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DEPARTMENT OF CLINICAL MICROBIOLOGY

EXTENDED-SPECTRUM-BETA-LACTAMASE PRODUCTION AMONG ESCHERICHIA COLI AND KLEBSIELLA SPECIES AT THE KOMFO ANOKYE TEACHING HOSPITAL IN KUMASI, GHANA.

THESIS SUBMITTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE MASTER OF SCIENCE DEGREE (MSC.) IN CLINICAL MICROBIOLOGY

BY

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DECLARATION

I declare that this thesis is my own work towards the award of an MSc in Clinical Microbiology and that, to the best of my knowledge, it does not contain any materials previously published by another person or accepted for the award of any other degree of any university, except where due acknowledgement has been made in the text.

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DEDICATION

I dedicate this work to God, my family and friends.

ABSTRACT

In recent times enterobacteriaceae including *Escherichia coli* and *Klebsiella spp.*, isolated at the Komfo Anokye Teaching Hospital (KATH) have shown significant resistance to 2nd and 3rd generation cephalosporins. In 2006, 18-32% of all enterobacteriaceae isolated from urine and blood were resistant to the cephalosporins cefuroxime, ceftazidime, cefotaxime and ceftriaxone. Microbial resistance to these antimicrobials if due to the production of Extended Spectrum-Beta-Lactamases (ESBLs) may also indicate resistance to the aminoglycosides and other antibiotics. This limits therapeutic options for the treatment of severe infections caused by these organisms. This study was undertaken to determine the prevalence of ESBL-production among *E. coli* and *Klebsiella spp.* at the KATH. A total of 300 non-selected, non-duplicate isolates were collected over a period of three months. ESBL production was phenotypically determined using the Combined Disc Method. Of the 300 isolates, 151 (50.33%) and 149 (49.67%) were *E. coli* and *Klebsiella spp.* respectively. Again, 149 (49.67%) out of the grand total were ESBL- producing *E. coli* (67; 44.37%) or *Klebsiella spp.* (82; 55.03%). These ESBL-producers were isolated from blood, urine, sputum, wound, pus, and other aspirates. ESBL-producing organisms were more common among in-patients (54.60%) than they were among out-patients (46.27%), although the differences were not statistically significant (odds ratio (OR), 1.40; 95% CI, 0.79 to 2.46; p=0.30). Again there was also no significant association between ESBL production and gender (OR, 0.99; 95% CI, 0.62-1.56; p=0.97). Meropenem, a carbapenem, emerged as the antimicrobial agent of choice for the treatment of serious infections associated with ESBL-producers on the basis of *in vitro* susceptibility testing carried out. Meropenem however, is expensive and its use in non-severe infections may be inappropriate. Nitrofurantoin seemed to be the agent of choice for urinary tract infections when ESBL- producers are susceptible to it. The high level of ESBL production found in these enterobacteriaceae with the resultant microbial resistance to the available cephalosporins and other agents may pose difficulties with the choice of therapeutic options for the treatment of severe infections. Efforts to prevent and/or control outbreaks of infections with ESBL-producing organisms must emphasize on the judicious use of all antibiotics as well as barrier precautions.

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CHAPTER ONE

1.0 Introduction

Extended-Spectrum-Beta-Lactamases (ESBLs) are enzymes which confer antibiotic resistance on certain bacteria in the family *Enterobacteriaceae* (Yan *et al.,* 2000). ESBLs are penicillin-destroying enzymes capable of efficiently hydrolyzing penicillins, narrow spectrum cephalosporins, many extended-spectrum cephalosporins, the oxymino group containing cephalosporins (e.g. cefotaxime and ceftazidime), and monobactams (Paterson and Bonomo, 2005). *Enterobacteriaceae* are Gram negative rods with peritirichous flagella all over their surface. They possess a complex antigenic structure, and produce a variety of toxins and other virulence factors. They also ferment a wide range of carbohydrates and produce carbon dioxide and other gases. They may be found in the intestinal tracts of animals and humans, in soil or in water. Examples are, *Salmonella spp., Escherichia coli*, *Klebsiella spp., Enterobacter spp. and Proteus spp.,* (Cheesbrough, 2000 and Brooks *et al.*, 2004). Of particular interest to this study are *Escherichia coli* and *Klebsiella spp.,* the most common Gram negative rods isolated in the laboratory (Brooks *et al.*, 2004).

It has become very important to study the prevalence of ESBL-producing organisms because of the increasing antimicrobial resistance and the decreasing number of new drugs available against such microbes (Kader *et al.*, 2006). Since the initial description of ESBLs, microbial isolates that are resistant to broad-spectrum cephalosporins are being increasingly recognized (Itokazu *et al.*, 1996).

The problem of antimicrobial resistance may be due to the fact that antibiotics can be obtained and used without medical authorization or supervision in developing countries (Hart and Kariuki, 1998), such as Ghana. This situation has led to inappropriate usage of antibiotics with patients taking the drugs for insufficient length of time or at suboptimal dosages, which may result in antimicrobial resistance.

The presence of an ESBL-producing organism in a clinical infection can cause significant treatment problems because ESBL-mediated resistance may result in treatment failure if any of the third generation cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) or a monobactam (aztreonam) are used (Kang *et al.*, 2004 and Drieux *et al.*, 2008). ESBL-producing organisms may also be difficult to detect because of the effect of their different levels of activity against various cephalosporins, thus making the choice of which cephalosporin to test critical. If an ESBL-producer is detected, it should always be reported as resistant to the penicillins, cephalosporins, and monobactams even if *in vitro* test results indicate susceptibility, since these may fail in treatment (Harbarth *et al.*, 2003 and Clinical and Laboratory Standards Institute, 2007).

It is worth noting that the therapeutic options for infections caused by ESBL-producing organisms are significantly limited because the organisms are also frequently resistant to other non beta-lactam drugs such as aminoglycosides, quinolones, trimethoprim or trimethoprim-sulfamethoxazole and tetracyclines (Kang *et al.*, 2004 and Pitout *et al.,* 2005). This is because the plasmids bearing the genes which encode for ESBLs frequently also carry genes encoding for resistance to these classes of antibiotics (Paterson and Bonomo, 2005).

1. 1 Problem statement and Justification of study

Microorganisms which produce ESBLs could be of great health concern at Komfo Anokye Teaching Hospital (KATH) not just because they have been reported worldwide but also because in 2006, 32.00% and 18.15% of all *Enterobacteriaceae* isolated from urine and blood respectively, from KATH, were resistant to cephalosporins (cefuroxime, ceftriaxone, cefotaxime and ceftazidime) (KATH Lab records, 2006). Again, records from the KATH Microbiology Laboratory in 2006 indicated that, of the total *Enterobacteriaceae* isolated from urine 40.48% were resistant to gentamicin (an aminoglycoside), 33.33% to ciprofloxacin (a fluoroquinolone) and 86.74% to tetracycline. Also, of the *Enterobacteriaceae* isolated from blood in that same year, 23.34% were resistant to gentamicin while 12.66% were resistant to ciprofloxacin and 63.94% were resistant to tetracycline. Some of these multi-resistant isolates could probably harbour ESBLs thus limiting therapeutic options. The delay in detecting and reporting ESBL-producers may lead to prolonged hospitalization of patients, increased morbidity and mortality as well as increased cost of health care (Lautenbach *et al.*, 2001).

