UNIVERSITY OF CAPE COAST

MOLECULAR EPIDEMIOLOGY OF EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING ENTEROBACTERICEAE FROM THREE TERTIARY HOSPITALS IN SOUTHERN GHANA

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BY

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Thesis submitted to the Department of Microbiology and Immunology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, in partial fulfillment of the requirements for the award of Master of Philosophy degree in Infection and Immunity

JULY, 2018

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature …..... Date ……..

Name: Awulley Lelen Amina Emmanuela

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast

Supervisor's Signature ……... Date ……

Name: Dr. (Mrs.) Dorcas Obiri-Yeboah

Co-Supervisor's Signature …… Date ……

Name: Dr. Anthony Ablordey

ABSTRACT

Antimicrobial resistance is fast becoming a global concern in both healthcare settings and in the community due to the rapid emergence of multi drug resistant organisms. High proportions of enterobacteria have developed resistance to the commonly prescribed antimicrobial drugs. Comprehensive data upon which to advocate control interventions are scanty. Hence this study determined the Molecular Epidemiology of ESBL producing enterobactericeae from three Tertiary Hospitals in Southern Ghana. A total of 167 non repetitive isolates consisting of 51 *E. coli* strains, 43 *K. pneumoniae,* 16 *P. mirabilis,* 21 *P. aeruginosa* ,12 *A. baumannii* and 24 other unspecified organisms were collected and tested for their antimicrobial susceptibility, ESBL production by double synergy method and the ESBL genotypes were determined by PCR.

Major beta-lactams to which resistance was found in this study included Ampicillin (94.7%), Cefuroxime (81.6%), Cefamandole (71.9%), Ceftriaxone (69.4 %,), Augmentin (66.7%), Cefpodoxime (78.1%), Cefotaxime (78.9%). All these beta-lactams registered more than 50% resistance. ESBL percent prevalence were; 6.7% for *Acinetobacter baumannii* followed by 8.3% *Proteus mirabilis*, 15.0% *Pseudomonas aeruginosa*, 18.3% *Klebsiella pneumoniae* and 40% for *E. coli*. Other isolates recorded 11.7%. ESBL genotypes (TEM, SHV and CTX-M) were found in 118 out of 167 ESBLs phenotypically identified. The overall prevalence of ESBL detected was (71.51 %). The high prevalence of ESBL calls for immediate intervention strategies to prevent further spread. Training of laboratory personnel on phenotypic testing of ESBLs in addition to training clinical staff and prescribers on ESBL issues are advocated.

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DEDICATION

To my lovely father, the late Mr. Awulley Abdul Razack, and my beautiful mother Awulley Ayisha

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LIST OF ABBREVIATIONS

ESBL	-Extended Spectrum Beta Lactamase
TEM	- Temoniera
SHV	- Sulfhydryl Variable Active Site
CTX-M	- Cefotaxime mediated resistance
Bla	- Beta-lactamase gene

CHAPTER ONE

INTRODUCTION

Chapter one captures the introduction and background of Extended Spectrum Beta-lactamase producing Enterobacteria, including their definitions, types, biological activities, their mechanism of drug resistance and their fate in the environment. The problem statement gives an insight into how ESBL contributes to drug resistance in Ghana. The problem statement further indicates the need for surveillance and continues monitoring which will serve as a basis for the disease control and monitoring. The justification section under this chapter elucidates some problems caused by ESBL in Ghana and globally. The chapter also raises some specific objectives and research questions to be answered in this study.

Bacterial resistance to antibiotics is increasing globally in both healthcare settings and in the community and this remains a global health threat due to the rapid emergence and spread of multi drug resistant organisms. In recent times, susceptible bacteria pathogens such as members of the *enterobacteriaceae* family are spontaneously developing resistance to these first-line choice drugs used for treatment of severe infections. This is an issue of due public health concern (Sangare *et al*., 2017).

Background

The members of the Enterobacteriaceae are a large family of Gram-negative bacteria that include many harmless symbionts and many of the more familiar pathogens, such as *Salmonella typhi, Escherichia coli, Yersinia pestis, Klebsiella spp,* and *Shigella spp.* Other disease-causing bacteria in this family include *Serratia, E. cloacae, Proteus mirabilis*, and *Citrobacter freundii*,

usually resident in the gastrointestinal tract. This group of organisms are responsible for common illnesses and cause various hospital acquired infections like gastrointestinal, urinary tract and pyogenic infections (Giddi *et al*., 2017). Options for treatment of infections due to enterobacteriaceae include beta-lactam antibiotics (Penicillin; Cephalosporins; Carbapenems; and the Monobactam, Aztreonam) or those beta-lactam antibiotics combined with beta-lactamase inhibitors, quinolones, TMP-SMX, Aminoglycosides, and Tigecycline (Richards *et al*., 2000). Enterobacteriaceae produce many different beta-lactamase enzymes with some having the capability to hydrolyze only Penicillins and 1st, 2nd and 3rd generation Cephalosporins. Overuse of drugs have been identified as a major cause of resistance to β-lactams among Gram-negative bacteria globally, a basis of the emergence of Beta-lactamases (Shaikh *et al*., 2015) and has been increasingly detected in resource limited regions such as Africa.

Beta-lactamases are hydrolytic enzymes which cleave the beta-lactam ring by hydrolyzing the amide bond of the beta-lactam ring and are the primary mechanism of conferring bacterial resistance to beta-lactam antibiotics especially in Gram-negative bacilli (Dhillon & Clark, 2012; Chaudhary & Aggarwal, 2004). The genes that code for the beta-lactamase associated resistance can be carried on bacterial chromosomes, an inherent resistant property of the organism or may be plasmid-mediated with the potential to move between bacterial populations, a clear implications regarding spread of infection (Shoorashetty *et al*., 2011). Beta-lactamases in present times have been detected in numerous countries and are known to hydrolyze the extended-Spectrum Cephalosporins and the monobactams (Thenmozhi *et*

al., 2014) hence these new beta-lactamases were coined extended-spectrum – lactamases (ESBLs) (Sirot *et al*., 1987). ESBLs are therefore a group of beta-lactamases rapidly evolving which share the ability to hydrolyze third-generation cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (eg. aztreonam) but do not affect cephamycins (e.g., cefoxitin) or carbapenems (e.g., meropenem or imipenem) and can hydrolyze all penicillins yet are inhibited by clavulanic acid (Livermore, 2012).

Beta-lactamases are classified by two different schemes; according to structural homology (Ambler's classification) (Ambler, 1980) and their functional property (Bush's and Jacoby's classification) (Bush *et al.*, 1995). ESBLs belong to the Ambler molecular class A as well as the Bush-Jacoby functional group 2be (Bush & Jacoby, 2010). These enzymes have been identified in organisms in different geographical areas particularly in Enterobacteriaceae and are significantly detected in various *E. coli* strains. Major antibiotics to which resistance has been detected include the ampicillin, tetracycline and cotrimoxazole which are capsules or tablets. Also many ESBL producers have become multi-resistant to non-beta lactam antibiotics, comprising fluoroquinolones and aminoglycosides, sulfonamides which most often is encoded by the gene concealed by the same plasmids that determine the ESBL type (Hijazi *et al*., 2016; Sangare *et al*., 2017). The main ESBL-producing organisms isolated globally remain *Klebsiella pneumonia* and *Escherichia coli* (Giddi *et al*., 2017) but has also been identified in several other members of the enterobacteriaceae family and in certain non-fermenters. The acquisition and spread of the genes that code for beta-lactamase enzymes among gram-negative bacterial species together with opportunistic bacteria

has led to widespread resistance to many beta-lactam agents, which is becoming an increasingly significant burden on human health (Iredell J. *et al*., 2016).

Over the years, emergence of resistance to beta-lactam antibiotics has increased exponentially. This began even before the first beta- lactam, penicillin was developed and was firstly identified in *Escherichia coli* prior to its release for medical use (Abraham *et al*.,1940). The introduction of the third-generation cephalosporins into clinical practice due to the increased prevalence of ESBLs in the early 1980s, signified a major breakthrough in the fight against beta-lactamase-mediated bacterial resistance to antibiotics (Paterson & Bonomo, 2005). These have broad spectrum activity and typically are effective against most beta-lactamase-producing organisms and had the major advantage of lessened nephrotoxic effects compared to aminoglycosides and polymyxins.(Paterson & Bonomo, 2005; Hijazi *et al*., 2016)

ESBLs possess strong and ubiquitous selection pressure and have seemingly been accompanied by a shift from "natural" resistance, such as inducible chromosomal enzymes, membrane impermeability, and drug efflux, to the modern paradigm of mobile gene pools that largely determine the epidemiology of modern antibiotic resistance. An in-depth account on this resistance mechanisms to each class of antibiotics by ESBLs have been reported (Doddaiah & Anjaneya, 2014). Also, modulation of the phenotype by host bacteria makes gene transmission less obvious. This in a way explains why tracking and control of ESBLs resistance has been particularly problematic in the enterobacteriaceae (Iredell J *et al*., 2016). Due to the selective pressure employed by beta-lactam producing bacteria, some soil

organisms found in the environment currently exhibit drug resistance (Ghuysen, 1991). Gram-negative bacteria mostly possess naturally occurring chromosomally mediated beta-lactamases but ESBLs are mostly plasmid related and these ESBL gene-encoded plasmids can be transmitted beyond bacterial species (Shibasaki *et al*., 2016). Plasmids coding for ESBLs may also carry additional beta-lactamase genes as well as genes conferring resistance to other antimicrobial classes (Carattoli, 2009). This can limit the chemotherapeutic options for ESBL-producing pathogens and facilitate the intra and interspecies dissemination of ESBLs (Bush & Fisher, 2011;Zahar *et al*., 2009).

The first report of plasmid-encoded beta-lactamases which is able to hydrolyze the extended-spectrum cephalosporins was discovered in 1965 in *Escherichia coli* isolated from a patient named Temoniera in Greece hence designated TEM and published in 1983 (Datta & Kontomichalou, 1965). The presence of TEM 1 on various plasmids and its association with a transposon aided the spread to other bacteria within a few years after its isolation and is now located in different species of the family Enterobacteriaceae globally (Bonnet, 2004). Subsequently, other beta-lactamases were soon discovered which were closely related to TEM-1 and TEM-2. These also possess the ability to confer resistance to the extended-spectrum cephalosporins (Thenmozhi *et al*., 2014). Another common plasmid mediated β-lactamase SHV-1(named after the Sulfhydryl-variable active site), has been identified in *Klebsiella spp* and *Escherichia coli* (Kilebe *et al*., 1985). This gene encoding the beta–lactamase showed a mutation of a single nucleotide likened to the gene encoding TEM-1. In the early 1980s, a *Klebsiella ozaenae* isolate from

Germany passed a beta-lactamase SHV-2 which efficiently hydrolyzed cefotaxime and to a lesser extent ceftazidime (Kilebe *et al*.,1985). Recently another type of ESBL (CTX-M) has been described which preferentially hydrolyze cefotaxime over ceftazidime and also hydrolyze cefepime with high efficiency (Bonnet,2004;Bush,2014). The majority of isolated ESBL-producing Enterobactericeae from studies has been established to have multiple genes (*bla*TEM, *bla*SHV, and *bla*CTX-M), where CTX-M are the predominant and CTX-M-9 and CTXM-15 are the most widespread ESBL types (Paterson & Bonomo, 2005). It has been found that most ESBLs were derivatives of TEM-1 type, TEM-2 type and SHV-1 type of beta-lactamase. And these are composed of one or several point code gene mutations that alter the amino acid configuration around the active site of these beta-lactamases with CTX-M-type produced by *Proteus Mirabilis* also emerging in recent times (Thenmozhi *et al*., 2014; Song *et al*., 2011). This extends the spectrum of beta-lactam antibiotics susceptible to hydrolysis by these enzymes.

An increasing number of ESBLs not of TEM or SHV lineage have recently been described. There are more than 1,600 known beta-lactamases, a list that is rapidly expanding with TEM, SHV, and CTX-M-type mostly (Bush, 2014). In recent times over 100 clinical strains of CTX-M encoding genes have been located on plasmid. These commonly vary in size from 7kb-260kb with majority of these plasmids being IncFII plasmids, either alone or in association with Inc FIA and FIB (Carattoli *et al*., 2008).Plasmids encoding *bla*CTX-M-15 are found mainly in *Enterobacteriaceae* and named recently as plasmids of resistance responsible for outbreaks due to their capacity to acquire and transfer genes of resistance among bacteria (Hijazi *et al*., 2016).

Intestinal colonization by ESBL producing isolates may thus represent a reservoir for ESBLs in the community not detected in clinical isolates (Fernandez-Reyes *et al.*, 2014). In recent times, clinical impact of ESBL-producing pathogens on morbidity and mortality in infectious diseases in adults, as well as their economic burden has been documented to be on the increase and that gram negative organisms are the most common cause of serious bacterial infection in young infants (Lukac *et al.*, 2015).

The ESBL prevalence varies across Africa. This emerging threat has been pointed out in numerous studies within communities in Africa. In North Africa, it was detected to be 16.4–77.8%, the highest and least in South Africa (8.8–13.1%). In East Africa, studies report a prevalence ranging from 37.4 to 62.8% (Kittinger *et al*., 2016). Sub-Saharan Africa reports considerably high intestinal ESBL carriage rates between 10% and 45%. ESBL within population and in animals is on the increase due to overuse of antimicrobials in veterinary medicine (Kittinger *et al*., 2016). In Ghana, it was reported from the largest tertiary care hospital (Korle-Bu Hospital, Accra) that 50% of the *Klebsiella pneumoniae* and 29% of the *Escherichia coli* bloodstream isolates were ESBL producers. However, this study did not distinguish between hospital or community acquired strains and genotyping for these isolates were not performed (Obeng-Nkrumah *et al*., 2013). Feglo and Opoku established that there is high prevalence of AmpC- and ESBL- producing *P. aeruginosa* and *P. mirabilis* strains circulating in the Komfo Anokye Teaching Hospital and in the community with higher antimicrobial resistance than the non AmpC and ESBL strains (2014). Also recently, a study conducted at Komfo Anokye Teaching hospital with three other tertiary hospitals in the Northern belt

indicates the prevalence of ESBL production to be 57.8% among the isolates; a significantly high level (Adu, 2016). This means that more than half of the Enterobacteria isolates tested produced ESBL. This exponential global spread of ESBLs conferring resistance to the majority of beta-lactam antibiotics, including third-generation cephalosporins, constitutes a major public health threat in both health care and community settings (Friedrich *et al.*, 2016, European Centre for Disease Prevention and Control, 2015). Implementing infectious disease management globally especially in developing countries is also challenging due to intestinal colonization and globalization.

Options for treatment of these infections are generally limited, and given that fewer antibiotics are approved for use in children, the problem is critically important to address. This brings to the fore the significance of the phenotypic and genotypic detection of ESBLs among Enterobacteriaceae species; an important contraption for epidemiological purposes as well as for limiting the spread of resistance mechanisms. This study therefore aims at addressing the emergence of ESBL-producing isolates in some tertiary hospitals of the southern belt in Ghana and comparing the molecular epidemiology between these institutions for informed decision on healthcare and community based infection prevention.

Problem Statement

The rapid emergence of multidrug resistant bacteria strains occurring globally is of public health concern. A major cause is the acquisition or production of genes that code for the beta-lactamase by ESBLS contributing to drug resistance in both hospital and community settings (Bradford, 2001; Hijazi *et al*., 2016). Beta-lactams have broad spectrum activity, low toxicity,

cost effective and currently are the major choices for the treatment of severe infections in Ghana (Saana *et al*., 2014). Resistance to these antibiotics through beta lactamase production by enterobacteria such as *Escherichia coli*, *Klebsiella spp*. *Pseudomonas aeruginosa* and *Proteus mirabilis* etc. is growing and increases the risk of infectious diseases (Moy & Sharma, 2017; Pitout, 2010). This poses a major obstacle in the therapeutic outcome of patients resulting in a significant clinical challenges if remained undetected (Hijazi *et al*., 2016; Upadhyay *et al*., 2015).

The rise in ESBL producers results from over expression of the naturally occurring cephalosporinase or acquired beta-lactamases hence previously susceptible microorganisms are currently developing resistance to these beta-lactams through the possession of the ESBLs (Moy & Sharma, 2017; Rodriguez-ban & Paterson, 2006). Also many ESBL producers have become multi-resistant to some non-beta lactam antibiotics, comprising fluoroquinolones, aminoglycosides and sulfonamides which most often is encoded by the gene concealed by the same plasmids that determine the ESBL type (Hijazi *et al*., 2016). Increased drug abuse due to inefficient control systems is on the increase hence this era of ESBL resistance (Adu, 2016). Furthermore, there is a rising number of anthropogenic antibiotic resistant bacteria (ARB) outside the clinical setting (Viens, 2017; Kittinger *et al*., 2016). Antibiotics and ARB released from many different sources like hospital and industrial effluents, municipal waste and farm houses are flushed into surface waters. This results in an emerging number of ARB in the environment (Bouki *et al*., 2013).

