescription from a doctor and about 26.4% obtain antibiotics from an informal dispenser (Vialle et al., 2012). Urinary tract infections are difficult to eradicate completely and thus a challenge to medical professionals (Padmapriya, 2012). There is an increasing trend of antimicrobial resistance in Gram-negative bacteria (GNB) and there has been little successful development of new antibiotic agents targeting this class of pathogens (Talbot et al., 2006). Furthermore, we are now in the presence of GNB that have 'extreme drug resistance', indicating complete resistance of strains to first-line antibiotics used for the treatment of GNB infections (amikacin, tobramycin, cefepime, ceftazidime, imipenem, meropenem, piperacillin- tazobactam, ciprofloxacin, and levofloxacin) plus second-line drugs such as tigecycline and polymyxins (Paterson, 2007). Rising antibiotic resistance rates among bacterial pathogens has resulted in increased morbidity and mortality as well as prolonged length of hospital and intensive care unit stay and increased expenses for the healthcare systems. Infections caused by drug resistant gram-negative bacteria are difficult to treat hence limiting the therapeutic options for treatment, and thus increased social benefit from disease prevention. Increasing rates of resistance lead many clinicians to treat patients empirically with multiple drugs.

Since antibiotics are commonly prescribed in hospitals and their use is one of the important contributors to the development and spread of resistance in the hospitals, an audit of their susceptibility patterns is important.

3.1. RESEARCH QUESTION

What were the antibiotic susceptibility patterns of *Enterobacteriaceae* isolated from both inpatient and outpatients' urine at the KNH microbiology laboratory in the period January 2012 to December 2012?

3.2. OBJECTIVES

3.2.1. Broad Objective

To determine the susceptibility patterns of *Enterobacteriaceae* isolated from both inpatient and outpatients' urine at the KNH Microbiology laboratory in the period January 2012 to December 2012 to different antibacterial agents.

3.2.2 Specific objectives

1. To determine the distribution of the various members of the *Enterobacteriaceae* family isolated.

2. To determine the antimicrobial susceptibility patterns of isolated *Enterobacteriaceae*.

3. To determine the prevalence of suspected ESBLs producing isolates.

4. To investigate the presence of carbapenems resistant *Enterobacteriaceae* (CRE).

CHAPTER 4: RESEARCH METHODOLOGY

4.1. Study site

The study was conducted at the Kenyatta National Hospital (KNH) medical microbiology laboratory.

4.2. Study design

The study was a cross sectional descriptive study involving review of patients' laboratory files.

4.3. Study population

Data were abstracted from the microbiology laboratory records of patients diagnosed with *Enterobacteriaceae* from urine specimens of both inpatients and outpatients during the period January 2012 to December 2012.

Inclusion criteria

- Laboratory records of patients from whose urine *Enterobacteriaceae* were isolated during the period January 2012 to December 2012**.**

Exclusion criteria

- Laboratory records with incomplete data.

4.4. Sample size determination

The sample size was estimated according to Fisher's formula (Fisher 1991)

$$n = \frac{z^2 \hat{p}(1-\hat{p})}{m^2}$$

n= $\underline{1.96^2 *0.5(1-0.5)}$

$\quad\quad 0.05^2$

n = 384

Where:

p = expected prevalence of *Enterobacteriaceae* infections in Kenya. This is unknown, therefore a 50% prevalence was assumed.

m = degree of precision or a tolerance error margin or width of the confidence interval (a measure precision of the estimate).

z = Z statistic for a level of confidence or is the normal distribution critical value for a probability of α/2 in each tail. For a 95% CI, z = 1.96

For this study, a specified level of confidence of 95% and an error margin of ±5% was considered acceptable, based on similar studies done elsewhere.

Since the study population was less than 10,000, finite population correction factor was used to adjust the sample size. Total number of laboratory records of patients diagnosed with *Enterobacteriaceae* from urine samples of both outpatients and inpatients during the period January 2012 to December 2012 was estimated to be N=5,000. The sample size that would be necessary was therefore given by;

$$n = \frac{n'}{1 + (n' + 1)/N}$$

Where; n is the adjusted sample size

n' is the sample size before adjustment,

      N is the total population.

The adjusted sample size was therefore given as;

n = 384 / (1 + (384 + 1) / 5000)

n = 356

4.5. Sampling technique

The sampling frame included laboratory records of patients' samples that had *Enterobacteriaceae* isolates in the KNH medical microbiology laboratory, and who met the inclusion criteria. Systematic random sampling was used to select the records. A sampling fraction k was obtained by dividing the 'total number of urine records with *Enterobacteriaceae* isolates, which were 1095, in 2012' by 356. The first record was selected randomly and the rest by subsequently adding the value of k, which was 3 for this study, until the sample size was achieved.

4.6. Data collection

After obtaining ethical approval (Appendix B) and permission from the KNH head of Laboratory medicine, data was obtained from the KNH microbiology laboratory records. A coded form (Appendix A) was used as the study instrument to abstract the information. Patients' names were left out for the sake of confidentiality. Data was extracted for the time period January 2012-December 2012. Only the investigator and the research assistant had access to the files for the purposes of the study.