ESBL-producers may be carried in the intestinal tracts for long periods of time and shed faecally, thus have the potential of spreading widely once introduced into a community (Louis, 2001), especially those with poor hygiene and sanitation.

It is worth knowing how common or widespread ESBLs are considering the problems mentioned above. This will aid doctors in the selection of antibiotics for patients use and also help in the planning of strategies to control the spread of ESBL-producers.

1. 2 Aim of study

The aim of this study is to investigate ESBL production among isolates of *E. coli and Klebsiella spp.,* two common Gram negative organisms isolated from blood and urine, at the Komfo Anokye Teaching Hospital.

The specific objectives are;

- To determine the antimicrobial susceptibility of *E. coli* and *Klebsiella spp.* at KATH.
- To determine if resistance to any of the ß-lactams *E. coli* and *Klebsiella spp.* is due to ESBL production.
- To determine the association between particular demographic factors and the presence of ESBL-producers.
- To make recommendations for the routine laboratory diagnosis of ESBL production in these organisms and their treatment.

CHAPTER TWO

2. 0 Literature review

2. 1 Antibiotic resistance

Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic (Brooks *et al.*, 2004). In the laboratory, an isolate is described as resistant to an antimicrobial when it is not inhibited by the usual concentrations of the agent with normal dosage schedules, and/or when it demonstrates zone diameters that fall in the range where specific resistance are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies (Clinical and Laboratory Standards Institute (CLSI), 2007).

Antibiotic resistance may develop naturally via natural selection through random mutation or through a process known as programmed evolution. Programmed evolution is a regulated process in which organisms switch on altered levels (usually higher) of mutagenesis. This is usually in response to sensed, non-mutagenic environmental cues. The three main mechanisms by which Gram- negative organisms exhibit resistance to antimicrobials are:

- Decreased permeability of the drug into the cell
- Hydrolysis of the drug by enzymes
- Decreased affinity of the target (Piddock *et al.,* 1997 and Cheesbrough, 2000).

Of these, the major mechanism of resistance in Gram negative organisms, which causes clinically significant infection, remains the expression of β-lactamases (Piddock *et al.,* 1997),

which are enzymes capable of hydrolyzing the β-lactam ring of penicillins, cephalosporins, and related antimicrobial drugs, rendering them inactive. There are a number of β-lactamases, which vary in substrate specificity and host range (Bush *et al.*, 1995).

Except for *Salmonella spp.*, all *Enterobacteriaceae* produce intrinsic chromosomal encoded beta-lactamases which are responsible for intrinsic resistance of individual species to some antibiotics (Susić, 2004).

2. 2 Antimicrobial Susceptibility Test (AST) for *E. coli* and *Klebsiella spp.*

Antimicrobial agents recommended for use in AST on Gram negative rods include a second and third generation cephalosporin, piperacillin, antipseudomonal penicillin, trimethoprim-sulfamethoxazole, fluoroquinolone and aminoglycoside. Nitrofurantoin, a quinolone, and a trimethoprim are included in the AST when the organism is isolated from urine (Brooks, 2004).

2. 3 Treatment of *E. coli* and *Klebsiella spp.* infections

E. coli and *Klebsiella spp.* are organisms of medical importance because they are responsible for causing several infections in humans, including urinary tract and blood stream infections (Kang *et al.*, 2004). At present, cephalosporins are recommended for the treatment of infections caused by *Klebsiella spp.* (Atlas, 1995 and Brooks *et al.*, 2004). The second-line treatment options are the trimethoprim-sulfamethoxazoles, aminoglycosides, carbapenems (imipenem/ meropenem), fluoroquinolones, penicillins (piperacillin and mezlocillin) or monobactam (aztreonem) (Atlas, 1995 and Brooks *et al.*, 2004).

Again a cephalosporin is the drug of choice for treating blood stream infections caused by *E. coli* (Brooks *et al*, 2004). A carbapenem, aminoglycoside or fluoroquinolone is used in cases where the *E. coli* is resistant to the cephalosporins (Brooks *et al*., 2004).

Urinary tract infections involving *E. coli* are treated with a fluoroquinolone or nitrofurantoin as the first-line options whereas the second-line treatment regiment involves the use of trimethoprim-sulfamethoxazole, fosfomycin or an oral cepholosporin such as cefuroxime (Brooks *et al*., 2004).

Of the eight classes of drugs recommended for the treatment of *E. coli* and *Klebsiella spp.* infections, five are regularly used in antimicrobial susceptibility testing at KATH either as first- or second-line drugs. These are the cephalosporins (cefotaxime, ceftriaxone, ceftazidime and cefuroxime), aminoglycosides (gentamicin and amikacin), fluoroquinolone (ciprofloxacin, norfloxacin and levofloxacin), trimethoprim-sulfamethoxazole (cotrimoxazole), nitrofuran (nitrofurantoin) and carbapenem (meropenem and imipenem) (KATH record, 2008).

2. 4 β-lactam antimicrobial agents

The β-lactam drugs derive their name from the characteristic ring structure that they all share (the β-lactam ring). The β- lactam drugs are a family of antimicrobial agents consisting of four major groups: penicillins; cephalosporins; monobactams; and carbapenems (Samaha-Kfoury and Araj, 2003).

All β-lactam drugs typically work by interfering with the synthesis of the bacterial cell wall which are made of a complex polymeric material called peptidoglycan, containing both amino acids and amino sugars. The two kinds of amino sugars found in the peptidoglycan layer are N-

acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). These two form a linear polymer of NAG alternating with NAM which is linked by a glycosidic bond between the number 1 and number 4 carbons (this is the linkage attacked by lysozyme). Four or five amino acids are attached to each NAM as side chains. These form covalent bonds with amino acids in adjacent chains (Brooks *et al.*, 2004).

2. 5 Mechanism of action of cephalosporins

Cephalosporins are bactericidal agents which have a broad spectrum activity and thus are used to treat severe systemic infections caused by Gram negative organisms (Atlas, 1995). They are also the drug of choice for patients who are allergic to penicillin, and for microbes that are resistant to penicillin (Atlas, 1995). Cephalosporins, just like penicillins, are β-lactam agents and work by attaching to the receptors of the wall and then inhibiting the formation of cross-linkages within the peptidoglycan backbone of the bacterial cell walls (Brooks *et al*, 2004). This is probably followed by the removal or deactivation of an inhibitor of autolytic enzymes in the cell wall (Atlas, 1995). This in turn activates the lytic enzyme and results in cell lysis if the environment is isotonic or hypotonic to the bacterial cell (Brooks *et al*, 2004).