In Ghana, there is scarcity of data on ESBLs, the available documented surveys demonstrated high prevalence of *Enterobacteriaceae* resistance to extended-spectrum cephalosporins and other non-beta-lactam antibiotics carried out in the Korle-Bu Teaching Hospital (KBTH) (Obeng-Nkrumah *et al*., 2013), and Komfo Anokye Teaching Hospital (KATH) (Feglo *et al*., 2013; Feglo & Opoku, 2014). The absence of routine surveillance and laboratory detection of beta-lactamases in many clinical laboratories in Africa, and Ghana in particular, further compounds the issues of antimicrobial resistance (AMR).

This study therefore is jeered towards determining the molecular epidemiology of extended-spectrum beta lactamases (ESBL) producing enterobacteriacae from three tertiary hospitals in Southern Ghana

Aim

To determine the molecular epidemiology of extended-spectrum beta lactamase (ESBL) producing enterobacteriacae from three tertiary hospitals in Southern Ghana.

Specific Objective

1. To identify bacteria isolates of the enterobacteriacae family and determine their antimicrobial susceptibility patterns.
2. To identify ESBL producing enterobacteriacae from these institutions in Ghana.
3. To determine the distribution of ESBL producing isolates in different patient care units at the various institutions.

4. To determine the prevalence of ESBL alleles among ESBL isolates from these institutions.

Significance of Study

Antibiotic resistance remains a major global health threat as previously susceptible bacterial pathogens have become resistant through acquisition of resistance mechanisms such as ESBLs, efflux etc. This era is significant because the pace of development of resistance is really outstripping the development of new drugs.

Beta-lactam antibiotics are the most important tools available for managing infectious diseases and are among the most often used antimicrobial agents. However an exponential increase of resistance to these drugs have been recorded globally especially in resource limited communities. This is of a great public health concern as beta lactamase producing organisms have shown resistance to other classes of antibiotics such as aminoglycosides, quinolones and cotrimoxazole resulting in narrowed therapeutic options. This causes increased mortality, higher treatment costs, disease spread, and increase duration of illness thereby posing significant challenge to clinical microbiologists, infection prevention and control practitioners, and clinicians in Africa.

In view of these, it is necessary to adopt immediate intervention strategies to prevent severe healthcare associated infections and to continuously monitor ESBL spread into the community (Adu, 2016).This also suggest the value for frequent surveillance to determine the circulating resistance strains to guide clinicians in their choice of antimicrobials to prescribe. The outcome of this study will contribute to the availability of a

local epidemiological data which will prove indispensable to patients' infection management whilst creating the necessary awareness on the clinical implications of beta-lactamase producing organisms in Ghanaian hospitals and communities. This will buttress the need for improved antimicrobial administration and inform public health interventions including routine beta-lactamase laboratory detection.

Delimitation

This study was carried out in three tertiary hospitals which could be extended but time constrains. Also the primers were chosen based common genes from previous study although there are other circulating ESBL genes.

Limitations

Due to financial constraints, sequencing and typing was not carried out. This could have helped to conclusively establish the epidemiology of these ESBLs. Despite these limitations the study achieved its objectives and recommendations were laid down.

Organization of the Study

The study is organized into five chapters. Chapter one is introduction which covers the background of the study, problem statement, objectives of the study, justification of the study, scope of the study and research questions. Chapter two focuses on the literature review, issues reviewed among others include the prevalence circulating ESBL genes in Ghana, the prevailing ESBL types and diversity, the common beta-lactams to which resistance is developing.

The present work is primarily towards determining the molecular epidemiology of these Extended spectrum beta-lactamase. Chapter three

provides the methodology. It deals with study area, sampling techniques, sample collection and instrumental analysis, analysis of results by statistical tools such as excel and SPSS. Chapter four includes results and discussion of the demographic characteristics of the study population, the antimicrobial susceptibility profiles of the organisms towards the drugs and the ESBL prevalence of the genotype.

Chapter five provides the summary of the research, conclusions and recommendations for further studies.

Summary

The production of ESBLs in Enterobacteria in clinical samples has contributed to prolonged hospital stay, increased hospital expenses and psychological stress on both the patient and relative. A recent report from some referral hospitals in Ghana indicate a high prevalence of ESBL genes circulating in Ghana. This enormous drug resistance towards the commonly used cephalosporins necessitates the development of routine infection prevention and control measures.

CHAPTER TWO

LITERATURE REVIEW

This chapter reviews several researches that has been done over the period concerning some enterobacteria, their mechanisms of drug resistance and the various ESBL genes responsible for these resistance. The enterobacteriacae are a large family of Gram-negative bacteria, inconsequentially referred to as enterobacteria or "enteric bacteria", as a number of these members live in the intestines of animals that include many harmless symbionts and many of the more familiar pathogens; *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella*, and *Shigella*. Other disease-causing bacteria in this family include *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter* (Jantsch, Chikkaballi, & Hensel, 2011).

Members of the Enterobacteriaceae are rod-shaped, typically 1–5 μm in length and appear as medium to large-sized grey colonies on blood agar, although some can express pigments (such as Serratia marcescens), facultative anaerobes, not spore-forming, mostly flagellated, ferments lactose to produce lactic acid and various other end products. Most also reduce nitrate to nitrite, although exceptions exist (e.g. *Photorhabdus*) with varied catalase reactivity (Fabrega &Vila, 2013;Ryan, 2004). Unlike most similar bacteria, Enterobacteriaceae generally lack cytochrome C oxidase, although there are exceptions (e.g. *Plesiomonas shigelloides*). Most members have peritrichous, type I fimbriae which is involved in the adhesion of the bacterial cells to their hosts. Some enterobacteria produce endotoxins and endotoxins which are released when the cell dies and the cell wall disintegrates, some when released into the bloodstream following cell lysis, cause a systemic inflammatory and

vasodilatory response. The most severe form of this is known as endotoxic shock, which can be rapidly fatal (Fabrega & Vila, 2013).

Escherichia coli

Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is usually found in the lower intestine of warm-blooded organisms (endotherms) (Tenaillon *et al*.,2010) . Most *E. coli* strains are innocuous forming part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 and preventing colonization of the intestine with pathogenic bacteria through symbiotic relationship. On the other hand, some serotypes cause serious food poisoning in their hosts, and are occasionally responsible for food contamination (Singleton, 1999). *E. coli* is a chemo heterotroph whose chemically defined medium must include a source of carbon and energy (Tortora, 2010) .*E. coli* and related bacteria possess the ability to transfer DNA via bacterial conjugation or transduction. This allows horizontal gene transfer through an existing population by employing the bacterial virus, a bacteriophage (Brüssow *et al*.,2004)

Several hundreds of complete genomic sequences of *Escherichia* and *Shigella* species have been documented. Comparison of these sequences shows a remarkable amount of diversity; only about 20% of each genome represents sequences present in every one of the isolates, while around 80% of each genome vary among isolates (Lukjancenko *et al*., 2010). According this research, each individual genome contained between 4,000 and 5,500 genes, but the total number of different genes among all of the sequenced *E. coli* strains (the pangenome) exceeded 16,000. This very large variety of

component genes was interpreted to mean that two-thirds of the *E. coli* pangenome originated in other species and arrived through the process of horizontal gene transfer (Zhaxybayeva & Doolittle, 2011). In February, 1997, the first complete DNA sequence of an *E. coli* genome (laboratory strain K-12 derivative MG1655) was published. It was found to be a circular DNA molecule 4.6 million base pairs in length, containing 4288 annotated protein-coding genes (organized into 2584 operons), seven ribosomal RNA (rRNA) operons, and 86 transfer RNA (tRNA) genes (Zhaxybayeva & Doolittle, 2011). Regardless of having been the subject of intensive genetic analysis for about 40 years, a large number of these genes are still unknown.

Klebsiella

Klebsiellae are non-motile, rod-shaped, Gram-negative bacteria with a prominent polysaccharide capsule which encases the entire cell surface resulting in the organism's large appearance on Gram stain and provides resistance against many host defense mechanisms (Ryan, 2004). Members of the *Klebsiella* genus typically express 2 types of antigens on their cell surface; O antigen (a lipopolysaccharide) and K antigen (a capsular polysaccharide). Both of these antigens contribute to pathogenicity. There are about 77 K antigens and 9 O antigens exist. The structural variability of these antigens forms the basis for classifying these bacteria into various serotypes but with similar virulence (Brisse *et al*.,2004; Shahab, 2017).

Three species in the genus *Klebsiella* are associated with illness in humans: *Klebsiella pneumoniae, Klebsiella oxytoca,* and *Klebsiella granulomatis*. Organisms previously known as *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis* are considered non-fermenting subspecies of *K*

pneumoniae that have characteristic clinical manifestations. With those exceptions, strains within this genus ferment lactose, most produce highly mucoid colonies on plates because of the production of a luxuriant polysaccharide capsule, and all are non-motile (Mandell, 2009). In recent years, *Klebsiella* have become important pathogens in healthcare associated infections commonly incriminated at sites including the urinary tract, lower respiratory tract, biliary tract, and surgical wound sites (Podschun & Ullmann, 1998). The spectrum of clinical syndromes includes pneumonia, bacteremia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, and meningitis (Nordmann *et al*., 2009; Ristuccia & Cunha, 1984)

Drug-resistant *Klebsiella* isolates remain an important hospital-acquired bacterial pathogen resulting significantly to prolonged hospital stays especially in high-impact medical areas such as intensive care units. This antimicrobial resistance is thought to be attributable mainly to multidrug efflux pumps (Ogawa *et al*., 2005). In addition, its ubiquitous nature and the ability to colonize the hospital environment, including carpeting, sinks, flowers, and various surfaces (Podschun & Ullmann, 1998), as well as the skin of patients and hospital staff, has been identified as a major factor in the spread of hospital-acquired infections (Jadhav *et al.*, 2012).

Salmonella

Salmonella are enterobacteria with cell diameters between about 0.7 and 1.5 µm, lengths from 2 to 5 µm, and peritrichous flagella (all around the cell body), mostly chemotrophs, obtaining their energy from oxidation and reduction reactions using organic sources. They are also facultative anaerobes,

able to generate ATP with oxygen when available employs other electron acceptors or fermentation anaerobically (Fabrega & Vila, 2013). Most subspecies of *Salmonella* produce hydrogen sulfide, which can readily be detected by growing them on media containing ferrous sulfate, such as is used in the triple sugar iron test (Hardy, 1999). These bacteria are not destroyed by freezing (Sorrells, Speck, & Warren, 1970) but UV light and heat at 55 °C (131 °F) for 90 min, or to 60 °C (140 °F) for 12 min (Beuchat & Heaton, 1975). This organism consists of two specie; *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is the typed species and is further divided into six subspecies (*S. e. enterica*, *S. e. salamae*, *S. e. arizonae*, *S. e. diarizonae*, *S. e. houtenae*, and *S. e. indica*. and include over 2,500 serotypes (defined on the basis of the somatic O, a lipopolysaccharide and flagellar H antigens by the Kauffman–White classification) (Gillespie & Hawkey, 2006; Su & Chiu, 2007). These serotypes can be divided into two main groups—typhoidal (include *Salmonella* Typhi and *Salmonella* Paratyphi A, which are adapted to humans and do not occur in other animals) and non-typhoidal *Salmonella* (usually causing self-limiting gastrointestinal disease and are the commonest). They usually cause typhoid fever, paratyphoid fever, *Salmonella* food poisoning of which symptoms resolve without antibiotics. However, in sub-Saharan Africa they can be invasive and cause paratyphoid fever. This can lead to life-threatening hypovolemic shock and septic shock and requires intensive care including antibiotics (Ryan, 2004). About 2,000 serotypes of non-typhoidal *Salmonella* are currently known a list that is continuously expanding, which may be responsible for as many as 1.4 million illnesses in the United States each year (Goldrick, 2003).

Mechanisms of infection differ between typhoidal and non- typhoidal serotypes. Non typhoidal serotypes preferentially enter M cells on the intestinal wall by bacterial-mediated endocytosis, a process associated with intestinal inflammation and diarrhoea and are also able to disrupt tight junctions, impairing the cells' ability to stop the flow of ions, water, and immune cells into and out of the intestine. This significantly contribute to the induction of diarrhoea (Haraga, Ohlson, & Miller, 2008). On the other hand, typhoidal serotypes are able to breach the intestinal barrier through phagocytosis and trafficking by CD18-positive immune cells and also are able to enter macrophages via macropinocytosis, factors that contribute towards achieving dissemination throughout the body via the mononuclear phagocyte system. These factors results in the low infective dose of this serotype (Haraga *et al*., 2008; Kerr *et al*., 2010). Furthermore a hallmark of *Salmonella* pathogenesis is due to resistance to oxidative burst, an ability of the bacterium to survive and proliferate in the presence of DNA damaging agents such as nitric oxide and oxygen radicals within phagocytes. These factors were documented indicating that mutants of *Salmonella enterica* lacking RecA or RecBC protein function are highly several articles sensitive to oxidative compounds synthesized by macrophages. Furthermore these findings added that successful systemic infection by *S. enterica* requires RecA and RecBC mediated recombinational repair of DNA damage (Buchmeier *et al*., 1993; Cano *et al*., 2002).

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram-negative bacterium of the member, Gamma proteobacteria class of bacteria, a non-fermenting, motile,

belonging to the Pseudomonadaceae family. This infectious bacterium has a rod-shaped and blue-green pigmented bacterium (Shovarani, 2008). *P. aeruginosa* is a multifaceted microorganism, ubiquitously distributed in terrestrial, aquatic, animal, human, and plant environments as well as a non-fastidious organism with a wide range of growth substrate and minimal nutrient requirements (Favero *et al.*, 1971; Sousa & Pereira, 2014). In addition, this bacterium is able to proliferate in temperatures as high as 50ºC and is capable of growing under aerobic conditions, as well as anaerobic conditions (Van Hartingsveldt & Stouthamer, 1973). There are a number of clinical diseases associated with *P. aeruginosa* infection. However, *P. aeruginosa* is an opportunistic organism infecting burns and causing pneumonia, otitis media, keratitis, gastrointestinal infections, cystic fibrosis, leukemic, transplant, neutropenic, long-term urinary catheters, and diabetic and immune compromised patients as well intravenous drug abusers (Alhazmi, 2015; Sousa & Pereira, 2014).

A bothersome characteristic of *P. aeruginosa* is its varied intrinsic resistance leading to low antibiotic susceptibility. This is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (e.g., *mexAB*, *mexXY*, etc.) and the low permeability of the bacterial cellular envelopes (Poole, 2004).This bacteria easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants. In 2008, a study have shown phenotypic resistance associated to biofilm formation in *P. aeruginosa* (Cornelis, 2008). These Mechanisms underlying antibiotic resistance have been found to include production of antibiotic-degrading or

inactivating enzymes, outer membrane proteins to evict the antibiotics and mutations to change antibiotic targets for example methylation of 16S rRNA to prevent aminoglycoside binding and modification of DNA, or topoisomerase to protect it from the action of quinolones. Also the presence of antibiotic-degrading enzymes such as extended-spectrum β-lactamases like PER-1, PER-2, VEB-1, AmpC cephalosporinases, carbapenemases like serine oxacillinases, metallo beta-lactamases, OXA-type carbapenemases, aminoglycoside-modifying enzymes, among others have been reported. *P. aeruginosa* has also been reported to possess multidrug efflux pumps like AdeABC and AdeDE efflux systems that confer resistance against number of antibiotic classes (Wong *et al*., 2012).

Proteus mirabilis

Proteus mirabilis is motile, facultatively anaerobic, Gram-negative rods belonging to the Enterobacteriaceae family with a significant characteristic of swarming on a growth medium (Ryan, 2004). *Proteus* species usually are non-lactose fermenters, but have shown to be capable of fermenting glucose depending on the species in a triple sugar iron (TSI) test. Since it belongs to the family Enterobacteriaceae, general characters are applied on this genus. It is oxidase-negative but catalase- and nitrate-positive. Specific tests include positive urease by producing urease to generate ammonia (Mobley & Belas, 1995; Rozalski *et al.*, 1997) (which is the fundamental test to differentiate *Proteus* from *Salmonella*) and phenylalanine deaminase tests (Jantsch *et al.*, 2011). These microorganisms are opportunistic pathogens, commonly responsible for urinary and septic infections, often healthcare associated infections. *Proteus mirabilis* comparable to *P.*

aeruginosa, is also a common cause of both community-acquired and catheter-associated UTI causing bacteremia, cystitis, pyelonephritis, prostatitis, wound infections, and burn infections, as well as respiratory tract infections, endophthalmitis, meningitis, and meningoencephalitis (Adler *et al.*, 2013; Lu *et al.*, 1999).