4.7. Data management and analysis

Data was abstracted from the patients file using a coded form (Appendix A) by the research assistant. All the filled forms were reviewed by the principle investigator to ensure they were completed appropriately. Data collected was entered into an Excel spreadsheet in a password protected computer. Back-up copies were stored in an external hard drive and compact disc which were in sole custody of principal investigator.

The filled forms were in safe custody of the principal investigator who filed and stored them in a lockable cabinet for verification during analysis.

Further cleaning was carried out after entry using frequency distributions and cross-tabulations until no more errors were detectable. The final step in the preparation for analysis was coding of the data and the creation of any composite variables from the cleaned data set. In order to achieve the objectives of the study, data analysis was done using Statistical Package for Social Sciences Programme (SPSS) version 17.0.

Univariate analysis involved frequency distributions for categorical variables and descriptive statistics for continuous variables, such as age.

Bivariate analysis was used to investigate any association between variables. The χ^2 test was to be used to test association between 2 variables if they were categorical and satisfied all the conditions. If some χ^2 conditions would not be met, Fisher's exact test was to be used instead. The student t-test was used for continuous variables.

Categorical variables were presented using bar charts and frequency distribution tables. Histogram was used to present age.

4.8. Ethical consideration

Permission to carry out the research was obtained from the KNH/U.o.N. Ethics and Research Committee (Appendix B) before conducting the research. Permission to extract data from the hospital registers and records was obtained from the Kenyatta National Hospital head of Laboratory medicine. The study was a minimal risk study since there was no direct patient involvement but a retrospective review of the records. For confidentiality, the patients' files were only used within the confines of the KNH microbiology laboratory and only the investigator with the assistance of the research assistants and laboratory personnel had the access to the files for the purposes of this study. The patient identifying information such as the name was not included in the data collection forms. All the filled forms were stored in lockable drawers. Raw data in form of filled forms, data stored in password protected computer or even the back-up copies in hard drives and compact disc were destroyed at the end of the study.

4.9. Study limitations

Completeness of files: A number of files were incomplete hence rendering the cases invalid. Important variables were left out like age of the patient, organism isolated and the sensitivity patterns.

Non molecular detection techniques: Both ESBL and Hodge confirmatory testing are poor substitutes for molecular methods for detection of ESBLs and carbapenemases.

Drugs used in the study site for sensitivity testing were not consistent and depended on the drugs available at that particular time.

CHAPTER 5: RESULTS

5.1.DEMOGRAPHICS

In the year 2012, there were 1095 records of *Enterobacteriaceae* isolated from urine samples of both inpatients and outpatients in KNH medical microbiology laboratory and, of these, 365 were sampled randomly.Urine samples were obtained from the General wards (36%), ICU (27%), Outpatient department (19%), Paediatric wards (4.5%), Burns unit (3.5%), renal unit (1.6%), and Private wing (0.3%).

Figure 1: Gender of the study population
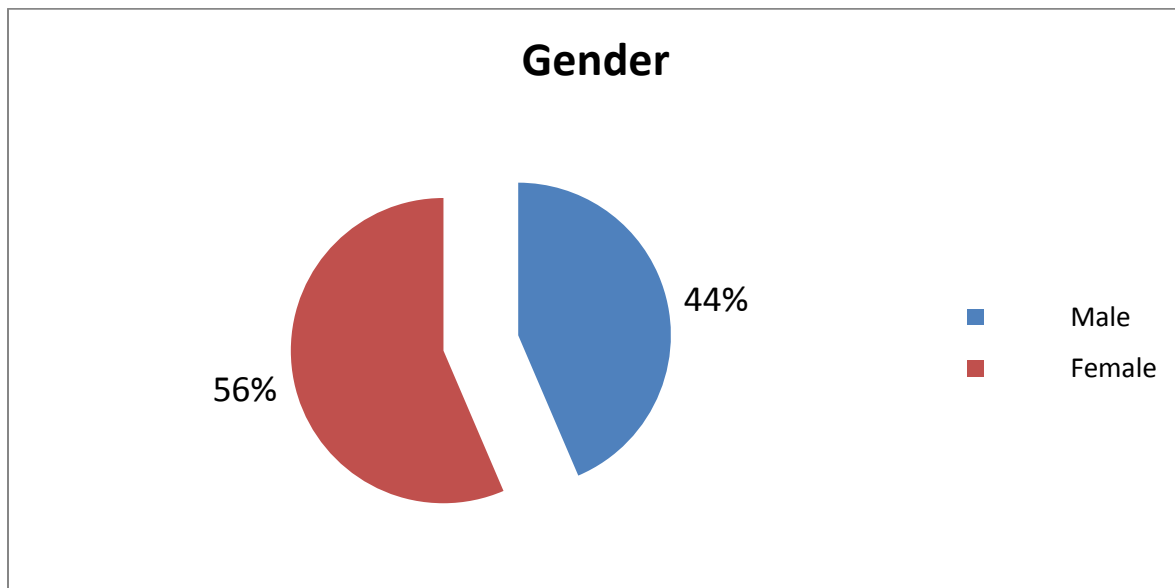
Females were 56% (206) while males were 44% (159)