In Ghana, ceftriaxone, cefuroxime and cefotaxime are recommended cephalosporins for the treatment of infections (Standard Treatment Guidelines (STG), 2004). These infections include meningitis, typhoid fever, UTIs, acute otitis media and sexually transmitted infections (STIs) such as gonorrhoea (STG, 2004).

2. 6 History of ESBLs

The first ß-lactamase was identified in *E. coli* before the release of penicillin for use in clinical practice (Knothe *et al.,* 1983 and Philippon *et al.,* 1989). The naturally occurring chromosomally mediated ß-lactamase of many Gram-negative bacteria was thought to have evolved from penicillin-binding proteins. This development was probably due to the selective pressure exerted by ß-lactam-producing soil organisms found in the environment (Rice *et al.*, 1990 and Ghuysen, 1999).

The first plasmid-mediated ß-lactamase in gram-negatives was however described in the early 1960s. It was named TEM-1, after the patient from whom the organism was isolated (Temoniera) (Knothe *et al.,* 1983 and Bradford, 2001). The TEM-1 enzyme spread worldwide within a few years after its isolation in Greece (Bradford *et al*, 1994 and Paterson *et al.*, 2003). Another common plasmid-mediated ß-lactamase found in *E. coli* and *Klebsiella pneumoniae* is the SHV-1 (for sulphydryl variable). The SHV-1 ß-lactamase is chromosomally encoded in the majority of isolates of *K. pneumoniae* but is usually plasmid mediated in *E. coli* (Bradford *et al.*, 1994 and Paterson *et al.*, 2003).

The introduction of new ß-lactam drugs in the treatment of patients has always been met by the emergence of new ß-lactamases that caused resistance to the new class. Oxymino-cephalosporins specifically ceftazidime, which was widely used in the 1980's for the treatment of serious Gram-negative infections, soon became ineffective as a results of the emergence of ESBL-producers (Bradford, 2001).

The first ESBL, SHV-2, was isolated from *Klebsiella ozaenae* in Germany and it was described in 1983 (Knothe *et al.,* 1983 and Pitout *et al*, 2005). Currently, over 200 different ESBLs have been described (Paterson and Bonomo, 2005) and these have been found in *Enterobacteriaceae* and *Pseudomonaceae* all over the world (Chanal *et al.*, 1996). However most of the ESBLs described have been isolated from *E coli* and *Klebsiella pneumoniae* (Pitout *et al*, 2005) which are major nosocomial pathogens which cause intra-abdominal infections, urinary tract infection, and primary bacteremia (Kang *et al.*, 2004).

It should be noted that ESBLs are not the sole β-lactamases in *E. coli* and *Klebsiella spp.* to confer resistance against the second and third generation cephalosporins, but they are the most important. Other modes of resistance to these cephalosporins include plasmid-mediated AmpC β-lactamases, in *Klebsiella spp.* and *E. coli* (rare) and hyperproduced K1 chromosomal β-lactamases in *K. oxytoca* (Standards Unit, Evaluation and Standards Laboratory (SUESL), 2005).

2. 7 Characteristic features of ESBL-mediated resistance

One characteristic feature of ESBL-mediated resistance in *Enterobacteriaceae* is the resistance to penicillins, as well as to second-generation and one or more of the third and fourth generation cephalosporins or aztreonam (Pitout *et al.*, 2005 and Drieux *et al.,* 2008). However TEM- and SHV-type ESBLs are not able to hydrolyze extended spectrum ß- lactam antibiotics as efficiently as their parent enzymes (Bradford, 2001).

Another feature of ESBL-mediated resistance is the increased susceptibility to ß-lactamase inhibitors particularly clavulanate (Bradford, 2001). This is due to the expansion of the active site of ESBL-producers which allows its increased activity against extended-spectrum

cephalosporins (Bradford, 2001). This characteristic feature is what makes possible a synergy between cephalosporins and β-lactamase inhibitor (Drieux *et al.*, 2008). Sulbactam and tazobactam are other examples of β-lactamase inhibitors. In general, clavulanic acid and tazobactam have better inhibitory activity than sulbactam against extended-spectrum β-lactamases and the enzymes from which they evolved (Payne *et al*, 1994).

2. 7. 1 Clavulanate

Clavulanate or clavulanic acid is a β-lactamase inhibitor sometimes combined with penicillin group antibiotics to overcome certain types of antibiotic resistance arising from β-lactamase enzymes in certain bacteria (Bradford, 2001). The most common type is the potassium salt, potassium clavulanate, which is combined with amoxicillin to form co-amoxiclav otherwise known as Augmentin. Clavulanic acid has a β-lactam ring that is characteristic of β-lactam antibiotics and this similarity in chemical structure allows the molecule to act as a competitive inhibitor of β-lactamases secreted by certain bacteria to confer resistance to β-lactam antibiotics. Clavulanic acid also has negligible intrinsic antimicrobial activity. This inhibition restores the antimicrobial activity of β-lactam antibiotics against β-lactamase-secreting resistant bacteria (Bradford, 2001).

2. 8 Classification of ESBLs

ESBLs are classified either according to the Ambler or the Bush system. The Ambler classification is based on the molecular structure of the enzyme, such as the amino acid sequence. The majority of ESBLs belong to Ambler class A (a few belong to class D) (Pitout *et*

al., 2005) and are characterized by an active serine site, a molecular mass of approximately 29,000 Da and the preferential hydrolysis of penicillins (Bradford, 2001).

The Bush classification (a more recent scheme) on the other hand, takes into account the biochemical properties of the enzyme, the molecular structure, and the nucleotide sequence of the gene to classify ß-lactamases into functional groups. According to the Bush classification, ESBLs belong to the functional group 2be (Aibinu *et al.*, 2003).

2.9 Types of ESBLs

2. 9.1 Cephalosporin-hydrolyzing mutants of TEM and SHV

Most of the ESBLs described currently are derivatives of TEM and SHV enzymes (Taşh and Bahar, 2005).

2. 9. 1. 1 TEM

TEM-3, the first TEM–type ß-lactamase to exhibit the ESBL phenotype, was first reported in 1989. There are currently more than 90 derivatives of the TEM-type that have been described (Bradford, 2001). They have been reported in the genera of *Enterobacteriaceae* such as *E coli*, *K. pneumoniae, Enterobacter aerogenes*, *Morganella morganii*, *Proteus mirabilis*, *Proteus rettgeri*, and *Salmonella spp.* (Perilli *et al,* 2000 and Bradford, 2001). TEM-type ESBLs have also been found in non-Enterobacteriaceae gram-negative bacteria. The TEM-42 ß-lactamase was found in a strain of *P. aeruginosa* (Perilli *et al.,* 2000 and Bradford, 2001).