Antibiotics and Antibiotic Resistance

Antibiotics (also known as antibacterials) are a type of antimicrobial drug used in the treatment and prevention of bacterial infections .These chemical substances can either be bacteriostatic or bactericidal depending on the bacterial growth phase, and often requires ongoing metabolic activity and division of bacterial cells (Mascio *et al.*, 2007). Some sources distinguish between antibacterial and antibiotic; antibacterials are used in soaps and disinfectants, while antibiotics are used as medicine. The first antibiotic produced was penicillin, discovered accidentally from a mold culture. Today, over hundreds of different antibiotics are available to cure minor, and life-threatening infections and this has revolutionized medicine in the 20th century (Gualerzi, Brandi, Fabbretti, & Pon, 2013). Antibiotics are among the most frequently prescribed medications in modern medicine. However, their effectiveness and easy access have also led to their overuse (Rollins *et al.*, 2016), prompting bacteria to develop resistance (Brooks, 2015). This widespread problems, prompted the World Health Organization to classify antimicrobial resistance as a "serious threat [that] is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country"(WHO, 2014).

Classification of antibiotics

There are various ways of classifying antibiotics. These classification scheme mostly are based on mechanism of action, spectrum of activity with the majority targeting bacterial functions or growth processes and their chemical structure (Calderon & Sabundayo, 2007). Based on their mechanism of action, they are categorized as bactericidal; those that target the bacterial cell wall (penicillins and cephalosporins) or the cell membrane (polymyxins), or the ones that interfere with essential bacterial enzymes (rifamycins, lipiarmycins, quinolones, and sulfonamides). Protein synthesis inhibitors (macrolides, lincosamides and tetracyclines) are usually bacteriostatic (with the exception of bactericidal aminoglycosides). Over 40-year discontinuity in discovering new classes of antibacterial compounds, four new classes of antibiotics have been brought into clinical use in the late 2000s and early 2010s: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), oxazolidinones (such as linezolid), and lipiarmycins (such as fidaxomicin) (Calderon & Sabundayo, 2007). Some common classes of antibiotics based on chemical or molecular structures include Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides and Oxazolidinones (Adzitey, 2015; Frank & Tacconelli, 2012; van Hoek *et al.*, 2011).

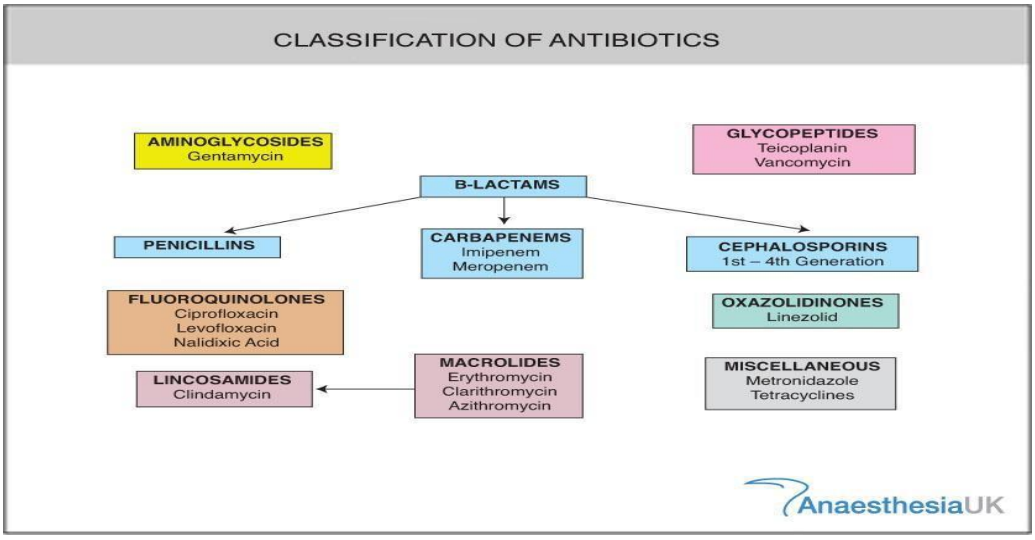

Figure 1: Classification of antibiotics

Beta-lactam antibiotics

Beta-lactam antibiotics are a large group of antibiotics, uniquely named due to the beta-lactam ring in their chemical structure. Members of this class of antibiotics contain 3 carbon and 1-nitrogen ring that is highly reactive (Tidwell, 2008). The beta-lactam antibiotics function by inhibiting transpeptidation, the last step in peptidoglycan synthesis hence interfering with the structural cross-linked latticework structure of peptidoglycans in bacterial cell walls (Gilbert, 2009). The lethality of penicillin for bacteria appears to comprise both lytic and non-lytic mechanisms. Penicillin's interference of the balance between PBP-mediated peptidoglycan assembly and murein hydrolase activity results in autolysis whiles the non-lytic killing by penicillin may involve holin-like proteins in the bacterial membrane that collapse the membrane potential (Bayles, Taylor, Bates, Hilton, & Law, 2001). The spectrum of action is against many gram-positive, gram-negative and anaerobic organisms. Beta-lactams include the penicillins and its derivatives, cephalosporins (cephems), monobactams and carbapenems (Figure 2.2-2.4). Because many of these drugs are well absorbed after oral administration, they

are clinically useful in the outpatient setting and are one of the most effective and commonly used agents in the treatment of infectious diseases (Byarugaba, 2010; Holten & Onusko, 2000). Beta-lactams are used globally. According to Livermore and Woodford (2006), beta-lactam antibiotics constitute about 60% of the worldwide antibiotic usage. Bacteria, in an attempt to survive, show resistance to these antibiotics via the production of beta-lactamases (Rice, 2012). However, the distribution of these enzymes responsible for resistance to oxyimino-cephalosporins varies (Miro *et al.*, 2005; Nordmann *et al.*, 2009).

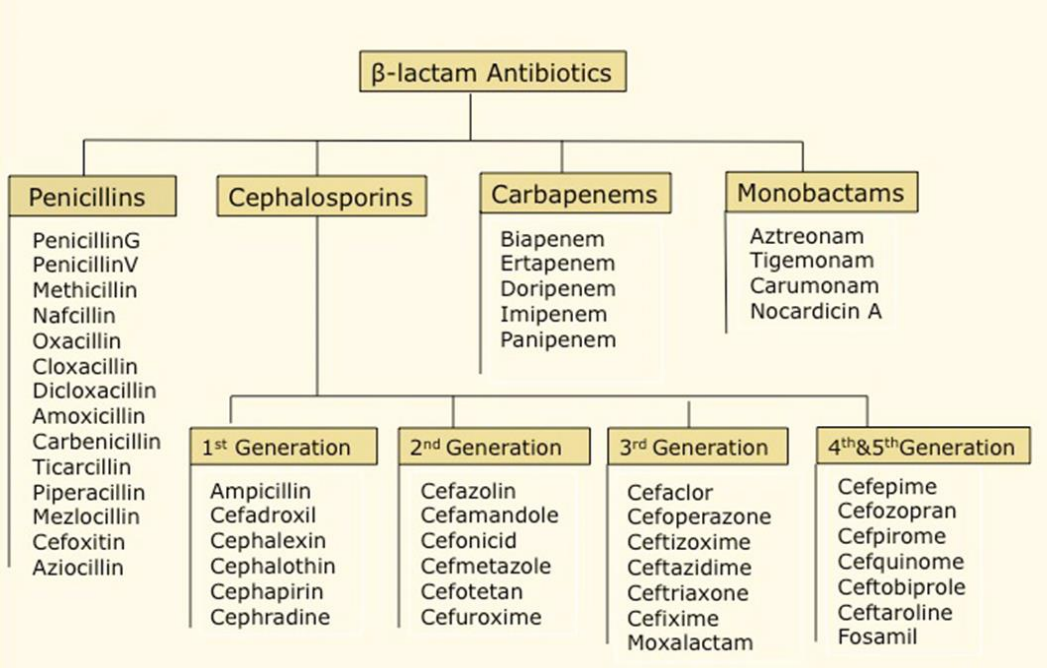

Figure 2: Classification of beta lactams (Kiiru *et al.*, 2012; Paterson & Bonomo, 2005).

Beta lactamase

Beta- lactamases also known as penicillinases are hydrolytic enzymes which cleave the beta-lactam ring of penicillin and other penicillinases-susceptible compounds into inactive penicilloic acid are the primary mechanism of conferring bacterial resistance to beta-lactam antibiotics, such as penicillins and cephalosporins. These enzymes can be carried on bacterial chromosomes, that is, inherent to the organism, or may be plasmid-mediated with the potential to move between bacterial populations. The beta-lactamases are an extremely diverse group of enzymes, and it has been shown that small amino acid sequence differences can make significant functional impacts, either in the substrate spectrum, the rate of hydrolysis, or the ability to be inhibited by clinically used beta-lactamase inhibitors (Mshana, 2011). Beta-lactamases can be classified according to two general schemes; the Ambler molecular classification scheme and the Bush-Jacoby-Medeiros functional classification system. The Ambler schemes rests upon the basis of protein homology and not phenotypic characteristics and divides beta-lactamases into four major classes: A to D. Class A, C and D are serine beta- lactamase while class B is metallo beta-lactamases. The Bush-Jacoby Medeiros scheme groups these enzymes according to functional similarity, substrate and inhibitor profile (Jacoby & Medeiros, 1991; Bush *et al*., 1995). In clinical practice and research, the Bush-Jacoby Medeiros classification scheme is of more relevance to physicians or microbiologists in diagnostic laboratory because it considers beta-lactamase inhibitor and beta-lactam substrates that are clinically relevant.

Table 1: Modified Bush –Jacoby Medeiros Classification of β-lactamases (Bush & Jacoby, 2010)

Functional group	Substrate profile	Molecular Class	Inhibitor	Example
1	Cephalosporinase	C	OXA	AmpC,MIR-1
2a	Penicillinase	A	Clav	*S.aureus*
2b	Broad spectrum	A	Clav	Tem-1/2,SHV-1
2be	Extended	A	Clav	Tem-3-29,Tem-46,Tem 104, SHV 2-28, CTX-M types
2br	Inhibitor resistant	A	-	Tem-30-41(IR 1-12)
2c	Carbenicillinase	A		AER-1 (C), CARB-3
2d	Oxacillinase	D	Clav	PSE-1
2e	Cephalosporinase	A	Clav	OXA-1, OXA-2,10
2f	Carbepenemase		Clav	IPM-1,NmcA, Smc1-3
3	Metalloenzymes	A	-	*S. maltophilia*
4	Penicillinase	B		*B. cepacia* (c)

Definition and classification of ESBLs

ESBLs are enzymes capable of conferring bacterial resistance to penicillin, first, second and third generation cephalosporins and aztreonam but not the cephamycins or carbapenems (Mshana, 2011). They have the ability to hydrolyze these antibiotics but are inhibited by β-lactamase inhibitors such as clavulanic acid (Bush *et al.*, 1995). The common ESBLs are mostly the Bush-Jacoby-Medeiros group 2be and those of group 2d which share most of the fundamental properties of group 2be enzymes (David & Bonomo, 2005). The group 2b enzymes hydrolyze penicillin and ampicillin, and to a lesser degree carbenicillin or cephalothin but are unable to hydrolyze extended-spectrum cephalosporins or aztreonam to any significant degree (Aktas *et al.*, 2002). Over time, it has been observed that the ESBLs derived from TEM-1, TEM-2,

or SHV-1 differ from their progenitors by as few as one amino acid. This results in a profound change in their enzymatic activity hence can now hydrolyze the third-generation cephalosporins or aztreonam. This activity results in the extension of spectrum compared to the parent enzymes (Aktas *et al*., 2002). According to the classification scheme of Ambler, with the exception of OXA-type enzymes (which are class D enzymes), the ESBLs are of molecular class A, and are able to hydrolyze the penicillins, narrow-spectrum and third-generation cephalosporins, and monobactams (Jacoby & Medeiros, 1991). They hydrolyze these antibiotic at least 10% that for benzylpenicillin but inhibited by clavulanic acid. This property differentiates the ESBLs from the AmpC-type β -lactamases (group 1) produced by organisms such as *Enterobacter cloacae* which have third-generation cephalosporins as their substrates but which are not inhibited by clavulanic acid (Bush *et al*., 1995). Extension of the spectrum of OXA-type β -lactamases (group 2d) towards the extended-spectrum cephalosporins has recently been observed, and regarded by many authorities as ESBLs (Medeiros, 1997).

ESBLs types and diversity

Temoniera (TEM)

These type of ESBLs are derivatives of TEM-1 and TEM-2. TEM -1 was first reported from the patient named Temoniera in 1965 hence the designation TEM (Datta & Kontomichalou, 1965). TEM-type enzymes which are less susceptible to the effects of beta-lactamase inhibitors have negligible hydrolytic activity against the extended-spectrum cephalosporins and are not considered ESBLs.TEM-1, 2 and 13 are not Extended Spectrum Beta-Lactamases. This is because TEM-1 though can hydrolyze ampicillin at

greater extent than oxacillin, carbenicillin or cephalothin and is inhibited by clavulanic acid, cannot hydrolyze extended spectrum cephalosporins such as ceftriaxone, cefotaxime, ceftazidime (Mshana, 2011). TEM-2 also has the same hydrolytic profile as TEM-1, but possesses more active native promoter and different isoelectric point of 5.6 compared to 5.4 of TEM-1. TEM-13 on the other hand exhibits a similar hydrolytic profile as TEM-1 and TEM-2 hence is considered non-ESBL. (Jacoby & Medeiros, 1991).

Currently over 100 TEM-type β-lactamases have been described of which most of them are ESBLs (http://www.lahey.org/Studies/temtable.asp). Their isoelectric points range from 4.2 to 6.5. Nevertheless, research has shown a unique TEM-derived enzyme, TEM-AQ, found in Italy. This enzyme has an amino acid deletion not seen in other TEM enzymes plus several amino acid substitution (Perilli *et al*., 1997).Also, interesting mutants of TEM -lactamases are being recovered that maintain the ability to hydrolyze third-generation cephalosporins but which also demonstrate inhibitor resistance. These are referred to as complex mutants of TEM (CMT-1 to -4)(Poirel *et al*.,2004)**.**

Sulfhydryl variable (SHV)

SHV refers to Sulfhydryl variable. This designation was made because it was thought that the inhibition of SHV activity by *p*-chloromercuribenzoate was substrate-related, and was variable according to the substrate used for the assay but never confirmed in subsequent studies (Sykes & Bush., 1982). The first SHV that hydrolyze extended spectrum β-lactam antibiotics was isolated from *Klebsiella ozaenae* in 1983 in Germany. This enzyme was found to differ with parent enzyme SHV-1 by replacement of glycine with serine at 238th

position and was designated SHV-2 (Kilebe, C. *et al*., 1985).The SHV types, used to be more frequently found in clinical isolates than any other type of ESBL. Within 15 years of the discovery of this enzyme, organisms harboring SHV-2 were found in every inhabited continent. This implies that selection pressure from third-generation cephalosporins in the first decade of their use was responsible for this variation (Paterson *et al*., 2003). SHV types of ESBLs have been detected in a wide range of enterobacteriaceae and outbreaks of SHV – producing *Pseudomonas spp* and *Acinetobacter spp* have been reported (Nuesch-Inderbinen *et al*., 1997). Contrasting TEM-type β-lactamases, there are few derivatives of SHV-1; more than 50 SHV varieties have been defined globally(Ulises Garza-Ramos & Esperanza Martínez-Romero, 2007).

Cefotaximase (CTX-M)

This is a recently described family of ESBLs. The name CTX reflects the potent hydrolytic activity of these beta-lactamases against cefotaxime and also cefepime with high efficiency but with minimal effect on ceftazidime. Organisms producing CTX-M-type beta-lactamases typically have cefotaxime MICs in the resistant range of 64 g/ml, while ceftazidime MICs susceptible range of 2 to 8 g/ml (Alobwede *et al*., 2003).Conversely, Tazobactam exhibits a better inhibitory effect towards CTX-M than sulbactam and clavulanate (Reynaud, Péduzzi, Barthélémy, & Labia, 1991)(Reynaud *et al*., 1991). Aztreonam MICs are variable. CTX-M type has been reported in most parts of the world, and it is believed that it might be the most frequent type of ESBLs in the world. Genes for these enzymes are positioned on the plasmids generally ranging from 7-260kb of size and are known to be acquired from

chromosomes of *Kluyvera spp* (Reynaud *et al*., 1991;Humeniuk *et al*., 2002). Studies currently indicate that more than 113 CTX-M varieties are known with the *bla*CTX-M-15 allele is considered to be predominant in many countries (http://www.lahey.org/Studies/other.asp).

Remarkably, identical beta-lactamases have been discovered in widely separated parts of the world (Mshana, 2011). Toho beta-lactamase discovered at the University School of Medicine Omori Hospital in Tokyo, where a child was hospitalized has recently been discovered. Toho-1 beta- lactamase discovered from *Escherichia coli* and Toho-2 are beta-lactamases related structurally to CTX-M-type beta-lactamases (Labia, 1999). Like most CTX-M-type -lactamases, the hydrolytic activity of the Toho-1 and Toho-2 enzymes is more potent against cefotaxime than ceftazidime (Ma *et al*., 1998)(Ma *et al*., 1998). Clonal spread of CTX-M-type -lactamase producing bacteria has been well-documented (Gniadkowski *et al*., 1998).