Figure 2: Age distribution of the study population.
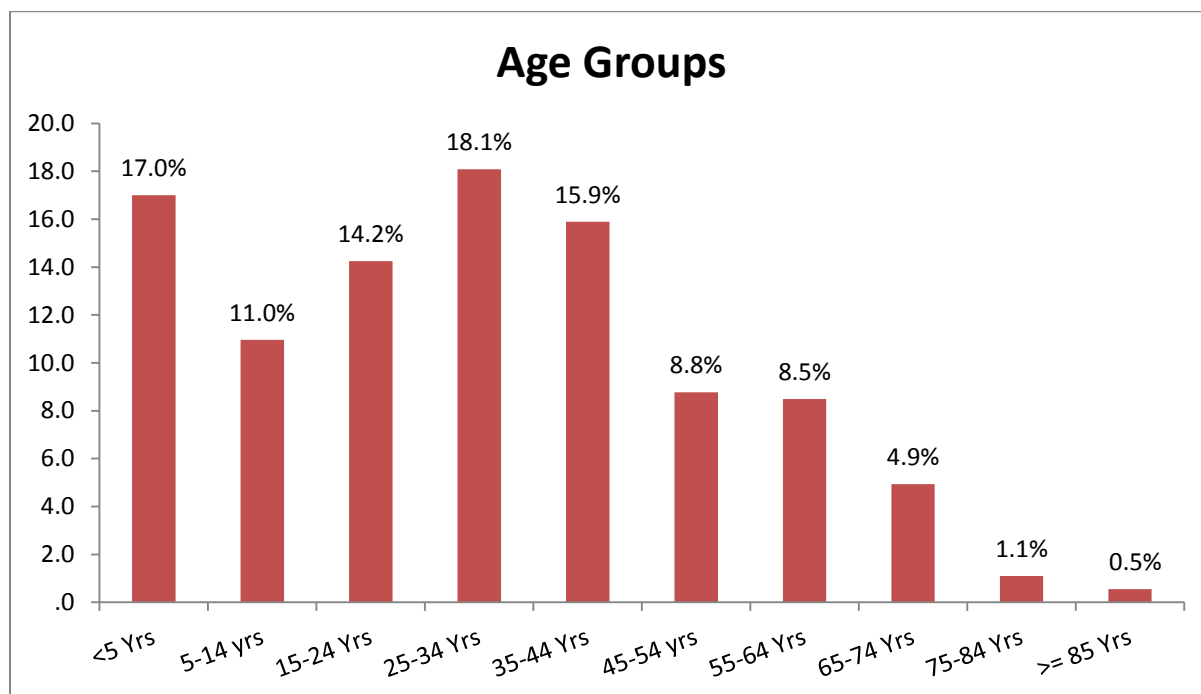
The patients' ages ranged from <1 year to 86 years. Median age was 29 years. Those aged between 25 to 34 years were the majority (18.1%) while those aged 5years and below were 17.0%. Those patients aged between 15 and 44 years were 48.2%.

5.2. DISTRIBUTION OF ORGANISMS ISOLATED

The Enterobacteriaceae isolated were: *E.coli* (46%), *Klebsiella* spp. (19.5%), *Citrobacter* spp. (15.9%), *Proteus* spp. (7.1%), *Enterobacter* spp. (5.8%), and *Acinetobacter* spp. (5.5%)

Table 1: Distribution of the isolated organisms

Organism	n=365	%
E.coli	168	46.0
Klebsiella spp.	71	19.5
Citrobacter spp.	58	15.9
Proteus spp.	26	7.1
Enterobacter spp.	21	5.8
Acinetobacter spp.	20	5.5

5.3. ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF URINE, *ENTEROBACTERIACEAE* ISOLATES

Nineteen antibiotics were tested against the isolated organisms. Overall the highest sensitivity was demonstrated by Meropenem (84.4%) and Levofloxacin (48.8%) while Augmentin (75.3%), Ampicillin (61.9%), Ceftriaxone (58.1%), Cefuroxime (55.6%) and Cefotaxime (42.2%) showed the highest resistance as shown in table 2.

Table 2: General sensitivity patterns for the drugs used.

Antibiotic	Sensitive (S) n(%)	Resistant (R) n(%)
Amoxycillin/Clavulanate (Augmentin)	81(22.2)	275(75.3)
Doxycycline	5(1.4)	14(3.8)
Cefuroxime	84(23.0)	203(55.6)
Ampicillin	11(3.0)	226(61.9)
Levofloxacin	178(48.8)	148(40.5)
Chloramphenicol	4(1.1)	6(1.6)
Gentamycin	19(5.2)	35(9.6)
Timentin	0	3(0.8)
Imipenem	73(20.0)	16(4.4)
Cefepime	3(0.8)	1(0.3)
Ceftriaxone	103(28.2)	212(58.1)
Cefotaxine	88(24.1)	154(42.2)
Cotrimoxazole	5(1.4)	31(8.5)
Nalidixicacid	13(3.6)	23(6.3)
Ceftazidime	6(1.6)	10(2.7)
Meropenem	308(84.4)	30(8.2)
Ciprofloxacin	18(4.9)	22(6.0)
Ofloxacin	1(0.3)	2(0.5)
Cefoxitin	1(0.3)	0

Resistance and Sensitivity of selected organisms

Among the top three isolated organisms *E.coli* (46%), *Klebsiella* (19.5%), and *Citrobacter* (15.9%), *E.coli* showed high resistance to Ampicillin (95.4%), Cotrimoxazole (94.1%) and Augmentin (69.9%) (Table 3), *Klebsiella* spp. had high resistance to Ampicillin (97.9%), Augmentin (78.3%) and Gentamicin (75%) (Table 4). *Citrobacter* showed high resistance to Ampicillin (94.4%), Augmentin (91.2%) and Cefuroxime (78.7%) (Table 5)