Amino acid substitutions occur within the TEM enzyme at limited number of positions. The combinations of these amino acid result in the different ESBL phenotypes. A number of amino

acid residues are especially important for producing the ESBL phenotype when substitutions occur at that position. They include glutamate to lysine at position 104, arginine to either serine or histidine at position 164, glycine to serine at position 238, and glutamate to lysine at position 240 (Perilli *et al*, 1997). A naturally occurring TEM-like enzyme, TEM-AQ, that contains a number of amino acid substitutions and one amino acid deletion that have not been noted in other TEM enzymes, has however been described (Perilli *et al*, 1997 and Bradford, 2001).

2. 9. 1. 2 SHV

Unlike TEM-type β-type-lactamases, there are relatively few derivatives of SHV-1 β-lactamase. Changes have occurred in few positions within the structural gene of the SHV β-lactamase to give rise to SHV ESBLs (Bradford, 2001). The majority of SHV ESBLs are characterized by the substitution of a serine for glycine at position 238. A number of variants related to SHV-5 also have a substitution of lysine for glutamate at position 240. It is interesting that both the Gly238Ser and Glu240Lys amino acid substitutions mirror those seen in TEM-type ESBLs. The serine residue at position 238 is critical for the efficient hydrolysis of ceftazidime, and the lysine residue is critical for the efficient hydrolysis of cefotaxime (Huletsky *et al*, 1993).

2. 9. 2 CTX-M type

These enzymes are also plasmid-mediated ESBLs but not closely related TEM or SHV β-lactamase. A phylogenic study of CTX-M ESBLs showed four major types: CTX-M-1type; CTX-M-2 type; Toho-2 type; and CTX-M-8. Kinetic studies have shown that the CTX-M-type β-lactamases hydrolyze cephalothin or cephaloridine better than benzylpenicillin and they preferentially hydrolyze cefotaxime over ceftazidime (Bradford *et al*, 1998 and Tzouvelekis *et*

al, 2000). Although there is some degree of hydrolysis of ceftazidime by these enzymes, it is usually not enough to provide clinical resistance to organisms in which they reside. CTX-M ESBLs have mainly been found in strains of *E coli* and *Salmonella enterica* serovar Typhimurium. It has been suggested that the serine residue at position 237, which is present in all of the CTX-M enzymes, plays an important role in the extended-spectrum activity of the CTX-M-type β-lactamases (Tzouvelekis *et al*, 2000).

Another unique feature of these enzymes is that they are inhibited better by the β-lactamase inhibitor tazobactam than by sulbactam or clavulanate (Bradford *et al.*, 1998 and Bradford, 2001). Strains expressing CTX-M-type β-lactamases have been isolated from many parts of the world, but have most often been associated with faecal outbreaks in Eastern Europe (Gazouli *et al.*, 1998), South America, and Japan (Ma *et al.*, 1998).

2. 9. 3 Obscure types

There are a number of other types of ESBL known as the obscure types. These are less common, and include OXA, PER, VEB, CME, TLA, SFO and GES types (Bradford, 2001).

2. 10 Risk factors associated with ESBL-producing organisms

Infections with ESBL-producing *E. coli* and *K. pneumoniae* have been reported to be associated with certain conditions or factors. Some of these include, length of hospital stay (Penã *et al.,* 1997) , time spent in the Intensive Care Unit (ICU) (Rice, 1999), hospitalization (Pitout *et al.*, 2005 and Melzer and Petersen, 2007), severity of illness - e.g. peritonitis, neutropenia, and severe sepsis (Kang *et al.*, 2004), intubation and mechanical ventilation (Penã *et al.,* 1997 and Bradford, 2001), urinal and arterial catheterization (Bradford, 2001), diabetes mellitus

(Colodner *et al.*, 2004), male gender over 60 years of age (Pitout *et al.*, 2005) and previous exposure to antibiotics (in terms of both total number of antibiotics and total duration of antibiotic treatment) especially extended spectrum cephalosporins, fluoroquinolones, aminoglycosides, cotrimoxazole, vancomycin and metronidazole (Lautenbach *et al.*, 2001 and Kang *et al.*, 2004).

It is believed that a series of events may be required for infection with ESBL-producing *E. coli* and *Klebsiella spp.* to develop (Lautenbach *et al.,* 2001). They may arise as a result of the selective effect of antibiotics, especially extended-spectrum cephalosporins or contact with a contaminated and/or a colonized source (Lautenbach *et al.,* 2001).

2. 11 Mode of spread of ESBL-producing organisms

Environmental sources of Gram negative rods, including ESBL-producers, are contaminated glass thermometer (Rogues *et al.*, 2000), blood pressure cuffs (Bureau-Chalot *et al.*, 2004), bronchoscope (Bradford *et al.,* 1998) and ultrasonography coupling gel (Gaillot *et al.*, 1998). Cockroaches have also been implicated as possible vectors of infections caused by ESBL-producers (D'Agata *et al.*, 1998). ESBL-producing organisms have been isolated from patients' soaps (Szabo *et al.*, 1999), sink basins (Hobson *et al.*, 1996) and babies' baths (Eisen *et al.*, 1995). An important means of transfer of ESBL-producers to patients in a health facility is through contact with a health care worker whose hands have been colonized by these organisms (Eisen *et al.*, 1995, Szabo *et al.*, 1999 and Paterson and Bonomo, 2005).

2. 12 Infections caused by ESBL-producers

Infections caused by ESBL-producing bacteria and other coliforms can be subdivided into various organs or systems as follows: urinary tract infection, bacteremia-primary or secondary, respiratory tract infection, gastrointestinal tract infection (intra-abdominal abscess, peritonitis and cholangitis), skin and soft tissue infection, catheter or device related infection, sinusitis and neurosurgical meningitis related to ventricular drainage catheters (Consensus Guidelines for the Management of Infections by ESBL-Producing Bacteria(CGMIEB), 2001).

2. 13 Epidemiology

The prevalence of ESBLs isolates varies from country to country and from institution to institution. The occurrence of ESBL production in *Enterobacteriaceae* stands at about 3% in the USA according to the Center for Disease Control and Prevention (Bradford, 2001).

In Europe, the prevalence of ESBL production among countries varies greatly. A survey of 11 hospital laboratories in Netherlands showed that less than 1% of *E. coli* and *K. pneumoniae* strains produced ESBLs (Bradford, 2001). Croatia has 4.7% and 36.8% as its prevalence rate for *E. coli* and *K. pneumoniae* respectively (Marija *et al.*, 2005). However in Turkey the prevalence of ESBL producers among *E. coli* is 17% and that among *K. pneumoniae* is 57.1% (Taşh and Bahar, 2005). It is important to note that while the proportion of ESBL-producers may be decreasing in some parts of Western Europe, a significant increase may be occurring in Eastern Europe (Sekowska *et al.*, 2002).