Oxacillinase (OXA) Beta lactamases

These classes of β-lactamases are so named because of their ability to hydrolyze oxacillin. They predominantly occur in *Pseudomonas spp*, but have been detected in many other gram negative bacteria (Bedenic *et al*.,2001). OXA β-lactamases are characterized by their ability to hydrolyze cloxacillin and oxacillin 50% more than benzyl penicillin but mostly do not hydrolyze extended spectrum cephalosporins to a significant degree hence are not ESBLs. OXA-10 weakly hydrolyze cefotaxime, ceftriaxone and aztreonam. Other OXA ESBLs derived from OXA-10 includes OXA-14, 16, 15, 18, 19, 28, 31, 32, and (Boras *et al*., 2001).

PER, VEB-1, BES-1, and Other ESBLs

A variety of other -lactamases which are plasmid-mediated or integron-associated class A enzymes have been recently discovered (Silva *et al.*, 2000).These types include PER 1&2, VEB-1&2, GES, SFO and IBC. The PER-type ESBLs share about 25 to 27% homology with known TEM- and SHV-type ESBLs. PER-1 was first detected in *Pseudomonas aeruginosa* (Neuhauser *et al.*, 2003) and later in *Salmonella enterica* serovar Typhimurium and *Acinetobacter* isolates. PER-2,which shares 86% homology to PER-1, has also been detected in *S.enterica* serovar Typhimurium, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, and *Vibrio cholerae* O1 El Tor (Petroni *et al*., 2002) PER-2 has only been found in South America so far (Bauernfeind *et al*., 1996) .

Other ESBLs types include VEB-1 & 2, GES, SFO and IBC. VEB-1 has greatest homology (38%) with PER-1 and PER-2 and confers higher level resistance to ceftazidime, cefotaxime and aztreonam, which is reversed by clavulanic acid (Bedenic *et al*., 2001). It was first isolated from a Vietnamese child hospitalized in France and also plasmid mediated (Randegger *et al.*, 2001). Other VEB enzymes have been described in Kuwait and China (Bell, *et al*., 2002). GES, SFO and IBC are examples of non-TEM, non –SHV ESBLs and have been found in a wide range of geographical locations (Ben *et al.*, 1990) .

Antimicrobial resistance mechanisms in ESBLs

Antimicrobial resistance is the ability of bacteria or other microbes to resist the effects of an antibiotic by adjusting in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to

cure or prevent infections. These bacteria survive and continue to multiply, causing more harm (Ventola, 2015). The persistence of bacteria to antibacterials often results from an inheritable resistance and also through acquisition which is likely to occur in locations of frequent antibiotic use (Dyer, 2003; Witte, 2004).Conversely, resistance can be obtained through biochemical process (antimicrobial inactivation) by hydrolysis [such as in ESBLs where the amide bond is targeted and cleaved off (Breton *et al.*, 2004)], redox process[through oxidation and reduction reaction of antibiotics. For instance the oxidation of tetracycline antibiotics by the TetX enzyme (Yang *et al*., 2004)], group transfer [where these enzymes inactivate antibiotics (aminoglycosides, chloramphenicol, streptogramin, macrolides or rifampicin) by chemical substitution (adenylyl, phosphoryl or acetyl groups are added to the periphery of the antibiotic molecule), target modification (Spratt, 1994) and through genetic modifications via mutations [like the mutations of the sequences of genes encoding the target of certain antibiotics (e.g. resistance to rifampicin and fluoroquinolones is caused by mutations in the genes encoding the targets of these molecules, RpoB and DNA-topoisomerases, respectively) (Ruiz, 2003)] and finally also via horizontal transfer (Woodford, Turton, & Livermore, 2011).

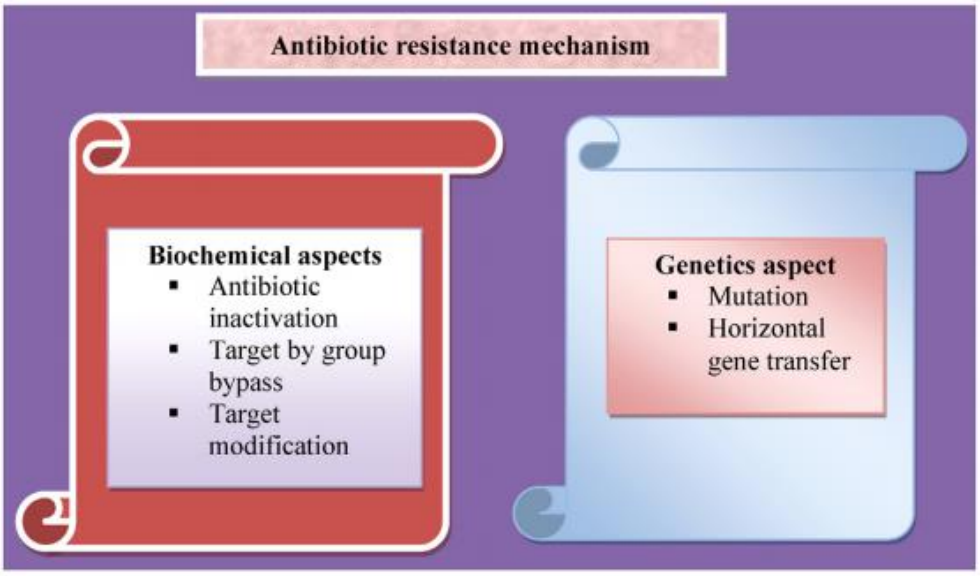

Figure 3: Biochemical and genetic aspects of antibiotic resistance mechanisms (Shaikh *et al*., 2015)

Rather than providing an extensive list of antibiotic resistance mechanisms of the various classes of drugs (which can be found elsewhere [Alekshun & Levy, 2007]), the common genetic and molecular mechanisms of resistance as related to ESBLs will be highlighted. Several of these molecular mechanisms of antibacterial resistance exist; intrinsic and acquired.

Intrinsic antibacterial resistance is as a result of the genetic makeup of bacterial strains and is due to lack of target sites or their ability to be naturally become impervious to the drugs mostly facilitated by their porin proteins (outer membrane) hence unaffected by them. This mechanism is mostly encoded in the microorganism's chromosome (Byarugaba, 2010; Strateva & Yordanov, 2009). In some ESBLs (*P. aeruginosa)*, the expression of membrane efflux pumps also facilitate resistance (Strateva & Yordanov, 2009).

Acquired resistance is due to the mutations in the bacterial chromosome or the acquisition of extra-chromosomal DNA (Alekshun & Levy, 2007; Pawlowski *et al.*, 2016). This acquired mechanisms involve mutations in genes targeted by the antibiotic and the transfer of these resistance determinants borne on plasmids, bacteriophages, transposons, and other mobile genetic material and the expression of the resistant gene taken up by the bacterium; leading to resistance in a naturally susceptible microorganism due to genetic transfer (Breidenstein *et al.*, 2011; Alekshun & Levy, 2015). Plasmids and transposons are examples of elements of DNA that can be passed from among bacteria spp. via transformation, transduction and conjugation which confer resistance to a wide range of antibiotics (Gellatly & Hancock, 2013). The spread of antibacterial resistance in ESBL producing microbes is mostly plasmid mediated and often occurs through vertical transmission of mutations during growth and by genetic recombination of DNA by horizontal genetic exchange (Gyles & Boerlin, 2014; Witte, 2004). Plasmids that carry several different resistance genes can confer resistance to multiple antibacterials. In addition, cross-resistance to several antibacterials may also occur when a resistance mechanism encoded by a single gene conveys resistance to more than one antibacterial compound (Baker-Austin *et al.*, 2007).

In addition to the above, environmental conditions can result in adaptability (adoptive resistance) of organisms to drugs. This occurs when environmental conditions such as exposure to low-dose antibiotic concentrations, swarming motility, biofilm formation, ion exchange as in *P. aeruginosa* causes resistance to cationic antimicrobial peptides (McPhee *et al.*,

2003). Also over-prescription and inappropriate use of antibiotics, availability of few new drugs, exposure to microbes carrying resistant genes, exposure to suboptimal levels of antimicrobials, exposure to broad-spectrum antibiotics, lack of hygiene in clinical environments contribute to resistance. The use of antibiotics in foods/agriculture, have been cited as some of the factors that promote resistance (Huttner *et al.*, 2013). These conditions upregulates genes that can confer resistance in bacteria (Breidenstein *et al.*, 2011).

Multi-drug resistance

Multi-Drug Resistance (MDR) refers to the acquisition of resistance to at least one agent in three or more antimicrobial categories (Basak *et al.*, 2016). Over the years, due to continued selective pressure by different drugs, organisms have developed additional kinds of resistance mechanisms that resulted in multidrug resistance (MDR)—novel penicillin-binding proteins (PBPs), mutated drug targets, enzymatic mechanisms of drug modification, enhanced efflux pump expression and altered membrane permeability (Michael & Levy, 2015). Plasmids and transposons are the commonly known DNA elements that carry several resistant genes; hence resistance to several antimicrobial agents may be acquired simultaneously, resulting in MDR organisms (Byarugaba, 2010; Farshad *et al.*, 2010).

Extensively-Drug Resistance (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) whereas Pan drug-resistance (PDR) is defined as non-susceptibility to all agents in all antimicrobial categories (i.e. no agents tested as susceptible for that organism) (Magiorakos *et al.*, 2012).

Determination of antimicrobial activity

Several methods which can differ between countries and Clinical Microbiology Laboratories have been used to screen and confirm the presence of Extended Spectrum β –Lactamase (Murray, Baron, Pfaller, Tenover, & Yolken, 1999). Determination of the antimicrobial activity in pathogenic bacteria can be performed by either the dilution or diffusion methods. The Clinical Laboratory and Standard Institute (CLSI) proposed disk diffusion methods for screening ESBL producing *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*(CLSI, 2016). This study used the Kirby-Bauer disc diffusion method. This method measures the susceptibility of bacteria using zone of inhibition.

Kirby-bauer disk diffusion method

This method operates on the principle that antibiotic-impregnated disk, placed on agar (Muller Hinton Agar) previously inoculated with the test bacterium, picks up moisture and the antibiotic diffuse radially outward through the agar medium creating an antibiotic concentration gradient. The concentration of the antibiotic at the edge of the disk is highest and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth (Tendencia, 2004). Reliable results can only be obtained with disk diffusion tests that use the principle of standardized methodology and zone diameter measurements correlated with minimal inhibitory concentrations (MICs) with strains known to be susceptible or resistant to various antimicrobial agents (CLSI, 2012).

Table 2: Interpretation Zones and MIC according to CLSI for enterobacteriacae

Antibiotic	Potency	Code	Zone diameter(mm) S I R			Break-points MIC ug/ml S	R
Ampicillin+ Sulbactam	10+10µg	SAM20	≥15	14-12	≤11	≤8/4	≥32/16
Amikacin	30ug	AM130	≥17	16-15	≤14	≤16	≥32
Cefotaxime	30ug	CTX30	≥26	25-23	≤22	≥1	≥4
Ceftazidime	30ug	CAZ30	≥21	20-18	≤17	≤4	≥16
Chloramphenicol	30ug	CL30	≥18	17-13	≤13	≤8	≥32
Ciprofloxacin	5ug	CIPR5	≥21	20-16	≤15	≤1	≥4
Gentamicin	10ug	GEN10	≥15	14-13	≤12	≤4	≥8
Ofloxacin	5ug	OFL5	≥15	16-13	≤12	≤2	≥8
Norfloxacin	10ug	NORFX	≥17	16-13	≤12	≤4	≥16
Tetracyclines	30ug	TET30	≥15	14-12	≤11	≤4	≥16
Levofloxacin	5ug	LEVOF	≥17	16-14	≤13	≤2	≥8
Ceftizoxime	30ug	ZOX30	≥25	24-22	≤21	≤1	≥2

S= sensitive, R= resistant, I= intermediate (CLSI 20th Inf. Suppl. M100-S20, 2010)

Laboratory detection of ESBLs

Observation of organisms harboring ESBLs provides clinicians with helpful information as treatment of infections caused by ESBL-producing organisms with extended-spectrum cephalosporins or aztreonam may result in treatment failure, even when the causative organisms appear to be susceptible to these antimicrobial agents by routine susceptibility testing (David L Paterson & Bonomo, 2005). The Clinical Laboratory Standards Institute (CLSI) recommended both standardized screening and confirmatory tests for ESBL detection. This guideline is based on the principle that most ESBLs hydrolyze third-generation cephalosporins although they are inhibited by clavulanate, and recommend initial screening with either 8 mg/L of cefpodoxime, 1 mg/L each of cefotaxime, ceftazidime, ceftriaxone, or

aztreonam. This is followed by confirmatory tests (including the E-test ESBL strips) with both cefotaxime and ceftazidime in combination with clavulanate at a concentration of 4 lg/mL(CLSI, 2016). In addition to these, automated systems that employ similar detection principles have proved to be popular in clinical laboratories, especially those in North America and certain European countries (Shaikh *et al*., 2015).

Screening for ESBL producing bacteria

i. Screening for ESBL using Disc Diffusion Method

The screening for ESBL producing bacteria as recommended by CLSI is done by the disc diffusion method (CLSI, 2016). In this test, either cefpodoxime (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg) or aztreonam (30 µg) is used as an indicator drug. Bacteria which produce zone of inhibition diameter of ≤ 21mm around cefpodoxime (10 µg), ≤ 17mm around ceftazidime (30 µg), ≤ 22mm cefotaxime (30 µg), ≤ 19mm around ceftriaxone (30 µg) or ≤ 17mm (30 µg) are suspected to produce ESBL and hence, need to be confirmed (CLSI, 2016).

ii. Screening for ESBL producers by Minimum Inhibition Concentration Method

The Clinical Laboratory Standards Institute (CLSI) has recommended dilution methods for screening for ESBL production by Enterobacteriaceae. Ceftazidime, aztreonam, cefotaxime and ceftriaxone could be used for screening ESBLs. Growth of bacteria at a screening concentration of ≥ 16 µg/ml for ceftazidime and aztreonam, ≥ 4µg/ml for cefotaxime and ceftriaxone are suspected to be ESBL producers and is an indication for the organism to be tested by a phenotypic confirmatory test (CLSI, 2016).

Confirmatory test for ESBL production

The confirmatory test for ESBL rest on indicating synergy between the third-generation cephalosporin and clavulanic acid to which the isolate is initially found resistant in the screening test. The following tests can be used to confirm ESBL production: Double Disc Synergy Test (DDST), Combined Disc Test (CDT), E-test and MIC broth dilution test (CLSI, 2016).

i. Double Disc Synergy Test (DDST)

In this test, the test inoculum (0.5 McFarland's turbidity) is spread onto Muller-Hinton Agar (MHA) by using a sterile cotton swab. A disc of Augmentin (30 µg ceftazidime and 30 µg cefotaxime + 10 µg of clavulanic acid) is placed on this MHA; then discs of cefotaxime (30 µg) and ceftazidime (30 µg) are kept 20mm apart from the Augmentin disc. The plate is incubated at 37^{o}C overnight. An increase of ≥ 5 mm in the inhibition zones of either cephalosporin in combination with clavulanic acid compared to the cephalosporin alone is inferred as ESBL production (Cockerill, 2012) as shown in the figure below.