Table 3: Antimicrobial sensitivity patterns among the *E.coli*

	Sensitive (S) n (%)	Resistant (R) n (%)
Augmentin	49 (30.1)	114 (69.9)
Ampicillin	5 (4.6)	104 (95.4)
Levofloxacin	71 (48.6)	75 (51.4)
Cefuroxime	47 (35.6)	85 (64.4)
Cefotaxime	54 (44.3)	68 (55.7)
Meropenem	148 (96.7)	5 (3.3)
Gentamicin	13 (50.0)	13 (50.0)
Imipenem	35 (97.2)	1 (2.8)
Ceftriaxone	57 (41.3)	81 (58.7)
Ciprofloxacin	9 (40.9)	13 (59.1)
Cotrimoxazole	1 (5.9)	16 (94.1)

Table 4: Antimicrobial sensitivity patterns among the *Klebsiella* spp.

	Sensitive (S) n (%)	Resistant (R) n (%)
Augmentin	15 (21.7)	54 (78.3)
Ampicillin	1 (2.1)	47 (97.9)
Levofloxacin	42 (68.9)	19 (31.1)
Cefuroxime	14 (26.4)	39 (73.6)
Cefotaxime	13 (30.2)	30 (69.8)
Meropenem	66 (95.7)	3 (4.3)
Gentamycin	4 (25.0)	12 (75.0)
Imipenem	13 (81.3)	3 (18.8)
Ceftriaxone	18 (31.0)	40 (69.0)
Ciprofloxacin	5 (83.3)	1 (16.7)
Cotrimoxazole	3 (30.0)	7 (70.0)

Table 5: Antimicrobial sensitivity patterns among the *Citrobacter* spp.

	Sensitive n (%)	Resistant n (%)
Augmentin	5 (8.8)	52 (91.2)
Ampicillin	2 (5.6)	34 (94.4)
Levofloxacin	26 (48.1)	28 (51.9)
Cefuroxime	10 (21.3)	37 (78.7)
cefotaxime	9 (23.1)	30 (76.9)
Meropenem	42 (79.2)	11 (20.8)
Gentamycin	1 (33.3)	2 (66.7)
Imipenem	11 (61.1)	7 (38.9)
Ceftriaxone	12 (21.4)	44 (78.6)
Ciprofloxacin	1 (25.0)	3 (75.0)
Cotrimoxazole	0 (0)	3 (100)

Figure 3: Antimicrobial sensitivity patterns of selected organisms
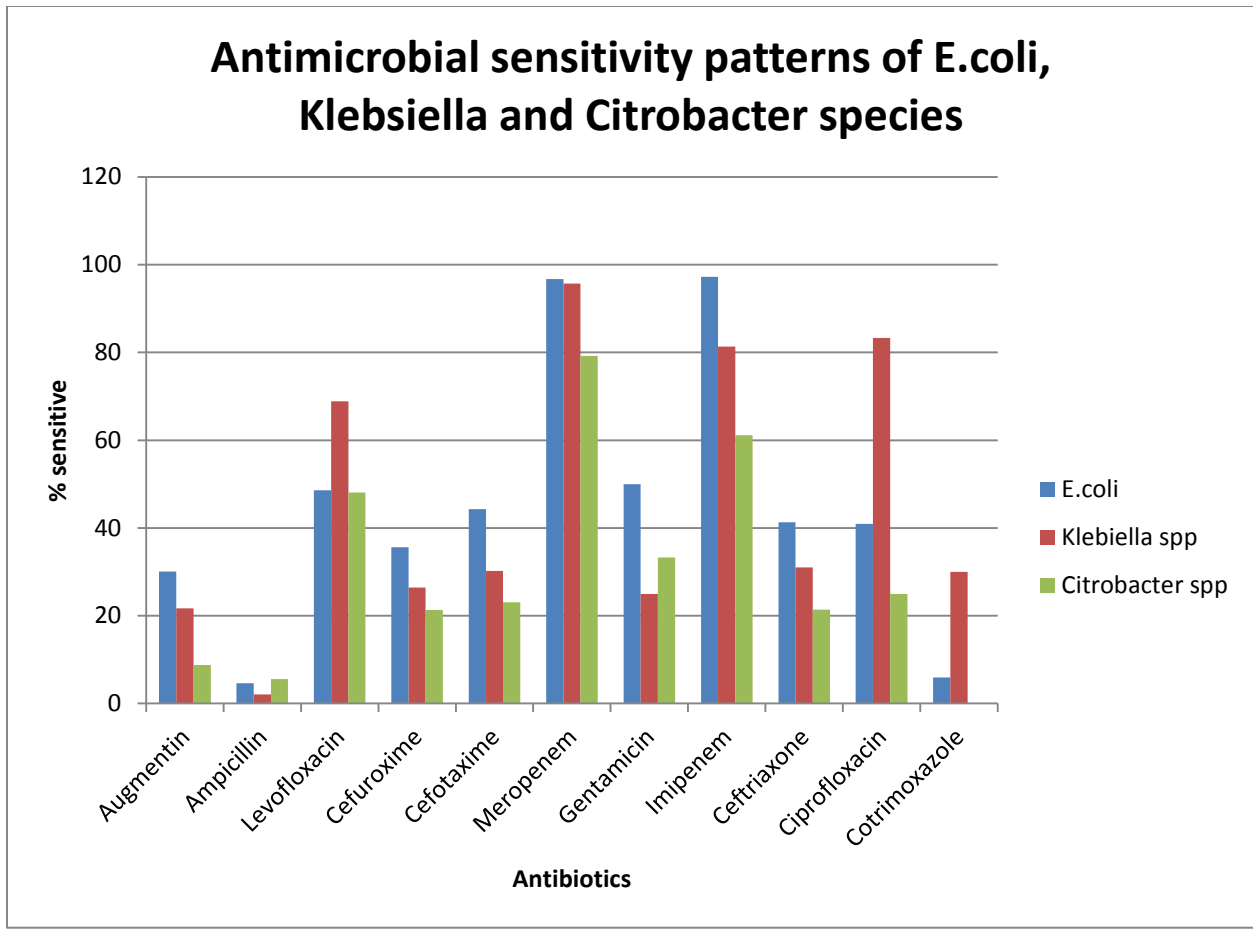
Majority of the isolates were sensitive to the Carbapenems (Imipenem and Meropenem).