The situation is no different in Asia. Japan has a very low prevalence level of <0.1 of *E. coli* and 0.3 of *K. pneumoniae* strains possessing ESBL (Yagi *et al.*, 2000) while Korea, on the other

hand, has 7.7% of *E. coli* and 15.9% of *K. pneumoniae* strains producing ESBLs (Kang *et al.*, 2004). In China, 16% of *E. coli* and 17% of *K. pneumoniae* were ESBL-producers (Ling *et al.*, 2006). ESBL production was found to be 41% in *E .coli* and 40% in *K. pneumoniae* in urine isolates in India (Babypadmini and Appalaraju, 2004).

ESBL producing organisms have also been isolated in Africa. South Africa and Kenya have reported several outbreaks of infections due to ESBL-producing *Klebsiella spp.* (Akindele and Rotilu, 1997 and Shipton *et al.,* 2001). A novel enzyme, CTX-M-12, has been found in Kenya (Kariuki *et al.*, 2001). Studies have reported that 14.7% of all *E. coli* and 20.8% of all *K. pneumoniae* strains in Nigeria are ESBL-producers (Aibinu *et al.*, 2003). Despite the many problems that may be associated with ESBL-producing *Enterobacteriaceae*, there is no information on their epidemiology in Ghana.

2. 14 ESBL detection methods

2. 14. 1 Disc Diffusion Method (DDM)

2. 14. 1.1 Screening for ESBL-producers

This method can detect the production of ESBLs by *Escherichia coli*, *Klebsiella spp.* and *Proteus mirabilis*. This is done by noting specific zone diameters which indicate a high level of suspicion for ESBL production (CLSI, 2007). Either cefpodoxime (10µ), ceftazidime (30µ), aztreonam (30µ), cefotaxime (30µ), or ceftriaxone (30µ) is used. However, the use of more than one of these agents for screening improves the sensitivity of detection. If an organism is resistant to any of the cephalosporins, a phenotypic confirmatory test is performed to ascertain the diagnosis.

2. 14. 1. 2 Confirmatory Test

The CLSI advocates use of cefotaxime (30 µg) and ceftazidime discs (30µg) with and without clavulanate (10 µg) for phenotypic confirmation of the presence of ESBLs (CLSI, 2007) in *E. coli* and *Klebsiella spp.* The CLSI recommends that the disc tests be performed with confluent growth on Mueller-Hinton agar. A difference of 5 mm or more between the zone diameters of either of the cephalosporin discs and their respective cephalosporin/clavulanate disc is taken to be a phenotypic confirmation of ESBL production (CLSI, 2007).

Figure 2. 1 A photograph of the CLSI confirmatory test (CGMIEB, 2001)

2. 14. 2 Dilution method

2. 14. 2. 1 Screening for ESBL-producers

Screening can be done by dilution antimicrobial susceptibility tests for ESBL production by *E. coli* and *Klebsiella spp.* Ceftazidime, aztreonam, cefotaxime, or ceftriaxone can be used at a screening concentration of 1 µg/ml (CLSI, 2007). Growth at this screening antibiotic

concentration (that is, minimum inhibition concentration (MIC) of the cephalosporin of 2µg/ml) is suspicious of ESBL production and is an indication that the organism should be tested by a phenotypic confirmatory test (CLSI, 2007).

2. 14. 2. 2 Confirmation test

This is performed by broth microdilution assays using ceftazidime (0.25 to 128 µg/ml), ceftazidime plus clavulanic acid (0.25/4 to 128/4 µg/ml), cefotaxime (0.25 to 64 µg/ml), and cefotaxime plus clavulanic acid (0.25/4 to 64/4 µg/ml). Broth microdilution is performed using standard methods. Phenotypic confirmation is considered as a three to two fold-serial-dilution decrease in MIC of either cephalosporin in the presence of clavulanic acid compared to its MIC when tested alone (CLSI, 2007).

2. 14. 3 Combination Disc Methods (CDM)

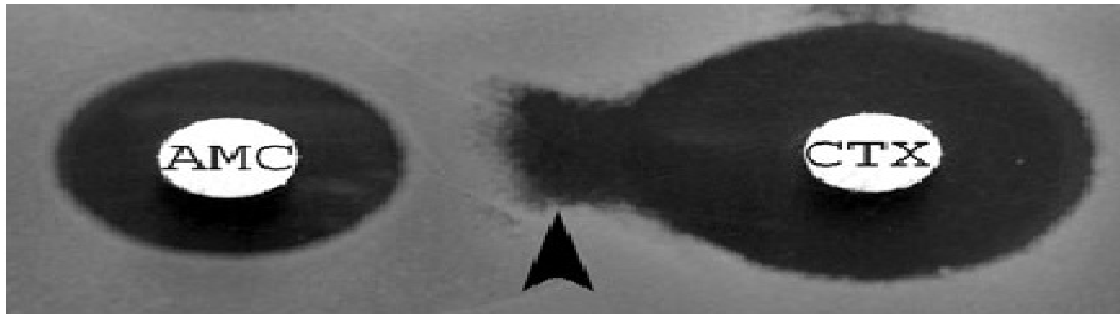
Just like the DDM this method compares the zone diameters of cephalosporin discs to those of the same cephalosporin plus clavulanate however in the CDM, screening for ESBL-producers is not performed. According to the instructions of the manufacturer, either the difference in zone diameters, or the ratio of diameters, is compared. A diameter increase of 5 mm / 50% or more in the presence of the clavulanic acid is indicative of an ESBL-producer (Carter *et al.*, 2000 and M'Zali *et al.*, 2000). This method has a sensitivity and specificity of 92.9% and 96.6% respectively for all species (Drieux *et al.*, 2008). It is also cheap and does not require critical disc spacing.

2. 14. 4 Double-disc diffusion test

In this method, a plate is inoculated as for a routine susceptibility test. Discs containing cefotaxime (30µg) and ceftazidime (30 µg) or cefpodoxime (10 µg) are applied 25-30 mm away on either side of a co-amoxiclav disc (20/10 µg) (Paterson and Bonomo, 2005). ESBL production is inferred when a synergy is observed between either of cephalosporin and the clavulanate. The sensitivity and specificity of this method are 94.1% and 81.4% respectively for all species (Drieux *et al.*, 2008).

The major advantages of the double-disc diffusion test is that is technically simple (Paterson and Bonomo, 2005) and cheap (SUESL, 2008). However, the interpretation of the test is quite subjective and the optimal disc separation varies with the strain and some producers may be missed (SUESL, 2008). Sensitivity may be reduced when ESBL activity is very low. This has been noted for *Proteus mirabilis* (Vercauteren *et al.,* 1997).