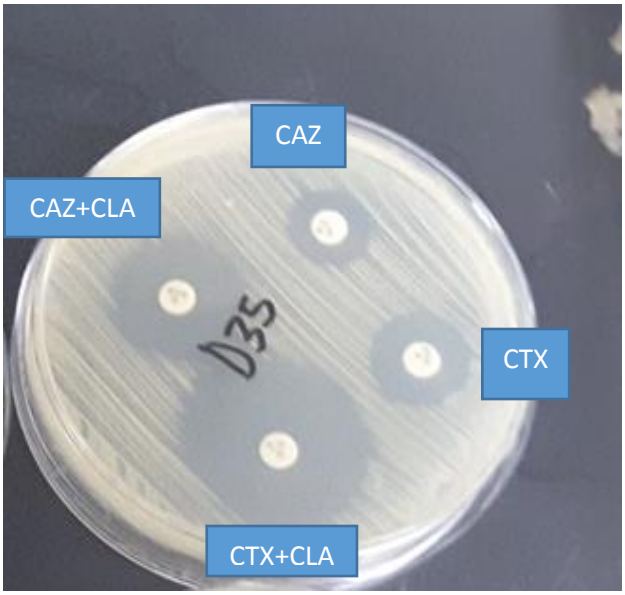

Figure 4: Double Disc Synergy Test (DDST) (Upadhyay *et al.*, 2010).

ii. MIC broth dilution test

In this method, the minimum inhibitory concentration (MIC) of a third-generation cephalosporin alone and in combination with clavulanic acid is compared. A decrease in the MIC of the combination of 3 two-fold dilution (8 times) indicates ESBL production (Joumana *et al.*, 2003).

iii. Combined Disc test (CDT)

CDT compares the zone diameters of cephalosporin discs to those of the same cephalosporin plus clavulanic acid. The test inoculum (0.5 McFarland's turbidity) is spread onto Muller-Hinton Agar (MHA) by using s sterile cotton swab. Cephalosporin discs alone and in the combination with clavulanic acid are applied on the Muller-Hinton agar plates and incubated at 37 ^{o}C overnight. ESBL production is confirmed if the difference in zone diameters around the cephalosporin discs containing clavulanic acid and the corresponding discs without clavulanate is $\geq$ 5 mm (CLSI, 2015).

iv. Disc Replacement Method

In this test, two Augmentin (Amoxycillin (20µg) + Clavulanic acid (10 µg)) discs are placed on Mueller-Hinton agar which has been inoculated with the test organisms. After one hr incubation at room temperature, the Augmentin discs are replaced with Cefotaxime and Ceftazidime discs on the same spot along with control discs placed 30mm apart and incubated at 37^{o}C for between 18-24 hours. A positive test is reported by an increased zone of inhibition of ≥ 5 mm for the discs which have replaced the Augmentin discs compared to the control discs (Al-Jasser, 2006).

v. ESBL NDP (Nordmann/Dortet/Poirel) Test

This is a rapid biochemical test used to identify ESBLs and is established on the in-vitro detection of cefotaxime hydrolysis that is inhibited by the addition of tazobactam. ESBL activity is indicated by a change in color from red to yellow of phenol red, an indicator. The sensitivity and specificity of ESBL NDP test are 92.6% - 98% and 99.8%-100% respectively (Dortet *et al.*, 2014; Nordmann *et al.*, 2012).

vi. E-test

This method involves the use of ESBL strips that have cephalosporin gradient at one end and cephalosporin and clavulanate gradient at the other. One side of the strip is calibrated with minimum inhibitory concentration (MIC) reading scale and the other side has two predefined antibiotic gradients. There are two strips used in this test and testing must be performed with both strips. One of the strips contains cefotaxime gradient (0.25 to 16µg/ml) at one end and ceftazidime/clavulanic acid gradient (0.016 to 1 µg/ml plus 4 µg/ml of

clavulanic acid) at the other end. The second strip contains ceftazidime gradient (0.5 to 32µg/ml) at one end and ceftazidime/clavulanic acid gradient (0.064 to 4 µg/ ml plus 4 µg/ml clavulanic acid) at the other end. The presence of ESBL is confirmed by the appearance of phantom zone below the cefotaxime or deformation of the ceftazidime inhibition ellipse or when the clavulanic acid causes a more than or equal to three doubling concentration decreases (ratio of ≥8) in the MIC values of cefotaxime and ceftazidime (Sridhar Rao, 2015).

Figure 5: Detection of ESBL carriage with an E-test ESBL strips. Ceftazidime MIC against isolate in A is > 32µg/mL in the absence of clavulanate and 0.125µg/mL in the presence of clavulanate (Sridhar Rao, 2015)

vii. Other Methods

There are automated ESBL detection methods. These include, Vitek ESBL cards(Spanu *et al*., 2006), Microscan panels and the BD Phoenix automated microbiology systems (Peer *et al.*, 2008).

Molecular Characterization of ESBL Genes

To determine whether a specific ESBL present in a clinical isolate is related to TEM and SHV enzymes is complicated due to point mutations around the active sites of the TEM and SHV sequences which have led to amino acid changes, increasing the spectrum of activity of the parent enzymes, such as in TEM1, TEM2, and SHV1(Bradford, 2001). The commonest molecular techniques used is the PCR followed by sequencing. Sequencing is required to differentiate between different ESBL enzyme isolates (TEM 3, SHV2 etc) and non-ESBL isolates (TEM 1, TEM 2, or SHV1). This method has the ability to differentiate between ESBLs and non-ESBLs making this system the method of choice (Fluit *et al.*, 2001). In addition, other molecular methods have been developed to characterize ESBLs. These include PCR with RFLPs (Chanawong *et al.*, 2000), PCR with single-strand conformational polymorphism (M'Zali *et al.*, 1996), ligase chain reaction (Kim & Lee, 2000), restriction site insertion PCR (Chanawong *et al.*, 2001), real-time PCR (Randegger & Hachler, 2001) and multiplex PCR (Woodford *et al.*, 2006).

Epidemiology of ESBL-producing organisms

ESBLs are plasmid-mediated enzymes that confer resistance to a wide range of β-lactams. These enzymes arise due to genetic mutation from natural β-lactamases among Gram negative bacteria, (Kiiru *et al.*, 2012; Pfaller & Segreti, 2006).

ESBLs have been identified among members of the family Enterobacteriaceae and Pseudomonadaceae in various parts of the globe. However, these enzymes are frequently observed in *Klebsiella pneumoniae and Escherichia coli* and have evolved as an important mechanism of

resistance amongst these bacteria (Bonnet, 2004; Mukherjee *et al.*, 2013).The first plasmid mediated ESBL was identified in 1983 when Knothe (Knothe *et al.*, 1983) observed a single nucleotide mutation in an SHV-type that represented the first plasmid-encoded beta lactamase that was capable of hydrolyzing the ESCs in an isolate of *K. ozaenae,* and this type was named SHV2. The ESBL family is diverse and heterogeneous and include CTX-M (cefotaximase), TEM (temoniera), SHV (sulfhydryl variable) and OXA (oxacillinase) (Canton & Coque, 2006). Among them, the CTX-M group is the one most commonly encountered ESBL and can be transferred using mobile genetic elements that enable cell-to-cell transmission (Canton & Coque, 2006; EUCAST, 2013). Other ESBL enzyme-types are being found such as the Toho, PER, VIM, VEB, BES, GES, TLA, SFO, and IBC (G.A. Jacoby & Munoz-Price, 2005). ESBLs exhibit varied activity against different oxymino-beta-lactams, but cannot inactivate cephamycins (such as cefotaxime and cefoxitin) and carbapenems (Paterson & Bonomo, 2005). The epidemiology of ESBL in different parts of the world varies.

In Europe, the first report of ESBL producer strains was from Europe, specifically Germany (Knothe *et al.*, 1983) and England (Du Bois *et al.*, 1995). The enzymes mostly responsible for ESBL production in Eastern European countries are: CTX-M-3, SHV-2 and SHV-5. It is evident that there is an increasing prevalence of CTX-M-15 and it constitutes the epidemiology of ESBL in all the European countries (Oteo *et al.*, 2006). The studies in the Middle East revealed a higher prevalence of ESBL than in other parts of the world. A survey on *E. coli* ESBL producers in Egypt, conducted during the period 1999 to 2000, indicated that, 38% of the *E. coli* tested positive for

ESBLs. In another study in Iran, undertaken between 2007 and 2008, 45% of the *K. pneumoniae* isolated from urinary tract infections were found to be ESBL producers (Ghafourian *et al.*, 2012). In the same study, it was detected that, 59.2% of *K. pneumoniae* of the clinical isolates from respiratory tract infections tested positive for ESBL production (Ghafourian *et al.*, 2011). In Iran, a one-year study on *E. coli* collected from urinary tract infections showed that 25% of the isolates were ESBL producers (Pakzad *et al.*, 2011). Also in Saudi Arabia, about 26% of *K. pneumoniae* isolated in 2008 produced ESBLs. In most of the isolates SHV-12, CTXM-15 and TEM-1 were responsible for resistance to 3rd generation cephalosporin (Tawfik *et al.*, 2011). Moreover, data collected over three years in Kuwait showed that the levels of ESBLs were lower in community with 25 isolates of *K. pneumoniae* (17%) and *E. coli* (12%) than in the corresponding hospital isolates (28% and 26%, respectively) (Al Benwan *et al.*, 2010).

In Asia, the first isolates of *K. pneumoniae* harboring SHV-2 were reported from China in 1988 (Rossi *et al.*, 2006). Recently the study of ESBLs in Asia showed a high prevalence among clinical strains. In 2001, the first CTX-M positive strains were reported in New Delhi (Karim *et al.*, 2001). It is estimated that 5 to 8% of *E. coli* isolates from Korea, Japan, Malaysia, and Singapore were positive for ESBL while it was 12 to 24% in Thailand, Taiwan, the Philippines, and Indonesia. However, the *K. pneumoniae* ESBL producers were less than 5% while in other Asian countries it was between 20 to 50% (Lewis *et al.*, 1999). The predilection of ESBLs for *K. pneumoniae* has never been clearly explained. As early as the mid-1990s, it was noted that 25% of the *Enterobacteriaceae* in Thailand were producing ESBLs, mainly

different SHV enzymes (Hawkey, 2008). Luvsansharav *et al.* (2012) analyzed stool samples from healthy volunteers in Thailand in 2009, and the results showed that 30–50% of these subjects in three different regions were ESBL carriers (CTX-M types). The first report of CTX-M-producing *Enterobacteriaceae* in New Delhi was published in 2001 (Karim *et al.*, 2001). Later, in 2006, Ensor *et al*. (Ensor *et al*., 2006) found that 66% of third-generation cephalosporin-resistant *E. coli* and *K. pneumoniae* from three medical centers in India harbored the CTX-M-15 type of ESBL, which was also the only CTX-M enzyme found, and an investigation of 10 other centers in that country showed that rates of ESBL producing *Enterobacteriaceae* reached 70% (Mathai *et al.*, 2002). In other recent studies, Sankar *et al. (2012)* observed ESBL rates of 46% and 50% in out- and inpatients, respectively, and Nasa and co-workers detected ESBL production in almost 80% of clinical isolates (Nasa *et al.*, 2012).

In the Southern and Central parts of America, a study revealed that ESBL positive were identified in 30 to 60% of *Klebsiella spp* in Brazil, Colombia, and Venezuela (Mendes *et al*., 2000). The prevalence of ESBL producer *E. coli* and *Klebsiella* in Latin America showed an increase in 2008 compared to the previous years. Generally, 26% of *E. coli* and 35% of *K. pneumoniae* in Latin American were ESBL producers in 2008. In 2003, 10% of *E. coli* and 14% of *K. pneumoniae* were positive for ESBL production, while in 2004, it was 10% of *E. coli* and 18% of *K. pneumoniae* (Rossi *et al*., 2006).The first ESBL positive reported in 1988 from North America, was *K. pneumoniae* with TEM-10 (Jacoby *et al.*, 1988). This was followed by TEM-12 and TEM -26 (Bush, 2008). In 2009, a survey on *E. coli* reported that 9%

of *E. coli* were ESBL producers (Bhusal *et al.*, 2011). The first occurrence of ESBL in France was in 1986, where of 54 patients from three intensive care units (ICUs) were found positive for ESBL (Burn-Buisson *et al.*, 1987). Subsequently, the vast majority of ESBL positive strains were found in France (Philippon *et al.*, 1989). In recent times, the frequency of ESBL among Enterobacteriaceae in France was under 1%, while there was an increasing prevalence of CTX-M (Galas *et al.*, 2008). The studies also showed that the prevalence of ESBL producer strains in 2005 was much lower than in previous years, for instance *P. mirabilis* (3.7% decreasing to1.3%), *Enterobacter aerogenes* (53.5% decreasing to 21.4%) and *K. pneumoniae* (9.4% decreasing to 3.71%).

In Africa, there have been some studies which showed a high prevalence of ESBL producing *K. pneumoniae* in South Africa (Cotton *et al.*, 2000). The first study on ESBLs in Tanzania was performed in 2001–2002 and analyzed blood isolates from neonates, and it was found that 25% of the *E. coli* and 17% of the *K. pneumoniae* produced ESBLs, mainly the CTX-M-15 and TEM-63 types (Blomberg *et al.*, 2005).

In Ghana, study set out to determine the prevalence of Extended-Spectrum Beta-Lactamases in one of the tertiary facilities in Ghana, reported an overall prevalence of 49.3% among 300 enterobacteria isolates. ESBL phenotype was highest among *Enterobacter cloacae,* followed by *K. pneumoniae, C. freundii, K. oxytoca,* and *E. coli* (Obeng-Nkrumah *et al.*, 2013). More so, a report by Oduro-Mensah *et al.* (2016) determined the prevalence of ESBL to be 37.96 % with an overall 62.7 % of isolates having

the *bla* $_{TEM}$ genes in Ghana, an indication of the ubiquitous nature of ESBLs and the urgency of surveillance and control.

Summary

Bacterial resistance to antibiotics is on the rise worldwide in healthcare settings and in the community resulting in a lot of challenges to the effective treatment of infections. Due to the broad antibacterial spectrum and excellent safety profile of β-lactam antibiotics, resistance of pathogenic bacteria to these drugs taken a great threatening dimension especially with the emergence ESBL enterobacteriaceae (Abdallah *et al.*, 2015). The ESBLs first identified in 1983 in Germany arose from a single nucleotide polymorphism in the blaSHV genes that altered specificity to oxyimino-cephalosporins. Overtime there has been a wide spread of ESBLs with an ever evolving ability to hydrolyze penicillins, first, second and third generation cephalosporins and monobactams but not carbapenems. In Africa, there are various reports of ESBLs implicated in infectious diseases across all ages. Several works stated high prevalence of ESBLs circulating worldwide and this is of a global concern.

CHAPTER THREE

MATERIAL AND METHODS

Effective methods for analyzing and assessing the molecular epidemiology of the extended spectrum Beta-lactams from CCTH, VRH, ERH and the detection of the prevalence of drug resistance in these three facilities is the primary focus of this study. Due to globalization and in effective drug monitoring, systems, high levels of organisms are developing resistance to previously effective and commonly used drugs. There is therefore the need to continually assess and monitor the antimicrobial resistant patterns in our various health facilities and the community at large. This chapter therefore describes the field works and procedures for the sample collection, sample preparation and processing and the analysis carried out to evaluate the levels and distribution of ESBLS in these facilities.

Study Area

This cross sectional study was conducted from September 2017 to June 2018 to determine the epidemiology of ESBL producing Enterobacteriaceae. Three major tertiary hospitals were chosen for the study. The enterobacteria isolates analyzed in this study were from Cape Coast Teaching Hospital (CCTH), Efia Nkwanta Regional hospital and Volta Regional Hospital (VRH). CCTH serves as a teaching facility for the University Of Cape coast and a referral hospital in the central region, with bed capacity of 400, Efia Nkwanta Regional hospital, a referral hospital in the Western region registering an average of about 115,958 hospital admissions and with an average annual OPD attendance of 131,235. Volta Regional Hospital popularly known as Trafalgar is the regional and teaching hospital in Ho in the Volta Region of

Ghana. It became a teaching hospital in 2015 to serve the University of Health and Allied Sciences with 240 bed-capacities. These facilities were chosen because they received the highest number of patients and cases in the city.

Isolates

A total of 167 clinical isolates were processed from the three facilities during the sampling period from September 2017 to June 2018. Among these, 4(2.40%) were anonymous pending further advanced identification.

Non-repeat isolates of enterobacteriaceae were collected from a variety of clinical specimens namely, blood, urine, sputum, stool, high vaginal swab, urethral swab, wound, pus and any other body fluid aspirates. These bacteria were identified by picking single colony from agar plates. The identification involved pre-screening for typical enterobacteria growth, lactose fermenting characteristic on MacConkey agar, Gram stain for Gram negative reaction using Giemssa stain.

Pure cultures of clinical isolates were identified using Uri*Select*™4, a non-selective chromogenic agar medium for the isolation, differentiation and enumeration of urinary tract pathogens. A set of in-house biochemical tests in addition to other protocols for identification of enterobacteria (Table 3.1) were employed. Isolates so identified exhibiting ambiguous taxonomic classification were confirmed with API 20E (bioMerieux SA, Marcy l'Etoile, France) following the manufacturer's instructions. In few cases Matrix Assisted Laser Desorption Ionization Time- Of-Flight (-MALDI-ToF) MS was used for confirmation of the isolates.

Table 3: Example of Biochemical tests which were used to identify Enterobacteriacae (Murray *et al*, 1999)

Species	Lac	KIA	ORN	LYS	IND	Ure	CIT	MOT	Glucose	Rhamnose
K .pneumoniae	+	I	-	+	-	+	+	-	+	+
K. oxytoca	+	I	-	+	+	+	+	-	+	+
Escherichia coli	+ d	I or II	d	+	+	-	-	+	+	+
Ent. aerogenes	+d	I or II	+	+	-	-	+		+	+
Ent cloacae	+ d	I or II	+	-	-	+ w	+		+	+
Citr diversus	+d	I or II	+	-	+	+	+		+	+
Serr marcescens	-	II	+	+	-	d w	+		10%	-
Serr liqufaciens	-	II	+	D	-	-	+		10%	-
Hafnia alvei	-	II	+	+	-	-	+		+	+
Prov rettgeri	-	II	-	-	+	+w	+	+	-	-
Prov stuartii	-	II	-	-	+	-	+	+	-	-
Morg. morganii	-	II	+	-	+	+w	-		d	-
Prot mirabilis	-	III	+	-	-	+s	d	+	+	-
Prot vulgaris	-	III	-	-	+	+s	-	+	d	-
Salmonella	-	III	+	+	-	-	d	+	+	+
Citro freundii	+	III	+	-	-	+	+	+	+	+

I= Yellow/Yellow, II= Red/ Yellow, III= Black coloration due to H_2S, D = Differential, W= few give positive results, Lact=Lactose, KIA=Kligler Iron Agar, ORN=Ornithine decarboxylase, LYS=Lysine decarboxylase, IND=Indole, CIT=Citrate

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by the Kirby Bauer disc diffusion method on Muller-Hinton agar (Thermofisher, UK). Phenotypic ESBL screening and confirmation was carried out as recommended by the Clinical and Laboratory Standard Institute (CLSI, 2016).