E.coli showed the highest sensitivity to Meropenem (96.7%) and Imipenen (97.2%) (Table 3).

Resistance was noted among the second line drugs Cefotaxime (E.coli-55.7%, *Klebsiella*-69.8% and *Citrobacter*-76.9%) and Ceftriaxone (*E.coli*-58.7%, *Klebsiella*-69% and *Citrobacter*-78.6%) (Tables 3, 4 and 5).

5.4. ANTIBIOTIC PATTERNS

There were variations in the sensitivity patterns along the months in 2012 with Meropenem and Levofloxacin showing consistently better activity against the isolates while most of the isolates were resistant to Ampicillin and Augmentin (Figure 4).

Figure 4: Monthly trends in sensitivity patterns of *E. coli* to selected antimicrobials in 2012
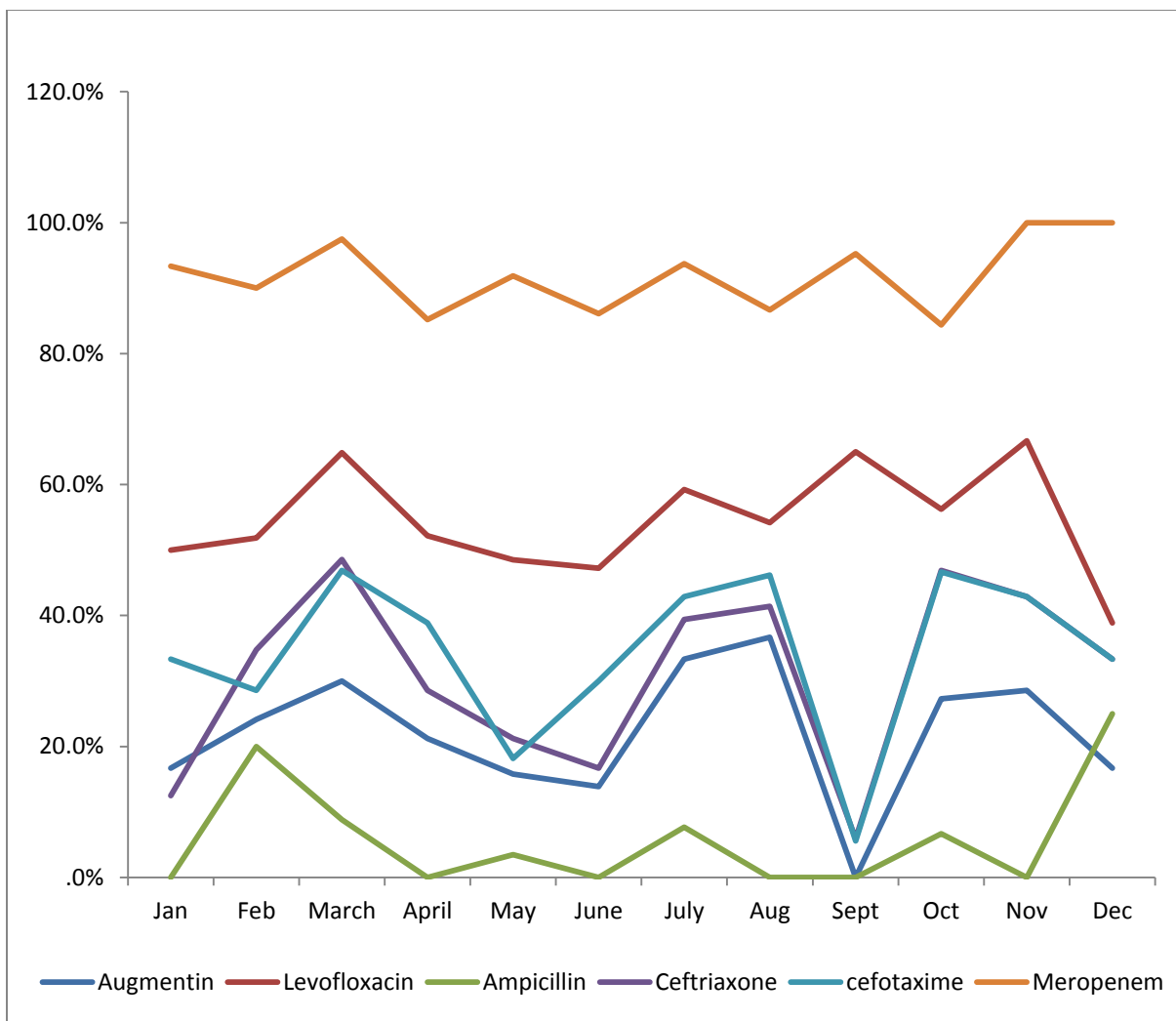
## 5.5. PREVALENCE OF ESBL PRODUCTION