Figure 2. 2 A Photograph showing the Double-disc diffusion method (Drieux *et al.*, 2008)



2. 14. 5 Disc replacement method

In this method three amoxicillin/clavulanate discs are applied to a Mueller-Hinton plate inoculated with the test organism. After 1 hour at room temperature, these antibiotic discs are removed and are replaced on the same spot by discs containing cefotaxime, ceftazidime and aztreonam. Control discs of these three antibiotics are simultaneously placed at least 30 mm from these locations. A positive test is indicated by a zone increase of 5 mm for the discs which have replaced the amoxicillin/clavulanate discs compared to the control discs (Paterson and Bonomo, 2005).

2. 14. 6. Agar supplemented with clavulanate

Ho *et al.* (1998) described a method by which Mueller-Hinton agar was supplemented with 4 µg/ml of clavulanate. Antibiotic discs containing ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30µg), and aztreonam (30 µg) were placed on the clavulanate containing agar and on regular clavulanate free Mueller-Hinton agar plates. A difference in β-lactam zone width of 10mm on the two media was considered positive for ESBL production. The difference in ceftazidime zone sizes was 93 to 96% sensitive and 100% specific in the hands of the originators of the test (Ho *et al.*, 1998), but somewhat less so according to other investigators (Bedenic *et al.*, 2001). A major drawback of the method is the need to freshly prepare clavulanate containing plates. The potency of clavulanic acid begins to decrease after 72 hours (Paterson and Bonomo, 2005).

2. 14. 7. E-Test for detecting ESBL-producers

These are plastic drug-impregnated strips, one end of which contains a gradient of ceftazidime (MIC test range 0.5 to 32µg/ml) and the other with a gradient of ceftazidime plus a constant concentration of clavulanate (4µg/ml). Similar strips containing cefotaxime and cefotaxime/clavulanate are also available. These strips are useful for both screening and phenotypic confirmation of ESBL production. The reported sensitivity of the method as a phenotypic confirmatory test for ESBLs is 94.1% for all species (Drieux *et al.*, 2008) and the specificity is 84.7% for all species (Drieux *et al.*, 2008). The sensitivity and specificity of the method depend on the ratio of MICs of the cephalosporin versus cephalosporin/clavulanate combination used –the manufacturer currently recommends an 8-fold reduction in cephalosporin MICs in the presence of clavulanate. Occasionally the MIC of the cephalosporin alone is difficult to read because the inhibition zone is distorted by the clavulanic acid diffusing from the opposite ends of the strip. The use of both cefotaxime and ceftazidime strips improves the ability to detect ESBL types which preferentially hydrolyze cefotaxime such as CTX-M-type enzymes (Paterson and Bonomo, 2005).

Figure 2. 3 A picture of an E-test strip used in the detection of ESBL-producers

(Guleri *et al.*, 2004)

2. 14. 8 VITEK ESBL test

This is an automated system which can be used for the detection of ESBL producing organisms. It is based on the simultaneous assessment of the antibacterial activity of cefepime, cefotaxime and ceftazidime, measured either alone or in the presence of clavulanate. This test relies on card wells containing 1.0 mg/L of cefepime, or 0.5 mg/L of cefotaxime or ceftazidime, either alone or associated with 10 or 4mg/L of clavulanate, respectively. After inoculation, cards are introduced into the VITEK machine, and for each antibiotic tested, turbidity is measured at regular intervals. The proportional reduction of growth in wells containing a cephalosporin combined with clavulanate is then compared with that achieved by the cephalosporin alone and is interpreted as ESBL-positive or ESBL-negative through a computerized expert system (Drieux *et al.*, 2008). Sensitivity and specificity of the method are 85.9% and 78.0% for all species respectively (Drieux *et al.*, 2008).

The principal advantage of the VITEK ESBL card is that it can be easily integrated into the workflow of laboratories already using the VITEK system (Paterson and Bonomo, 2005). The disadvantages of this method are that laboratories using conventional VITEK cards risk incorrectly reporting ESBL-producing organisms as susceptible to cephalosporins when MICs are 8 µg/ml (Queenan *et al.*, 2004). Again false-negative results have been observed in *Klebsiella pneumoniae* isolates producing both an ESBL and an AmpC-type β-lactamase (Tzouvelekis *et al.*, 1999). Most importantly the reliability of the VITEK to detect ESBLs in species other than *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Escherichia coli* is unknown (Paterson and Bonomo, 2005).

2. 14. 9. BD Phoenix automated microbiology system

This is a short-incubation system for bacterial identification and susceptibility testing (Leverstein-van Hall *et al.,* 2002, Sanguinetti *et al.*, 2003, Sturenburg *et al.*, 2003). This test uses growth response to cefpodoxime, ceftazidime, ceftriaxone and cefotaxime, with or without clavulanic acid, to detect the production of ESBLs. The test algorithm has been delineated by Sanguinetti *et al.* (2003). Results are usually available within 6 hours. This method has a sensitivity of 98.8% but a low specificity of 52.2% for all species according to Drieux *et al.* (2008). The advantage of this method is that, it correctly detects ESBL production by *Enterobacter*, *Proteus,* and *Citrobacter spp.*, in addition to *Klebsiella spp.* and *Escherichia coli* (Sanguinetti *et al.*, 2003).

2. 14. 10. Microscan panels

These are dehydrated panels for microdilution antibiotic susceptibility testing. The disadvantage of this method is that, panel users reliably report ESBL producers as ceftazidime resistant but are less likely to report cefotaxime or ceftriaxone as resistant (Sturenburg, *et al.,* 2004). Studies with large numbers of ESBL-producing isolates showed that Microscan panels which contained combinations of ceftazidime or cefotaxime plus β-lactamase inhibitors were highly reliable (Thomson *et al.*, 1999, Komatsu *et al.*, 2003and Sturenburg *et al.*, 2004).

2. 14. 11 Three-dimensional test

The three-dimensional test gives phenotypic evidence of ESBL-induced inactivation of extended-spectrum cephalosporins or aztreonam without relying on demonstration of inactivation of the β-lactamases by a β-lactamase inhibitor. In this test, the surface of the

susceptibility plate is inoculated by standard methods for disc diffusion testing, but additionally a circular slit is cut in the agar concentric with the margin of the plate. A heavy inoculum of the test organism (10^9 to 10^{10} CFU of cells) is pipetted into the slit. β-lactam-impregnated discs are then placed on the surface of the agar 3 mm outside of the inoculated circular slit. β-lactamase-induced inactivation of each test antibiotic is detected by inspection of the margin of the zone of inhibition in the vicinity of its intersection with the circular three-dimensional inoculation. The presence of β-lactamase-induced drug inactivation is visualized as a distortion or discontinuity in the usually circular inhibition zone or the production of discrete colonies in the vicinity of the inoculated slit (Paterson and Bonomo, 2005).