E. coli ATCC 25922 was used as positive control for susceptibility testing. Susceptibility was tested against imipenem (10 µg), meropenem (10 µg), doripenem(10 µg), ertapenem(10 µg), ampicillin (10 µg), augmentin (20/10µg), tetracycline (30µg), gentamicin (10 µg) , penicillin (10 µg), SXT

(1.25/23.75 µg), ciprofloxacin (5 µg), cefpodoxime (10 µg), ceftazidime (30 µg), cefepime (30 µg),ceftriaxone (30 µg), and cefotaxime (30 µg), (BD BBL, USA). All isolates resistant to multiple cephalosporins were confirmed for ESBL production using double disk synergy (Disk approximation method). Bacterial colonies were suspended in sterile double distilled water to a turbidity of 0.5 McFarland standards and then inoculated on a Muller Hinton agar plate. An already prepared disc of ceftazidime+clavulanic acid (30 /10 µg) and cefotaxime + clavulanic acid (30 /10 µg) discs were placed at a distance of 25 mm apart on the Petri dish already inoculated with the test strain while cefotaxime (30 µg) and ceftazidime (30 µg) discs were placed at a distance of 20 mm (centre to centre) from the clavulanic acid discs on the same dish. These plates were then incubated aerobically at 37°C for 18- 20 h. An enhanced zone of inhibition (>5mm) towards the ceftazidime+clavulanic acid (30 /10 µg) and cefotaxime + clavulanic acid (5 /10 µg) disk indicated positive ESBL production (Wayne, 2006; Yves *et al*., 2003).

Amplification of ESBLs genes

All isolates with ESBL phenotypes were screened for blaTEM, blaSHV and blaCTX-M genes using Polymerase Chain Reaction (PCR). The DNA of the isolates was extracted using the boiling technique (Holmes & Quigley, 1981). Two to three colonies of the pure culture on nutrient agar were inoculated in 400 µL sterile nuclease free water. The suspension was incubated for 10 minutes at 95^{o}C to lyse the cells, and then centrifuged at full speed for 10 minutes to remove cellular debris. The supernatant containing the DNA was aliquoted serving as the template DNA in the PCR reaction (Karisiki *et al*., 2006; Kiratisin, Apisarnthanarak, Laesripa, & Saifon, 2008).

Amplification of TEM, SHV and CTX-M genes using PCR were performed as described previously using primers in Table 4. For amplification, 5 µL of template DNA was added to a 45 µL master mix containing 200 µM of dNTP mixtures (Roche, Switzerland) 0.4 µM of each primer, 2.5U taq polymerase (Invitrogen, USA) and appropriate buffer (0.2 µL $MgCl_2$, 2.5 µL KCL, 0.5 µL 10% Tween 20, 1µL of Gelatin and 3.8 µL of pure water). The reaction was performed in Gene Amp PCR system 9700 thermo cycler (Applied Biosystems, USA) under the following conditions: Initial denaturation at 94^{o}C for 5 minutes followed by 30 cycles of 1 minute denaturation at 94^{o}C, annealing at 58^{o}C for 1 minute, 1 minute for extension at 72^{o}C, and a final elongation at 72^{o}C for 10 minutes. PCR products were detected with ethidium bromide fluorescence using the Dym-Bact image system after 1 hr electrophoresis in 2% TBE agarose gel.Positive controls for TEM, SHV and CTX-M of a previously confirmed isolate with the three genes were used in every run.

Table 4: Primers used for amplification and sequence of ESBL genes

Resistant genes	Primer name	Primer Sequence (5' to 3')	Band size	Cycling conditions
TEM,	MultiTSOT_for	FP:CATTTCCGTGTCGCCCTTATTC	800	Initial denaturation at 94ºC for 5min,30 cycles of 94ºC for 1min, 57ºC for 1min and 72ºC for 1 min and final elongation 72ºC for 10min
	MultiTSO-T_rev	RP:CGTTCATCCATAGTTGCCTGAC		
SHV	MultiTSO-S_for	FP:AGCCGCTTGAGCAAATTAAAC	713	
	MultiTSO-S_rev	RP:ATCCCGCAGATAAATCACCAC		
CTX-M	MultiTSO-O_for	FP:GGCACCAGATTCAACTTTCAAG	909	
	MultiTSO-O_rev	RP:GACCCCAAGTTTCCTGTAAGTG		

Data analysis

All data were entered in laboratory log book and then unto the computer using excel sheets. The data was manually cleaned and the final analysis done in accordance with the study objectives. Statistical Package for Social Sciences (SPSS) V21 was used. Chi square/Fisher's exact (values below 5 counts) was be used to explore the association between clinical and epidemiological characteristics of the infected patients and the resistant genes. Further, Odds ratio (OR) and confidence interval (CI) at 95% was used to estimate the degree of association. Cohen's Kappa values for agreement between confirmatory test and PCR was calculated with their 95% CI. Graphs were also used to illustrate data where necessary.

Epidemiological analysis

Categorical variables were described using frequencies and percentages. Continuous variables were defined using medians and their corresponding interquartile ranges (IQRs). Case fatality was compared among different populations using the odds ratio (OR) along with the 95%-confidence interval (CI).

Quality control

A set of in-house biochemical tests in addition to other protocols for identification of enterobacteria (Table 3) were employed. Isolates so identified exhibiting ambiguous taxonomic classification were confirmed with API 20E (bioMerieux SA, Marcy l'Etoile, France) following the manufacturer's instructions. In few cases Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-ToF) MS was used for confirmation of the isolates. In all

tests the use of positive and negative controls was adhered to and it was ensured that reading of tests was done by more than two people to avoid bias. Quality control strains were used as described in methodology section. All isolates have been preserved for future use and for further identification if needed.

Ethical Consideration

The study obtained clearance from Research and Ethical Review Committee of the University of Cape Coast, Noguchi Memorial Institute for Scientific and Medical Research for review and approval. Consent from the various institutions and parents/guardians was also obtained.

Summary

Chapter three examines the methods available for analyzing the prevalence of ESBLs. The field work was conducted in three tertiary hospitals which were carefully selected in the southern belt. Sample processing, treatment, antimicrobial identification and susceptibility were also discussed. DNA extraction, PCR and PFGE of the amplified genes were carried out to determine clonality or diversity among stains. The chapter finally examines the prevalence of the ESBL genes circulating in these facilities.

CHAPTER FOUR

RESULTS

Several parameters have been taken into consideration and evaluated for the validation of the analytical methods for quantitative determination of the molecular epidemiology of Extended Spectrum beta-lactamase producing enterobacteriacae. Analyses of the relation between the ages, gender, and patient care area from where the isolates were obtained at the various facilities were determined. The prevalence of the resistance to the various antibiotics phenotypically and genotypically was determined. Reproducibility and accuracy of the techniques employed were tested and confirmed with controls in order to ensure quality results.

General Characteristic

A total of 167 clinical isolates were obtained out of which 4(2.40%) were anonymous pending further advanced identification. From the various facilities, VRH recorded the highest number of isolates (65) followed by CCTH (61) and then ERH recorded the least (41). Majority of the isolates were *Escherichia coli.* 51(30.54%), followed by *Klebsiella pneumoniae* 43(25.7%), *Pseudomonas aeruginosa* 21(12.58%), *Proteus mirabilis* 16(9.50%), *Acinetobacter baumannii* 12(7.17%). Other organisms which include *Providentia spp, citrobacter, Enterobacter* etc, were 20(11.98%).

Socio-demographic characteristics of the Study Population

Age

The study included all patients who presented at the laboratory from the OPD and on Admission. The mean age of (±SD) of all patients was 43.4±22.58. Those infected with *E. coli* alone was 44.90±20.32 years,

Klebsiella pneumoniae alone was 43.37±22.78 years, *Pseudomonas aeruginosa* alone was 45.48±22.66 years, *Proteus mirabilis* alone was 52.50±24.07, *Acinetobacter baumannii* 27.58±18.07 with the others having average age of 36.45±25.24. The minimum age of the patients was 6 months with the highest been 88 years. The age did not have any significance on the isolates when extrapolated (p. value =0.444).

Gender

In this study more samples were obtained from females 106(63.3%) than males 61(36.5%). Out of these 35(68.6%) females and 16(31.4%) males were infected with *E.coli, K. pneumoniae* were isolated from 28(62.2%) females and 17(37.8%) males. Out of a total of 16 patients who were infected with P. *mirabilis*, 9(52.9%) were females. *A baumannii* was isolated from (75.0%) females and 3(25.0%) males. Equal amount of *P. aeruginosa* 11(50.0%) were isolated from both male and female. There was no significant difference between the isolates in relation to sex (p. value =0.520)

Department

The study involved Out-Patients-Department and In-Patients (Ward). Majority 124(74.3%) were from OPD and 43(25.7%) were on Admission. There was No Significant difference (p. value=0.054 with 95% CI) between the out patients (OPD) and those on admission (ward) when compared with the clinical isolates

Specimen

Isolates from blood, urine, wound, HVS and Others (pleural aspirate, ear swab, Urethral etc.) were collected. Majority of the isolates 89(53.3%)

were from urine, followed by 21(012.6%) from wound, 13(7.8%) from sputum. Equal number of isolates 2(1.2%) were isolated both from blood and HVS. Forty clinical isolates representing 24.0% were isolated from the other specimen. The highest number of isolates were recorded from urine; 33(64%) of *E. coli*, 23(51.1%) of *Klebsiella pneumoniae* followed by *P.aeruginosa* 11(50%), then *P. mirabilis* *7*(41.2%), and then *A. baumannii* representing 4(33.3%). The remaining organisms (Others) registered 11(55.0%) in total. In all, the least organisms were isolated from blood; 1(5.9%) of *P. mirabilis* and 1(4.5%) *P.aeruginosa.* There was no significant difference (p. value = 0.074) between the specimen in relation to the various organisms identified.

Continuous data are presented as mean ± Sd and compared to each other using unpaired t-test whilst categorical data presented as proportion and compared to each other using Chi-square analysis.

Facility

VRH recorded the highest number of isolates (65) followed by CCTH (61) and then ERH recorded the least (41). From VRH, majority of the isolates were *K. pneumoniae* 22(66.7%) followed by *P. mirabilis* 8(47.1%) and then *E. coli* 19 (41.3%). In CCTH, a total of 23 (50%) *E. coli* was isolated followed by 7(41.2%) *P. mirabilis* and then *K. pneumoniae* and *A. baumannii* recording 11(33.3%) and 4(33.3%) respectively. In ERH, no *K. pneumoniae* was isolated during the period but *P.aeruginosa* 9(39.1%), *A. baumannii* 4(33.3%), *P. mirabilis* 2(11.8%), *E. coli* 4 (8.7%) with the others constituting 9(45%) respectively. There was significant difference between the isolates from the various facilities (*p*. value=0.005).

Table 5: General characteristic of the studied population stratified by enterobacteria isolate

Demographic	*A. baumannii* N=(12)	*E. coli* N=(51)	*K. pneumoniae* N=(43)	*P. mirabilis* N=(16)	*P. aeruginosa* N=(21)	Others N=(24)	P-value(sig)
Age(yrs)	27.58±18.07	44.90±20.32	43.37±22.78	52.50±24.07	45.48±22.66	36.45±25.24	
Gender							**0.444**
Female	9(75.0%)	35(68.6%)	28(62.2%)	9(52.9)	11(50.0%)	14(70.0%)	
Male	3(25.0%)	16(31.4%)	17(37.8%)	8(47.1%)	11(50.0%)	6(30%)	***0.520***
Department							
OPD	6(50.0%)	37(72.5%)	34(75.6%)	15(88.2%)	20(90.9%)	12(60%)	
WARD	6(50.0%)	14(27.5%)	11(24.4%)	2(11.8%)	2(9.1%)	8(40%)	***0.054***
Specimen							
Blood	0(0.0%)	0(0.0%)	0(0.0%)	1(5.9%)	1(4.5%)	0(0.0%)	
Sputum	1(8.3%)	2(3.9%)	3(6.7%)	3(17.6%)	3(13.6%)	1(5.0%)	
urine	4(33.3%)	33(64.7%)	23(51.1%)	7(41.2%)	11(50%)	11(55.0%)	
Wound	4(33.3%)	3(5.9%)	5(11.1%)	3(17.6%)	0(0.0%)	6(30.0%)	
HVS	0(0.0%)	7(3.9%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
other	3(25%)	11(21.6%)	14(31.1%)	3(17.6%)	7(31.8%)	2(10%)	***0.074***
Facility							
CCTH	4(33.3)	23(50.0)	11(33.3)	7(41.2)	6(26.1)	10(50)	
Effiankwanta	4(33.3)	4(8.7)	0(0.0%)	2(11.8)	9(39.1)	9(45)	
Volta	4(33.3)	19(41.3)	22(66.7)	8(47.1)	8(34.8)	1(5)	***0.005***

Continuous data were presented as mean ± Sd and categorical data presented as proportion. Continuous data were compared to each other using One way ANOVA whilst categorical data compared to each other using Chi-square analysis.

Antibiotic susceptibility profiles of the isolates

A total of 167 Enterobacteria were tested against 18 diverse antibiotics including 12 beta lactams namely imipenem, dorepenem, ertapenem, meropenem, cefotaxime, ceftriaxone, cefuroxime, cefpodoxime, cefepime, cefoxitin, ampicillin, ceftazidime and other non beta-lactams such as augmentin, gentamicin, ciprofloxacin, co-trimoxazole, tetracycline and chloramphenicol. High proportions of isolates were resistant to the β-lactam antibiotics ranging from 6.5% for ertapenem to 94.7% for ampicillin. The proportion of isolates resistant to other antibiotics (non β-lactams) tested ranged from 1.9%in gentamycin to 78.3% in augmentin been registered in *P.aeruginosa* (Table 6).The antibiotic susceptibility patterns of the beta-lactams among the various organisms were mostly significant except for ampicillin (p. value=0.656) and penicillin (*p.* value=0.265)

Table 6: Proportion of Enterobacteria isolates stratified by resistance to antibiotics

Antibiotic	Resistance pattern	*A. baumannii* N=(12)	*E. coli* N=(51)	*K.pneumoniae* N=(43)	*P. mirabilis* N=(21)	*P. aeruginosa* N=(16)	Others N=(22)	P. value
Beta- lactams								
Meropenem	R	2(22.2%)	1(11.1%)	0(0.0%)	0(0.0%)	2(22.2%)	4(44.4%)	
	I	0(0.0%)	0.00%	4 (66.7%)	0(0.0%)	1(16.7%)	1(16.7%)	
	S	10(6.6%)	0(0.0%)	0(0.0%)	17(11.2%)	19(12.5%)	15(9.9%)	
								0.009
Ertapenem	R	7(22.6%)	5(16.1%)	5(16.1%)	2(6.5%)	6(19.4%)	6(19.4%)	
	I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	4(100.0%)	0(0.0%)	
	S	5(3.8%)	46(38.8%)	40(30.3%)	15(11.4%)	12(9.1%)	14(10.6%)	
								0
Imipenem	R	0(0.0%)	1(16.7%)	0(0.0%)	0(0.0%)	2(33.3%)	3(50.0%)	
	I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
	S	12(7.5%)	50(31.1%)	45(28.0%)	17(10.6%)	20(12.4%)	17(10.6%)	
								0.028
Dorepem	R	0(0.0%)	1(16.7%)	0(0.0%)	0(0.0%)	2(33.3%)	3(50.0%)	
	I	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
	S	12(7.5%)	50(31.1%)	45(28.0%)	17(10.6%)	20(12.4%)	17(10.6%)	
								0.028
Cefepime	R	4 (15.4%)	6(23.1%)	10(38.5%)	3(11.5%)	1(3.8%)	2(7.7%)	
	I	0(0.0%)	13(65.0%)	4(20.0%)	2(10.0%)	1(5.0%)	0(0.0%)	
	S	8(6.6%)	32(26.4%)	31(25.6%)	15(12.4%)	15(12.4%)	20(16.5%)	
								0.021