Among the *Enterobacteriaceae* isolated, the largest proportion of ESBL producers was in *Proteus* spp. (88.5%). The rate of ESBL production for the other isolates was as shown

Table 6: Rate of ESBL production among the isolated organism

Organism n = 365		ESBL production n (%)	No ESBL production n (%)
E.coli	168	115(68.5)	53(31.5)
Klebsiella	71	55(77.5)	16(22.5)
Citrobacter	58	42(72.4)	16(27.5)
Proteus	26	23(88.5)	3(11.5)
Enterobacter	21	16(76.2)	5(23.8)
Acinetobacter	20	13(65.0)	7(35.0)

5.6. PREVALENCE OF CARBAPENEM RESISTANT *ENTEROBACTERIACEAE*

Figure 5: Resistance of isolated organisms to Carbapenems
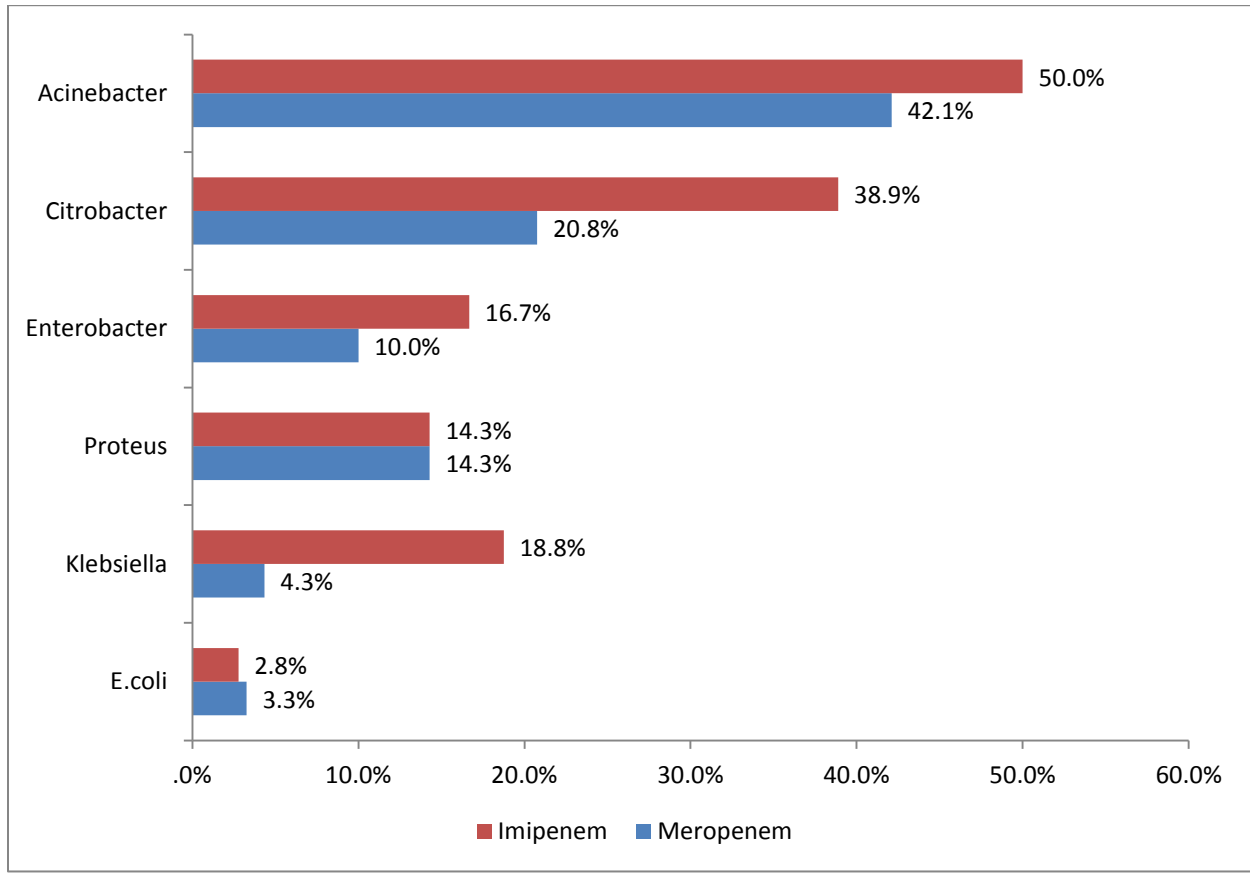
The carbapenems used in this study were meropenem and imipenem. Resistance to imipenem was generally higher than meropenem with *Acinetobacter* and *Citrobacter* spp. leading.