Disadvantages of the test include the need for an additional indirect test if inhibition zones are narrow or absent. The indirect test is performed by inoculating the surface of the agar with a fully susceptible strain, such as *E. coli* ATCC 25922, and then inoculating the circular cut in the agar with the suspension of the test organism. This precludes simultaneous determination of antibiotic susceptibilities but does permit investigation of the β-lactamases of organisms for which inhibition zones are very small. If the indirect test is used, the three-dimensional test is more sensitive than the double-disc diffusion test (Vercauteren *et al.*, 1997), but if the indirect test is not used, sensitivity declines (Bedenic *et al.*, 2001).

2. 14. 12 Molecular detection methods

There are several molecular techniques that are used in the detection of ESBL-producers. Some of these include the DNA probes method, PCR with oligonucleotide primers method, Oligotyping method, PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) method, PCR-Single-Strand Conformational Polymorphism (PCR-SSCP) method, Ligase Chain Reaction

(LCR) test and Nucleotide sequencing, the gold standard (Bradford, 2001). The disadvantages of the molecular methods are that they are labor intensive and technically more challenging than the phenotypic detection methods (Bradford, 2001).

2. 15 Treatment of infections due to ESBL-producing organisms

Infections due to ESBL-producing organisms, as already stated, present a major therapeutic dilemma as the choice of antibiotics is extremely limited (CGMIEB, 2001, Kang *et al.*, 2004 and Pitout *et al.,* 2005).

At the moment the carbapenems are the most reliable antibiotics to use for the treatment of infections caused by ESBL-producing organisms especially blood stream and intra-abdominal infections (CGMIEB, 2001 and Paterson and Bonomo, 2005). The second-line treatment for intra-abdominal infections is a fluoroquinolone plus matronidazole (CGMIEB, 2001 and Paterson and Bonomo, 2005)

Meropenem should be regarded as the drug of choice in meningitis infections caused by ESBL-producer and intrathecal polymyxin B, reserved as the second line of therapy (Segal-Maurer *et al.*, 1999 and Paterson and Bonomo, 2005).

The fluoroquinolones are recommended for the treatment of complicated urinary tract infections (UTI) caused by ESBL-producer whenever the organisms are susceptible to them (CGMIEB, 2001). The second-line treatment for ESBL-producers causing UTI is a β-lactam/β-lactamase inhibitor (e.g. Amoxicillin/clavulanate) (Paterson and Bonomo, 2005). However in areas where there is a high prevalence of infections with ESBL-producers, nitrofurantoin is recommended for the treatment lower UTIs (Melzer and Petersen, 2007).

It is important to distinguish between colonization and significant infections by microorganisms including ESBL-producers before commencing antibiotic therapy as some of these organisms have a propensity to colonize the upper respiratory tract and skin of seriously ill patients (CGMIEB, 2001).

2. 16 Control of ESBL producing organisms

An infection control program in a hospital begins with the identification of patients infected with ESBL producing organisms (Weinstein and Hayden, 1998). Screening of patients in high risk units should be considered (Weinstein and Hayden, 1998 and CGMIEB, 2001). If ESBL-producers are isolated from any sample, the ward is promptly informed (Weinstein and Hayden, 1998). Patients who are colonized or infected with ESBL-producing organisms are separated to decrease the risk of transmission of these organisms (CGMIEB, 2001).

Once ESBL-producers are isolated, all protocols that address preventing cross-infection, such as wearing of gloves and proper disinfection of hands and hospital equipment, are meticulous observed (Tolzis and Blumer, 1995, Garner, 1996 and Society for Healthcare Epidemiology of America and Infectious Disease Society of America Joint Committee on the Prevention of Antimicrobial Resistance, 1997)

CHAPTER THREE

3. 0 Materials and methods

3. 1 Study site

This study was performed at the Microbiology laboratory of the Komfo Anokye Teaching Hospital (KATH) in Kumasi, a tertiary referral centre.

3. 2 Ethical clearance

Ethical clearance for this study was obtained from the Committee on Human Research, Publications and Ethics, School of Medical Sciences/KATH, Kumasi.

3. 3 Isolates

All *E. coli* and *Klebsiella spp.* isolated within the study period, i.e. 18th March 2008 to 17th June 2008, were included in the research. A total of 300 non-duplicate isolates were collected over the period of three months. The organisms were isolated from blood, urine, sputum, wound, ear, pus and aspirates from both in-patients and out-patients.

For this study the "in-patient" was described as an organism isolated from a sample which was received from a patient on admission at the wards while all others were considered as "out-patients".

3.4 Isolation and identification of microorganisms

3.4.1 Isolation

3. 4. 1. 1 Blood specimens

Blood was received in the microbiology laboratory from both in-patients and out-patients in a 25 ml brain-heart infusion broth. The bottles were then incubated aerobically overnight at a

temperature of 35-37°C. After overnight incubation, the blood cultures were then subcultured on blood and MacConkey agar plates (first subculture).

The plates were then incubated overnight under aerobic conditions. On the third day, the first subculture was observed for growth, and any growth identified. The samples that did not record any growth were re-incubated for another 24 hours under the same conditions.

Up to three subcultures were performed similar to the procedure mentioned above if there was no growth from previous subcultures. The blood specimens were discarded on the firth day after the third subcultures were observed.

3. 4. 1. 2 Urine specimens

About 20ml of mid-stream-urine was collected into sterile universal bottles from patients. The urine was mixed by rotating the container and then inoculated onto Cysteine Lactose Electrolytes Deficient (CLED) agar with a 2mm diameter sterile calibrated wire loop and incubated aerobically at 37°C overnight. The plates were then examined the following day and any growth identified.

3. 4. 1. 3 Sputum specimens

Patients were given clean, dry, wide-neck, leak-proof containers into which they were asked to produce sputum by coughing. The appearance of the sputum was described. The sputum was then inoculated on chocolate, blood and MacConkey agar plates. The MacConkey plates were incubated aerobically, whilst the chocolate and blood agar plates were incubated in a carbon dioxide jar, at 35-37°C overnight and were examined for growth on the second day. Colonies were then identified.

3. 4. 1. 4 Pus, wound, ear and aspirated specimens

Specimens were collected with sterile cotton wool swabs or syringes by a medical doctor or an experienced nurse and brought to the laboratory. The specimens were macroscopically examined, described and inoculated on blood and MacConkey agar and then incubated aerobically overnight at 35-37^0 C. The cultured plates were examined after 24 hours of incubation. Isolates were then identified.

3. 4. 1. 5 Demographic data

The age, gender and location (i.e. in- or out-patient) of patient from whom isolates were obtained were recorded.

3. 4. 2 Identification

3. 4. 2. 1 Colonial morphology

Isolates that appeared as circular, convex colonies with smooth distinct edges on blood agar, pink or yellow colonies on MacConkey or CLED agar respectively were considered to be potential *E. coli* (Cheesbrough, 2000).