Table 6 Continues

Cefoxitin	R	9(14.5%)	11(17.7%)	8(12.9%)	15(24.2%)	2(3.2%)	8(100.0%)	
	I	0(0.0%)	3(37.3%)	2(25.0%)	1(5.9%)	1(12.5%)	97(12.5%)	
	S	3(3.1%)	37(38.1%)	35(36.1%)	4(4.1%)	14(14.4%)	4(4.1%)	
								0
Cefamandole	R	11(10.3%)	44(41.1%)	18(16.8%)	8(7.5%)	6(5.6%)	20(18.7%)	
	I	0(0.0%)	1(16.7%)	1(16.7%)	3(50.0%)	1(16.7%)	0(0.0%)	
	S	1(1.9%)	6(11.1%)	26(48.1%)	9(16.7%)	10(18.5%)	2(3.7%)	
								0
Cefuroxime	R	11(11.1%)	27(27.3%)	22(22.2%)	14(14.1%)	7(7.1%)	18(18.2%)	
	I	0(0.0%)	3(75.0%)	0(0.0%)	0(0.0%)	1(25.0%)	0(0.0%)	
	S	1(1.6%)	21(32.8%)	23(35.9%)	6(9.4%)	9(14.1%)	4(6.3%)	
								0.022
Ceftriaxone	R	10(11.9%)	27(32.1%)	22(26.2%)	11(13.1%)	5(82.6%)	9(70.0%)	
	I	0(0.0%)	5(21.7%)	4(17.4%)	2(8.7%)	3(13.0%)	9(39.1%)	
	S	2(3.5%)	19(33.3%)	18(31.6%)	6(10.5%)	9(15.8%)	3(5.3%)	
								0.003
Cefpodoxime	R	10(10.1%)	30(30.3%)	20(20.2%)	13(13.1%)	8(8.1%)	18(18.2%)	
	I	1(12.5%)	1(12.5%)	1(12.5%)	2(25.0%)	1(12.5%)	2(25.0%)	
	S	1(1.7%)	20(33.3%)	24(40.0%)	5(8.3%)	8(13.3%)	2(3.3%)	
								0.02
Cefotaxime	R	10(10.5%)	31(32.6%)	22(42.2%)	12(12.6%)	6(6.3%)	14(14.7%)	
	I	1(9.1%)	0(0.0%)	1(9.1%)	2(18.2%)	1(9.1%)	6(54.5%)	
	S	1(1.6%)	20(32.8%)	22(36.1%)	6(9.8%)	10(16.4%)	2(3.3%)	
								0
Ceftazidime	R	3(5.6%)	21(38.9%)	17(31.5%)	7(13.0%)	3(5.6%)	3(5.6%)	
	I	4(33.3%)	5(41.7%)	2(16.7%)	1(8.3%)	0(0.0%)	0(0.0%)	
	S	5(5.0%)	25(24.8%)	26(25.7%)	12(11.9%)	14(13.9%)	19(18.8%)	

Table 6: Continues

Cefoxitin	R	9(14.5%)	11(17.7%)	8(12.9%)	15(24.2%)	2(3.2%)	8(100.0%)	
	I	0(0.0%)	3(37.3%)	2(25.0%)	1(5.9%)	1(12.5%)	97(12.5%)	
	S	3(3.1%)	37(38.1%)	35(36.1%)	4(4.1%)	14(14.4%)	4(4.1%)	
								0
Cefamandole	R	11(10.3%)	44(41.1%)	18(16.8%)	8(7.5%)	6(5.6%)	20(18.7%)	
	I	0(0.0%)	1(16.7%)	1(16.7%)	3(50.0%)	1(16.7%)	0(0.0%)	
	S	1(1.9%)	6(11.1%)	26(48.1%)	9(16.7%)	10(18.5%)	2(3.7%)	
								0
Cefuroxime	R	11(11.1%)	27(27.3%)	22(22.2%)	14(14.1%)	7(7.1%)	18(18.2%)	
	I	0(0.0%)	3(75.0%)	0(0.0%)	0(0.0%)	1(25.0%)	0(0.0%)	
	S	1(1.6%)	21(32.8%)	23(35.9%)	6(9.4%)	9(14.1%)	4(6.3%)	
								0.022
Ceftriaxone	R	10(11.9%)	27(32.1%)	22(26.2%)	11(13.1%)	5(82.6%)	9(70.0%)	
	I	0(0.0%)	5(21.7%)	4(17.4%)	2(8.7%)	3(13.0%)	9(39.1%)	
	S	2(3.5%)	19(33.3%)	18(31.6%)	6(10.5%)	9(15.8%)	3(5.3%)	
								0.003
Cefpodoxime	R	10(10.1%)	30(30.3%)	20(20.2%)	13(13.1%)	8(8.1%)	18(18.2%)	
	I	1(12.5%)	1(12.5%)	1(12.5%)	2(25.0%)	1(12.5%)	2(25.0%)	
	S	1(1.7%)	20(33.3%)	24(40.0%)	5(8.3%)	8(13.3%)	2(3.3%)	
								0.02
Cefotaxime	R	10(10.5%)	31(32.6%)	22(42.2%)	12(12.6%)	6(6.3%)	14(14.7%)	
	I	1(9.1%)	0(0.0%)	1(9.1%)	2(18.2%)	1(9.1%)	6(54.5%)	
	S	1(1.6%)	20(32.8%)	22(36.1%)	6(9.8%)	10(16.4%)	2(3.3%)	
								0
Ceftazidime	R	3(5.6%)	21(38.9%)	17(31.5%)	7(13.0%)	3(5.6%)	3(5.6%)	
	I	4(33.3%)	5(41.7%)	2(16.7%)	1(8.3%)	0(0.0%)	0(0.0%)	
	S	5(5.0%)	25(24.8%)	26(25.7%)	12(11.9%)	14(13.9%)	19(18.8%)	

Socio-demographic characteristics of *E.coli*, *K. pneumoniae* and *P. aeruginosa*.

The antibacterial resistance patterns for the three common isolates were examined. There was significant difference in all the beta-lactams except Imipenem and Doripenem (p-value=0.080) respectively and ampicillin (p-value= 0.551).

The adjusted P. values for the significant resistance were determined to ascertain where the specific differences occurred. It was detected that most of the significant resistance pattern in the cephalosporins were detected in Pseudomonas *aeruginosa* with *E.coli* showing only Cefepime been significant in the intermediate (p-value= 0.0028).

Table 7: Proportion of E.coli, K. pneumoniae and P.aeruginosa isolates stratified by resistance to antibiotics with their adjusted P. values (aP. value)

Antibiotic	Resistance pattern	*E. coli* N=(51)	*K. pneumoniae* N=(43)	*P. aeruginosa* N=(21)	P. value	aP. Value E.	aP. Value K	aP. Value P
Beta-lactams								
Meropenem	R	1(33.30%)	0 (0.00%)	2(66.70%)		0.6892	0.1615	0.0278
	I	0(0.00%)	4(80.00%)	1(20.00%)		0.0455	0.0455	0.9203
	S	50(45.50%)	41(37.30%)	19(17.30%)		0.0719	0.4839	0.1615
					0.047			
Ertapenem	R	5(31.3%)	5(31.3%)	6(37.5%)		0.2985	0.5419	0.0373
	I	0(0.0%)	0(0.0%)	4(100.0%)		0.075	0.1101	0.0000
	S	46(46.9%)	40(40.8%)	12(12.2%)		0.0711	0.1845	0.0001
					0.000			
Imipenem	R	1(33.3%)	0(0.0%)	2(66.7%)				
	I	50(43.5%)	45(39.1%)	20(17.4%)				
	S	51(43.2%)	45(38.1%)	22(18.6%)				
					0.080			
Dorepem	R	1(33.3%)	0(0.0%)	2(66.7%)				
	I	50(43.5%)	45(39.1%)	20(17.4%)				
	S	51(43.2%)	45(38.1%)	22(18.6%)				
					0.080			
Cefepime	R	6(33.3%)	10(55.6%)	2(11.1%)		0.3577	0.0984	0.3727
	I	13(76.5%)	4(23.5%)	0(.0%)		0.0028	0.1802	0.0329
	S	32(38.6%)	31(37.3%)	20(24.1%)		0.1151	0.7866	0.0192

Table 7: Continues

					0.013			
Cefoxitin	R	11(30.6%)	8(22.2%)	17(47.2%)		0.0658	0.0184	0.0000
	I	3(50.0%)	2(33.3%)	1(16.7%)		0.7308	0.8037	0.8984
	S	37(48.7%)	35(46.1%)	4(5.3%)		0.1070	0.0172	0.0000
					0.000			
Cefamandole	R	44(53.7%)	18(22.0%)	20(24.4%)		0.0006	0.0000	0.0156
	I	1(50.0%)	1(50.0%)	0(0.0%)		0.8452	0.7275	0.4947
	S	6(17.6%)	26(76.5%)	2(5.9%)		0.0004	0.0000	0.0235
					0.000			
Cefuroxime	R	27(40.3%)	22(32.8%)	18(26.9%)		0.4627	0.1743	0.0086
	I	3(100.0%)	0(0.0%)	0(0.0%)		0.0443	0.1684	0.401
	S	21(43.8%)	23(47.9%)	23(47.9%)		0.9234	0.0701	0.0172
					0.027			
Ceftriaxone	R	27(46.6%)	22(37.9%)	9(15.5%)		0.5747	1.0000	0.4694
	I	5(27.8%	4(22.2%)	9(50.0%)		0.1322	0.1351	0.0001
	S	19(47.5%)	18(45.0%)	3(7.5%)		0.5779	0.255	0.0414
					0.003			

Table 7: Continues

Cefpodoxime	R	30(44.1%)	20(29.4%)	18(26.5%)		0.8185	0.0229	0.0109
	I	1(25.0%)	1(25.0%)	2(50.0%)		0.4542	0.5821	0.1014
	S	20(43.5%)	24(52.2%)	2(4.3%)		0.9639	0.0121	0.0014
					0.009			
Cefotaxime	R	31(46.3%)	22(32.8%)	14(20.9%)		0.4436	0.1743	0.4717
	I	0(0.0%)	1(14.3%)	6(85.7%)		0.0173	0.1804	0
	S	20(45.5%)	22(50.0%)	2(4.5%)		0.7056	0.0408	0.0024
					0			
Ceftazidime	R	21(51.2%)	17(41.5%)	3(7.3%)		0.2006	0.5871	0.0211
	I	5(71.4%)	2(28.6%)	0(0.0%)		0.1203	0.5912	0.1916
	S	25(35.7%)	26(37.1%)	19(27.1%)		0.0469	0.7885	0.0042
					0.041			
Ampicillin	R	23(37.7%)	21(34.4%)	17(27.9%)		0.2109	0.3908	0.0078
	I	14(63.6%)	6(27.3%)	2(9.1%)		0.0321	0.2448	0.2021
	S	14(40.0%)	18(51.4%)	3(8.6%)		0.6465	0.0535	0.0681
					0.551			
Non beta-lactams								
Chloramphenicol	R	23(38.3%)	20(33.3%)	17(28.3%)		0.2757	0.2747	0
	I	1(20.0%)	2(40.0%)	2(40.0%)		0.2841	0.9301	0.2102
	S	27(50.9%)	23(43.4%)	3(5.7%)		0.1262	0.2881	0.0011
					0.022			
Penicillin	R	5(43.5%)	44(38.3%)	21(18.3%)				

Table 7: Continues

	I	1(50.0%)	0(0.0%)	1(50.0%)				
	S	51(43.6%)	44(37.6%)	22(18.8%)				
					0.399			
Augmentin	R	23(37.7%)	21(34.4%)	17(27.9%)				
	I	14(63.6%)	6(27.3%)	2(9.1%)				
	S	14(40.0%)	18(51.4%)	3(8.6%)				
					0.027			
Ciprofloxacin	R	30(62.5%)	16(33.3%)	2(4.2%)		0.0005	0.3738	0.0008
	I	8(53.3%)	6(40.0%)	1(6.7%)		0.3974	0.8736	0.2024
	S	13(23.6%)	23(41.8%)	19(34.5%)		0.0001	0.4416	0
					0			
Gentamycin	R	17(38.6%)	23(52.3%)	4(9.1%)				
	I	3(33.3%)	3(33.3%)	3(33.3%)				
	S	31(47.7%)	19(29.2%)	15(23.1%)				
					0.081			

Socio-demographic characteristics of the study population in relation to ESBL phenotype

The socio-demographic characteristics of the participants were taken (Table 5) and antibiotic resistance prevalence was stratified by ESBL production phenotypically with antibiotics (Table 6). The mean age of the patients infected with ESBL was 49.17±22.89 whereas that of non-ESBL infected patients was 40.35±22.04. Out of 167 isolates, majority of ESBL producers were from females 70 (61.4%). ESBL prevalence determined phenotypically was 68% in 114 samples which is on a higher side. Among the specimens collected, 64(56.1%) isolated from urine were ESBL positive followed by isolates from wound 12(10.5%), sputum 8(7.0%) and then Blood 2(1.8%). There was no significant difference between proportion of female patients infected with ESBL and the male patients (p value=0.511). A total of 114 isolates were detected as ESBL positive with the highest ESBL prevalence determined at the OPD 71.9% whiles the in-patients exhibited 28.1%. The differences were not significant when the specimens were compared with prevalence of ESBL isolates production (p. value= 0.454) (Table 3.5).

ESBL production of the isolates as a phenotypic predictor of antimicrobial resistance

The antimicrobial resistance pattern for ESBL positive isolates and the non-ESBL phenotypes were compared to ascertain if ESBL production influenced the antimicrobial resistance prevalence. Most ESBLs were resistant to beta lactams with majority having intermediate resistance towards the carbapenems. With exception of Imipenem and doripenem to which almost all

isolates were sensitive to with no significant resistant pattern (P. value=0.085), considerably higher proportions of ESBL-producers were resistant to the antibiotics tested having their antibiotic resistance patterns being significant (P-value <0.05). Details are shown in Table 8

Table 8: Socio-demographic characteristic, sample type, ward and antibiotic resistance in relation to ESBL phenotype

Variable		ESBL (n=114)	Non-ESBL (n=53)	Total (n=167	P-value
Age		49.17±22.89	40.35±22.04		0.511
Gender					
Female		70(61.4%)	36(67.9%)	106(63.5%)	
Male		44(38.6%)	17(32.1%)	61(36.5%)	
					0.349
Department					
OPD		82(71.9%)	42(79.2%)	124(74.3%)	
WARD		32(28.1%)	11(29.4%)	43(25.7%)	
					0.471
Specimen					
Blood		2(1.8%)	0(0.0%)	2 (1.2%)	
Sputum		8(7.0%)	5(9.4%)	13(7.8%)	
Urine		64(56.1%)	25(47.2%)	89(53.3%)	
Wound		12(10.5%)	9(17.0%)	21(12.6%)	
Other		28(24.6%)	14(26.4%)	40(24.0%)	
					0.454
Antibiotics					
Meropenem	R	9 (7.9%)	0(0.0%)	9 (5.4%)	
	I	6(5.3%)	0(0.0%)	6(3.6%)	
	S	99(86.8%)	53(100%)	152(91.0%)	
					0.019
Ertapenem	R	28(24.6%)	3(5.7%)	31(18.6%)	
	I	3(2.6%)	1(1.9%)	4(2.4%)	
	S	83(72.8%)	49(92.5%)	132(79.0%)	
Imipenem	R	6(5.3%)	0(0.0%)	6(3.6%)	0.033
	I	0(0.0%)	0(0.0%)	0(0.0%)	
	S	08(94.7%)	0(0.0%)	161(96.4%)	
					0.085
Doripenem	R	6(5.3%)	0(0.0%)	6(3.6%)	
	I	0(0.0%)	0(0.0%)	0(0.0%)	
	S	108(94.7%)	53(100%)	161(96.4%)	
					0.085
Cefepime	R	24(21.1%)	2(3.8%)	26(15.6%)	
	I	19(16.7%)	1(1.9%)	20(12.0%)	
	S	71(62.3%)	50(94.3%)	121(72.5%)	

Table 8: Continues

					0.001
Cefoxitin	R	53(46.5%)	9(17.0%)	62(37.1%)	
	I	8(7.0%)	0(0.0%)	8(4.8%)	
	S	53(46.5%)	44(83.0%)	97(58.1%)	
					0
Augmentin	R	76(66.7%)	14(26.4%)	90(53.9%)	
	I	16(14.0%)	11(20.8%)	27(16.2%)	
	S	22(19.3%)	28(52.8%)	50(29.9%)	
					0
Cefamandole	R	82(71.9%)	25(47.2%)	107(64.1%)	
	I	4(3.5%)	2(3.8%)	6(3.6%)	
	S	28(24.6%)	26(49.1%)	54(32.3%)	
					0.003
Ceftriaxone	R	77(69.4%)	7(13.2%)	84(51.4%)	
	I	14(12.6%)	9(17.0%)	23(14.0%)	
	S	20(18.0%)	37(69.8%)	57(34.8%)	
					0
Cefuroxime	R	93(81.6%)	6(11.3%)	99(59.3%)	
	I	3(2.6%)	1(1.9%)	4(2.4%)	
	S	18(15.8%)	46(86.8%)	64(38.3%)	
					0
Cefpodoxime	R	89(78.1%)	10(18.9%)	99(59.3%)	
	I	4(3.5%)	4(7.5%)	8(4.8%)	
	S	21(18.4%)	39(73.6%)	60(35.9%)	
					0
Cefotaxime	R	90(78.9%)	5(9.4%)	95(56.9%)	
	I	9(7.9%)	2(3.8%)	11(6.6%)	
	S	15(13.2%)	46(86.8%)	61(36.5%)	
					0
Ceftazidime	R	53(46.5%)	1(1.9%)	54(32.3%)	
	I	12(10.5%)	0(0.0%)	12(7.2%)	
	S	49(43.0%)	52(98.1%)	101(60.5%)	
					0
Ampicillin	R	108(94.7%)	42(79.2%)	150(89.8%)	
	I	2(1.8%)	1(1.9%)	3(1.8%)	
	S	4(3.5%)	10(18.9%)	14(8.4%)	
					0.005
NON BETA LACTAM					
Tetracycline	R	68(61.3%)	28(52.8%)	96(58.5%)	
	I	15(13.5%)	3(5.7%)	18(11.0%)	
	S	28(25.2%)	22(41.5%)	50(30.5%)	
					0.036
Ciprofloxacin	R	57(50.0%)	9(17.0%)	66(39.5%)	
	I	8(7.0%)	11(20.8%)	19(11.4%)	
	S	49(43.0%)	33(62.3%)	82(49.1%)	
					0
Gentamycin	R	51(44.7%)	10(18.9%)	61(36.5%)	
	I	7(6.1%)	7(13.2%)	14(8.4%)	
	S	56(49.1%)	36(67.9%)	92(55.1%)	
					0.001

Table 8: Continues

Penicillin	R	111(98.2%)	51(96.2%)	162(97.6%)	
	I	0(0.0%)	0(0.0%)	4(2.4%)	
	S	2(1.8%)	2(3.8%)	166(100%)	
					0.45
Chloramphenicol	R	68(51.5%)	18(34.0%)	86(51.5%)	
	I	4(3.5%)	3(5.7%)	7(4.2%)	
	S	42(36.8%)	32(60.4%)	74(44.3%)	
					0.004

Continuous data were presented as mean ± Sd and categorical data presented as proportion. Continuous data were compared to each other using unpaired t-test whilst categorical data compared to each other using Chi-square analysis.