E. coli showed the least resistance (figure 5).

CHAPTER 6: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1. DISCUSSION

Findings from this study showed that antimicrobial resistance is a problem in our setting. In line with other studies done that showed that urinary tract infections are common in females than males, urine specimens from females (56%) were higher than those from males (44%). This could be explained by the fact that the female urethra appears to be less effective in preventing entry of bacteria (Padmapriya et al., 2012). The reason behind this high prevalence of UTIs in females could also be due to the close proximity of the urethral meatus to the anus, shorter urethra, sexual intercourse, incontinence, and bad toilet habits (Ochei et al., 2007). Higher incidence of gram negative bacteria, related to *Enterobacteriaceae*, in causing UTIs is due to many factors which are responsible for their attachment to the uroepithelium. They are able to colonize the urogenital mucosa with adhesins, pili, and fimbriae.

This study's results are in line with another study done that showed that the common uropathogens isolated were *E. coli* (21.95%), *Klebsiella* spp. (12.19%), p*roteus spp.* (9.75%) (Padmapriya et al., 2012) whereas (Prakash et al., 2013), investigated a total of 155 bacterial uropathogens of which *E. coli* was found to be the dominant bacteria, among all isolated uropathogens, with the prevalence rate of 42.58%. The second most prevalent isolate was *Klebsiella pneumonia* (18.71%) followed by *Proteus* spp. (9.03%), and *Enterobacter* spp. (7.10%).

Carbapenems used in this study were found to be among the most effective drugs against all isolated pathogens; *E. coli* (Meropenem, MRP; 96.7% and Imipenem, IMP; 97.2%), *Klebsiella* (MRP; 95.7% and IMP; 81.3%), *Citrobacter* (MRP; 79.2% and IMP; 61.1%). This is in line with

a study done in India which showed that most sensitive drug against all uropathogens was MRP (92.26%) and IMP (84.52%), (Prakash et al., 2013). Resistance to Imipenem was generally higher than that to Meropenem with imipenem showing lesser activity against *Acinetobacter* spp., *Citrobacte*r spp. and *Klebsiella* spp. (50%, 38.9% and 18.8% respectively) while meropenem showed the highest activity among *Acinetobacter* spp., *Citrobacte*r spp., and *Proteus* spp. (42.1%, 20.8% and 14.3% respectively) (Figure 5). A surveillance study done in two Mexican referral hospitals (Morfin-Otero et al., 2012), showed that more than 60% of the *Acinetobacter* isolates were resistant to all antibiotics tested, except imipenem (36.4% resistance), and meropenem (37.4% resistance). Their study also showed that all (100.0%) *Enterobacter* spp. tested were susceptible to imipenem and meropenem which was not the case in our study that showed a resistance of (MRP; 10% and IMP; 16.7%).

Enterobacteriaceae isolates in this study showed high resistance against the cephalosporins used. *E.coli* (Cefuroxime; 64.4%, Cefotaxime; 55.7% and Ceftriaxone; 58.7%), *Klebsiella* (Cefuroxime; 73.6%, Cefotaxime; 69.8% and Ceftriaxone; 69%), *Citrobacter* (Cefuroxime; 78.7%, Cefotaxime; 76.9% and Ceftriaxone; 78.6%). Another study showed that Gram negative bacilli were highly sensitive to meropenem but showed high resistance to cephalosporins (Goel et al., 2009).

ESBL production was high among the isolated organisms and predominantly among *proteus* spp. (88.5%). A study done in Tanzania showed that a significant proportion of ESBL producing strains were found to be resistant to antimicrobial agents including amoxicillin / clavulanic acid (90.9%), doxycycline (81.5%), gentamicin (72.7%) and trimethoprim/sulfamethoxazole (90.9%), ceftriaxone (100%) and cefuroxime (100%) (Ndugulile et al., 2005). Although the data linking

ESBL production to resistance was not available, ESBL production would have contributed to the resistance. Most samples were obtained from the General wards (36%) and ICU (27%) which could have contributed to the resistance especially due to hospital acquired infections.

In this study, the organisms isolated showed high resistance to ciprofloxacin; *E. coli* (59.1%), *Klebsiella* (16.1%), *Citrobacter* (75%). Resistance of *Klebsiella spp.* to fluoroquinolones was not in line with other studies done by (Prakash et al., 2013), that showed high resistance of *Klebsiella spp.* to fluoroquinolones. This high rate of resistance against fluoroquinolones was also suggested by other studies done in Spain, Europe and Iran (Gorbernado et al., 2007; Rashedmarandi et al., 2008).

Another study done in Spain also showed the reduced susceptibility of *E. coli* isolates from patients with UTI to fluoroquinolones (16%) (Gorbernado et al., 2007). McEwen et al., 2003 found that 37% of physicians actually prescribe trimethoprim-sulphamethoxazole closely followed by fluoroquinolones (32%) and the average duration of antibiotic therapy is 8.6 days in the United States. Contrary to the findings of other studies that showed fluoroquinolones to be the most effective against *Enterobacteriaceae*, our study did not really support that, although Levofloxacin showed better activity than most of the other molecules. The empiric use of fluoroquinolones should be restricted and strategies against the increasing resistance of pathogens to these antibiotics should be developed and implemented.

Another finding in this study was the high resistance of the isolated organisms to third-generation cephalosporins. This is an indication that many of the organisms are ESBL producers evident from our findings which showed that ESBL production was high, especially in *Proteus*

spp. (88.5%), *Klebsiella* spp. (77.5%), *Enterobacter* spp. (76.2%) and *Citrobacter* spp. (72.4%). The other possible explanation behind this situation is that the 3rd generation cephalosporins have been in use for a long period and may have been abused, and over this time organisms have developed resistance mechanisms. The inappropriate usage of wide spectrum antibiotics, insufficient hygiene, immunosuppression, and a prolonged stay in the hospital are some other major etiological factors that elevate the chances of Multi Drug Resistant (MDR) infections (Manjunath et al., 2011).

6.2. CONCLUSION

Carbapenems were found to be more effective than other drugs against the isolated pathogens. However, they are expensive and may not be suitable for use in resource-poor settings. Levofloxacin also showed good activity and is cheaper than the carbapenems but it cannot be used in children and expectant or lactating women. Augmentin, Ampicillin and Cotrimoxazole were less effective. Nitrofurantoin is commonly used for treatment of urinary tract infections especially in expectant women and is a cheaper alternative but it was not available for sensitivity testing in 2012.

Carbapenem resistant *Enterobacteriaceae* were isolated with *Acinetobacter* spp. and *E. coli* showing higher and lower resistance respectively. Resistance to imipenem was generally higher than that to meropenem.

E. coli was the most common *Enterobacteriaceae* isolated.

ESBL production among the *Enterobacteriaceae* isolated was found to be high (> 60%) with *Proteus* spp. being the most prevalent ESBL producers.

6.3. RECOMMENDATIONS

1. Regular antimicrobial audits and reviews of laboratory data (surveillance) should be done so as to have proper documentation of drug resistance patterns and timely updates of antibiotic formularies.

2. Information about drug resistance should be properly communicated to those prescribing antimicrobials and adequate guidelines regarding the selection of drugs should be availed. Additionally, adequately documented local retrospective data should be availed on the benches of health care providers to guide good antibiotic stewardship.

3. Antimicrobial stewardship programs and antibiograms should be developed by healthcare institutions to reduce inappropriate antimicrobial use, improve patient outcomes and reduce adverse consequences of antimicrobial use.

4. Antimicrobial research in Kenya should be emphasized and adequately funded.

5. As shown in this study, and resources allowing, Carbapenems are the choice drugs in treatment of *Enterobacteriaceae* infections. The government, and other stakeholders, should consider subsidizing the cost of these drugs.

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APPENDICES

APPENDIX A: DATA COLLECTION FORM

TOPIC: **Antimicrobial susceptibility patterns of the *Enterobacteriaceae* isolated from urine in KNH Microbiology Laboratory in the period January 2012 to December 2012**

QUESTIONNAIRE NUMBER: …………………………….

SOCIO DEMOGRAPHIC CHARACTERISTICS

Age of patient: ………..Years

Sex   Male   [ ]

             Female   [ ]

Month in which the organisms were isolated

January	[ ]	July	[ ]
February	[ ]	August	[ ]
March	[ ]	September	[ ]
April	[ ]	October	[ ]
May	[ ]	November	[ ]
June	[ ]	December	[ ]

Area from which the specimen was obtained

ICU ⬜ General ward ⬜ Burns unit ⬜

Paediatric ward ⬜ Renal unit ⬜ Out-patient department ⬜

Others (Specify) ⬜ ……………………………………….…..

Organism isolated

E. coli ⬜

Klebsiela ⬜

Salmonella ⬜

Shigella ⬜

Proteus ⬜

Enterobacter ⬜

Citrobacter ⬜

Others ⬜ Specify...

Is this organism a suspected ESBL producer? Yes ⬜ No ⬜

Antibiotic susceptibility patterns

Antibiotic	Sensitive (S)	Resistant (R)
Amoxy/Clav (Augmentin)		
Doxycycline		
Cefuroxime		
Ampicillin		
Levofloxacin		
Chloramphenical		
Gentamycin		
Piperacillin		
Timentin		
Imipenem		
Cefepime		
Amikacin		
Ceftriaxone		
Cefotaxime		
Cotrimoxazole		
Nalidixic acid		
Nitrofurantion		
Ceftazidime		
Meropenem		

APPENDIX B: APPROVAL
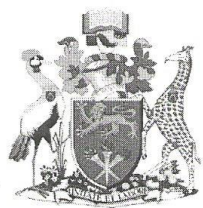
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Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/314

Link:www.uonbi.ac.ke/activities/KNHUoN

10th October 2013

Dr. Simon K. Njiru
W64/69495/2011
UNITID
College of Health Sciences
University of Nairobi

Dear Dr. Njiru

RESEARCH PROPOSAL: ANTIMICROBIAL SUSCEPTIBLITY PATTERNS OF *ENTEROBACTERIACEAE* ISOLATED FROM URINE AT THE KENYATTA NATIONALHOSPITAL MICROBIOLOGY LABORATORY (P361/07/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 10th October 2013 to 9th October 2014.

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 **www.uonbi.ac.ke/activities/KNHUoN.**

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