Proportion of enterobacteria isolates stratified by ESBL phenotype and genotype

Phenotypically, ESBLs were found in high proportions in all the clinical isolates tested. Generally ESBL production was detected among 114 (68.26%) isolates. ESBL production was detected in 10(12.8%) *A. baumannii*, 28(34.7%) *E. coli*, 17(24.6) *K. pneumoniae*, 12(15.6%) *P. .mirabilis*, 5(17.5%) *P. aeruginosa and* 13(36.16%) in the other organisms.

ESBL genotypes were significantly present in almost all the isolates phenotypically detected except that BlaSHV was absent in *Acinetobacter baumannii* and *Proteus mirabilis.* Also only *Bla*TEM was present in *Proteus mirabilis* in this study. *Bla*CTX-M and *Bla*TEM genotypes were more prevalent among the isolates than the *Bla*SHV. *Bla*TEM prevalence was significantly higher in *K. pneumoniae* relative to the other organisms.

Some of the isolates had multiple genes, where both *Bla*CTX-M and *Bla*TEM were found in 14 of the isolates, but equal number (9) in both *Bla*TEM with BlaSHV and *Bla*SHV with *Bla*CTX-M. The percentages of the genotypes that expressed *Bla*CTX-M, *Bla*TEM, *Bla*SHV were 28.14%,

35.93% and 19.76% respectively. All the three genes (*Bla*SHV, *Bla*CTX-M and *Bla*TEM) were found in 9 of the isolates (Table 5).

PFGE results for ESBL genes amplified

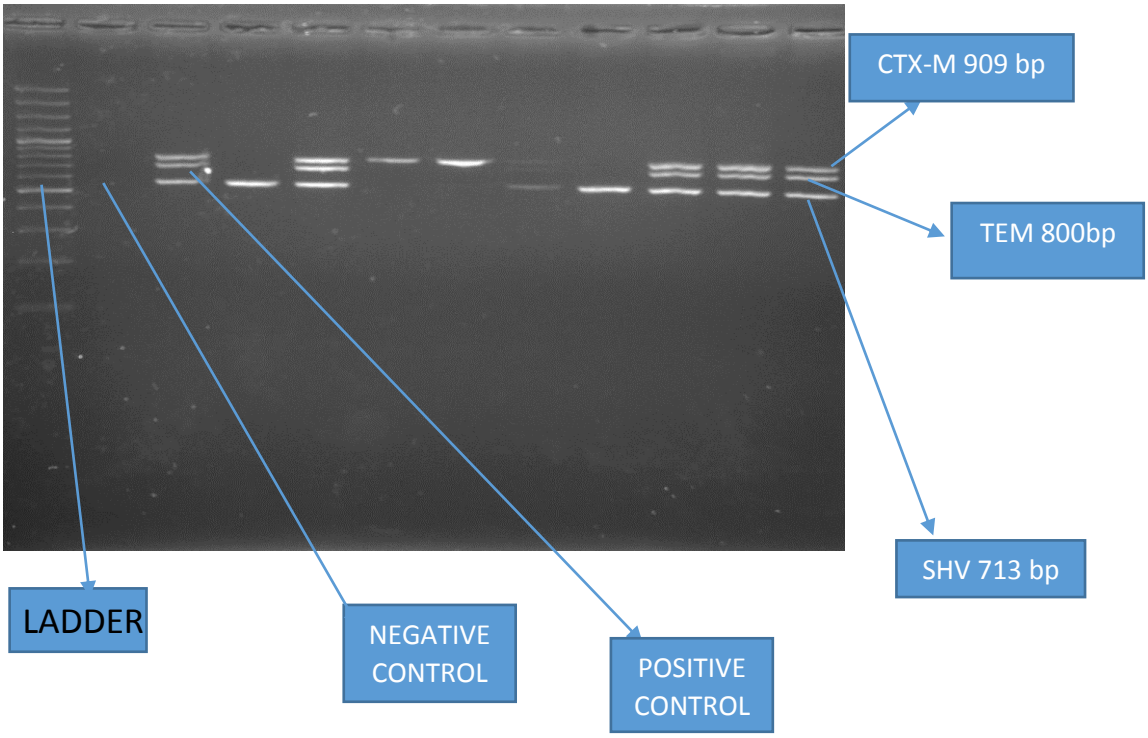

Table 9: Proportion of Enterobacteria isolates stratified by ESBL phenotype and genotype

	Resistance pattern	A. baumannii N=(12)	E. coli N=(46)	K. pneumoniae N=(33)	P. mirabilis N=(20)	P. aeruginosa N=(16)	Others N=(22)
ESBL phenotype	R	10(12.8%)	28(34.7%)	17(24.6)	12(15.6%)	5(17.5%)	13(36.16%)
ESBL genotype	TEM	4(6.7%)	24(40.0%)	11(18.3%)	5(8.3%)	9(15.0%)	7(11.7%)
	SHV	0(0.0%)	11(33.3%)	18(54.5%)	0(0.0%)	3(9.0%)	1(3.0%)
	CTX	3(6.4%)	18(38.2%)	17(36.2%)	0(0.0%)	6(12.8%)	3(6.4%)

Table 10: Prevalence of multiple ESBL genotypes and their distribution among the isolates from the various facilities

	N(%) of isolates with multiple ESBL genes (n=41)							
		Single gene (n=118)			Double genes (n=32)			All three genes present
Facility	All genes absent	TEM only	SHV only	CTX-M Only	TEM+ SHV	TEM+ CTX-M	SHV+ CTX-M	TEM+SHV+ CTX-M
CCTH	25(52.1%)	17(33.3%)	8(35.3%)	15(34.8%)	4(44.4%)	3(21.4%)	4(44.4%)	2(22.2%)
VRH	15(31.3%)	16(31.4%)	8(33.3%)	14(32.6%)	4(44.4%)	4(28.6%)	4(44.4%)	2(22.2%)
ERH	8(16.7%)	18(35.3%)	8(33.3%)	14(32.6%)	1(11.1%)	7(50.0%)	1(11.1%)	5(55.5%)
TOTAL	48(100%)	51(100%)	24(100%)	43(100%)	9(100%)	14(100%)	9(100%)	9(100%)

Discussion

Drug resistance is a global concern. Understanding the epidemiology and routes of transmission in these locations is key to prevention and control. This study has demonstrated high prevalence of ESBLs among the isolates obtained from the Clinical samples from these three facilities. The Out-Patients at CCTH, VRH, and ERH registered high prevalence relative to the In-patients, this could be due to the easy access and abuse of drugs in the community. Although sensitivity was registered in some antibiotics especially the Carbapenems (Meropenem, Ertapenem, Dorepenem and Imipenem), resistance was registered in these isolates with majority showing intermediate resistance. High proportions of the isolates were resistant to almost all the other antimicrobials tested. This prevalence was demonstrated in *E.coli*, showing (34.7%), followed by *K, pneumoniae (24.6%) A. baumannii (12.8%)* and 36.2% for the other isolates.

The resistant proportion to cephalosporins ranged from (46.5%) for ceftazidime and cefoxitin to 94.7% in Ampicillin. Resistance to cefepime, a fourth generation cephalosporins, and alternative antibiotics was 21.1% and 19 isolates showing (16.7%) intermediate resistance. Only 62.3% were sensitive with 37.7% showing multiple resistances thus are multidrug resistant. In 2008, research indicated that in-vitro susceptible cefepime treatment of ESBL producing *E. coli* and *Klebsiella pneumoniae* was associated with a failure rate of 23-83% (Projan, 2008). Most previously susceptible enterobacteria are now developing resistance to these antimicrobials due to their intrinsic resistances and their horizontal and vertical gene transfer (Zhang *et al.*, 2016). Documented evidences attributed these high proportion of resistance to misuse

of drugs by patients (Eibach *et al*., 2016; Obeng-Nkrumah *et al*., 2013) and indiscriminate use of Substandard drugs over the years (Azanu *et al.,* 2016). Also anthropogenic factors such as farmers employing antibiotics in animal production has been recorded in many developing countries including Ghana (Osei-Safo, Egbo, Nettey, Konadu, & Addae-Mensah, 2016). In addition to the above, environmental conditions can result in adaptability (adoptive resistance) of organisms to drugs. This occurs when environmental conditions such as exposure to low-dose antibiotic concentrations, swarming motility, growth states such as biofilm formation, ion exchange such as in *P. aeruginosa* causes resistance to cationic antimicrobial peptides (McPhee *et al*., 2003), availability of few new drugs, exposure to microbes carrying resistant genes, exposure to suboptimal levels of antimicrobials, exposure to broad-spectrum antibiotics, lack of hygiene in clinical environments as well as use of antibiotics in foods/agriculture, have been cited as some of the factors that promote resistance (Huttner *et al*., 2013). Another alarming factor for these high levels of resistance is due to misdiagnoses, over-prescription and inappropriate use of antibiotics**,** and lack of trained personnel to accurately perform antimicrobial susceptibility test. Due to these, most antibiotics have been abused and are now ineffective for the treatment of preciously susceptible organism.

Major beta-lactams to which resistance were found in this study included Ampicillin (94.7%), Cefuroxime (81.6%), Cefamandole (71.9%), Ceftriaxone (69.4 %,), Augmentin (66.7%), Cefpodoxime (78.1%), Cefotaxime (78.9%). All these, registered more than 50% resistance. Although most of them were of the 2nd and 3rd generation cephalosporins and are mostly

injectable, high resistance to these indicate that their abuse is on the increase, hence control measures ought to be put in place since they are major choices for the treatment of severe infections in most facilities in Ghana (Saana, Adu, Gbedema, & Duredoh, 2014). Comparing the resistance pattern between the three common organisms; *E.coli K, pneumoniae and Pseudomonas aeruginosa,* there was significant difference in the antimicrobial resistance. In order to ascertain where the specific significance occurred, an adjusted P. value was calculated between the resistance, intermediate and sensitive patterns, it was observed that most of the significant resistance occurred in *Pseudomonas aeruginosa*. These organisms are opportunistic and mostly associated with health care associated infections. Due to the multifaceted nature of these organisms, serious infection prevention and control practices must be encouraged in the various facilities (Favero *et al.*, 1971; Sousa & Pereira, 2014).

ESBL prevalence detected phenotypically ranged from 6.7% in *Acinetobacter baumannii* followed by 8.3% *Proteus mirabilis,* 15.0% *Pseudomonas aeruginosa*, 18.3% *Klebsiella pneumoniae* and 40% for *E. coli.* Other isolates recorded 11.7%. This higher prevalence in *E. coli* could be due to the higher urine samples since this organism is the commonest cause of urinary tract infection. Upon molecular detection, ESBL genotypes TEM, SHV and CTX-M were found in 118(71.51%) out of 167 ESBLs genotypically identified. The percentages of the genotypes that expressed *Bla*CTX-M, *Bla*TEM, *Bla*SHV were 28.14%, 35.93% and 19.76% respectively. The overall prevalence of ESBL detected was 71.51 %. Comparing this value to previous works done in Ghana, in 2013, Obeng-Nkrumah and co established

that 50% of the *Klebsiella pneumoniae* and 29% of the *Escherichia coli* bloodstream isolates. In 2014, Feglo and Opoku established that there is high prevalence of AmpC- and ESBL- producing *P. aeruginosa* and *P. mirabilis* strains circulating in the Komfo Anokye Teaching Hospital and in the community with higher antimicrobial resistance than the non AmpC and ESBL strains. Also a study conducted at Komfo Anokye Teaching hospital with three other tertiary hospitals in the Northern belt indicates the prevalence of ESBL production to be 57.8% among the isolates; a significantly high level (Adu, 2016). Comparing these prevalence with this study, there is actually an increasing rate of drug resistance in these tertiary hospitals. This deserves future surveillance studies because treatment of these multi-drug resistant organisms is a therapeutic challenge.

The high prevalence of ESBL call for immediate intervention strategies to prevent further spread. Training of laboratory personnel on phenotypic testing of ESBLs in addition to training clinical staff and prescribers on ESBL issues are advocated.

Summary

This research was set to determine the molecular epidemiology of ESBLs. In study, ESBL genotypes were significantly present in almost all the isolates phenotypically detected except that *Bla*SHV was absent in *Acinetobacter baumannii* and *Proteus mirabilis.* Also only *Bla*TEM was present in *Proteus mirabilis* in this study. *Bla*CTX-M and *Bla*TEM genotypes were more prevalent among isolates than the *Bla*SHV. *Bla*TEM prevalence was significantly higher in *K. pneumoniae* relative to the other organisms.

Some of the isolates had multiple genes, where both *Bla*CTX-M and *Bla*TEM were found in 14 of the isolates, but equal number (9) in both *Bla*TEM with *Bla*SHV and *Bla*SHV with *Bla*CTX-M. All the three genes (*Bla*SHV, *Bla*CTX-M and *Bla*TEM) were found in nine of the isolates. Upon molecular confirmation, the overall prevalence of ESBL detected was 71.51 %.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Though a number of studies have been done on ESBLS in Ghana, this research has immensely added to knowledge concerning the prevalence in the Cape Coast Teaching Hospital, Volta Regional Hospital and Efia Nkwanta Regional Hospital. This study has indicated the varied demographical epidemiology of these institutions and their relative drug resistant patterns.

Conclusion

Generally, the outcome of this study detected high antimicrobial resistance amongst enterobacteria to the commonly prescribed antimicrobial drugs at CCTH, VRH AND ERH. These resistance levels, perhaps are attributed to ESBL production by these isolates obtained from both the community and the hospital wards. The isolates demonstrated higher sensitivity against Doripenem, imipenem, Ertapenem, Cefamandole and Chloramphenicol whiles most beta-lactams demonstrated significant resistance and intermediate reactivity to these organism. All three ESBL genes (*Bla*CTX-M, *Bla*TEM and *Bla*SHV) tested for were prevalent among the isolates and there was significant difference between the prevalence from the various facilities.

The high levels of antimicrobial resistance and the widespread prevalence of ESBL producing enterobacteria especially in *Pseudomonas aeruginosa* highlights the necessity to adopt immediate intervention strategies to prevent severe healthcare associated infections in the hospital and to continuously monitor ESBL spread the community. Training of laboratory staff on phenotypic testing of ESBLs and other clinical staff and prescribers on ESBL issues are necessary and advocated.

Recommendation

This study has led to the identification of key areas that require further research because there are questions that remained unasked or unanswered. Also there are areas that need to be delved into. These include:

1. Plasmid analysis, phylogenetic analysis and MLST should be used to compare the molecular epidemiology of ESBL isolates from these institutions since there were relatively high levels of multidrug resistance in the isolates.

2. Policy makers should put strict measures in place to control the use of drugs especially antimicrobials in the country.

3. The Health ministry should work with the stake holders to train and refresh health professionals and Healthcare industries on the accurate, effective diagnosis and treatment of ESBLs.

4. Screening of ESBLs and carbapenem resistance should be incorporated in routine laboratory analysis.

5. Agricultural sector should also put measures in place to control the use of antibiotics by farmer.

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