On the other hand, isolates that appeared as large, grey-white, mucoid and viscous colonies on blood agar, pink- or yellow-mucoid colonies on MacConkey agar or CLED agar respectively were considered to be potential *Klebsiella spp.* (Cheesbrough, 2000).

3. 4. 2. 2 Confirmation of isolates

These potential *E. coli* and *Klebsiella spp.* were confirmed using the API 20E (bioMérieux, France).

3. 5 Storage of isolates

All isolates were stored in tubes containing 1.5 ml Brain-Heart Infusion broth with 20% v/v glycerol at -70°C until further analysis was performed.

3. 6 Sub Culturing

Stored frozen isolates were thawed at room temperature and subcultured on MacConkey.

3. 7. 1 Antimicrobial Susceptibility Testing (AST)

AST of each isolate was determined by the disc diffusion method, employing the British Society of Antimicrobial Chemotherapy (BSAC) standards (BSAC, 2008).

3. 7. 2 Preparation of AST plates

Mueller-Hinton agar (Cypress Diagnostics, Belgium) was prepared according to the manufacturer's instructions. This was done by pouring about 25ml of molten agar into sterile Petri dishes to a depth of 4±0.5mm. The molten media was then allowed to solidify. The surface of the agar was dried to remove excess moisture before use. Plates that were not used immediately were stored at 4-8^{0}C (BSAC, 2008).

3. 7. 3 Inoculum preparation

At least four morphologically similar colonies of *E coli* or *Klebsiella spp.* were touched with a sterile bacteriological loop. The growth was then transferred into a bijou bottle containing distilled water. The bottle was then shaken to uniformly mix the inoculum. The inoculum was compared and made up to 0.5 McFarland standard using a densimat. The suspension was used within 15 minutes of preparation (BSAC, 2008).

3. 7. 4 Plate inoculation

A sterile cotton-wool swab was dipped in the suspension and the excess liquid removed by turning the swab stick against the side of the bottle. The inoculum was spread evenly on the Mueller-Hinton agar plate by swabbing in three directions. The plates were covered and allowed to dry on the bench before applying the discs (BSAC, 2008).

3. 7. 5 Disc application

3. 7. 5. 1 Disc application on AST plate

Antibiotic discs were placed on the agar plate within 15 minutes of inoculation of isolates. The antibiotic discs used in this study were cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefuroxime (30µg), ampicillin (10µg), amikacin (30µg), gentamicin (10µg), nitrofurantoin (300 µg), nalidixic acid (30µg), norfloxacin (10µg), ciprofloxacin (1 µg or 5µg), cotrimoxazole (1.25/23.75 µg), tetracycline (30µg), chloramphenicol (30µg), pipemidic acid (20µg), meropenem (10µg), imipenem (10µg) and levofloxacin (5µg).

Antibiotic discs used in this study were same as those used in the KATH laboratory.

3. 7. 5. 2 Disc application on ESBL test plate

Cefotaxime (30 µg) and ceftazidime discs (30µg) or cefpodoxime (30µg) disc with and without clavulanate (10 µg) were applied on swabbed Mueller-Hinton agar plate according to Combined Disc Method (CDM) for ESBL detection.

The CDM was selected for the detection of ESBL-producers because it is the only phenotypic method that has a sensitivity and specificity for the detection ESBL producing *E. coli* and *Klebsiella spp.* greater than 90.0% (i.e. 94.4% and 97.0% respectively) (Drieux *et al.,* 2008).

3. 7. 6 Incubation

Both the plate for antimicrobial susceptibility testing and ESBL-testing were incubated at 35-37^{0}C aerobically for 18-20 hours.

3. 7. 7 Observations of plates

3. 7. 7. 1 Antimicrobial Susceptibility Test (AST)

Sizes of the zones of inhibition of all discs were measured with a ruler, recorded and compared with the values of the CLSI standards (CLSI, 2007).

3. 7. 7. 2 ESBL test

A difference of 5 mm or more between the zone diameters of either of the cephalosporin discs and their respective cephalosporin/clavulanate combination disc was considered to be a phenotypic confirmation of ESBL production (CLSI, 2007 and SUESL, 2008).

3. 7. 8 Quality control

3. 7. 8. 1 AST

Quality control for the antimicrobial susceptibility test was performed using *E. coli* ATCC 25922 (CLSI, 2007) every week.

3. 7. 8. 2 ESBL test

The ESBL test performance was monitored by the following control strains; *E.coli* NCTC 10418 (negative for ESBL), *E. coli* NCTC 13352 (positive for ESBL), *E. coli* NCTC CTX (positive for ESBL), *E. coli* NCTC 13351(positive for ESBL) and *K. pneumoniae* NCTC 165032 (positive for ESBL) every week.

3. 8 Data analysis

SPSS 14 Evolution (SPSS Inc.) software was used to analyse the epidemiological data and risk factors for the isolation of an ESBL-producer. It was also used to generate;

- Distribution of isolates used in the study
- Gender distribution of patients from whom isolates were obtained
- Age distribution of individuals who had *E. coli* or *Klebsiella spp.* isolated from their sample
- Distribution of the sample source of isolates
- Distribution of the source of isolates (location of patient)
- Antimicrobial susceptibility profile of *E. coli* isolated from blood at KATH
- Antimicrobial susceptibility profile of *E. coli* isolated from urine at KATH
- Antimicrobial susceptibility profile of all *E. coli* at KATH
- Antimicrobial susceptibility profile of *Klebsiella spp.* isolated from blood at KATH
- Antimicrobial susceptibility profile of *Klebsiella spp.* isolated from urine at KATH
- Antimicrobial susceptibility profile of all *Klebsiella spp.* isolated from KATH
- Antimicrobial susceptibility profile of *E. coli* and *Klebsiella spp.* at KATH
- Distribution of ESBL-producers and Non ESBL-producers
- Distribution of ESBL-producers among male and female patients
- Distribution of the sample source of ESBL-producers
- Distribution of ESBL-producers among age groups
- ESBL-producers from in-patients and out-patients

- Antimicrobial susceptibility profile of ESBL producing *E. coli* isolated from blood
- Antimicrobial susceptibility profile of ESBL producing *E. coli* isolated from urine
- Antimicrobial susceptibility profile of all ESBL producing *Klebsiella spp.*
- Antimicrobial susceptibility profile of ESBL-producers isolated from blood
- Antimicrobial susceptibility profile of ESBL-producers isolated from urine
- Antimicrobial susceptibility profile of ESBL-producers isolated from sputum
- Antimicrobial susceptibility profile of ESBL-producers isolated from wound
- Antimicrobial susceptibility profile of ESBL-producers isolated from pus
- Antimicrobial susceptibility profile of ESBL-producers isolated from aspirates
- ESBL-producers which were 'susceptible' to cephalosporins
- Susceptibility profile of all Isolates, non ESBL-producers and ESBL-producers
- Predictors of ESBL production

CHAPTER FOUR

4. 0 Results

4. 1 Number of isolates

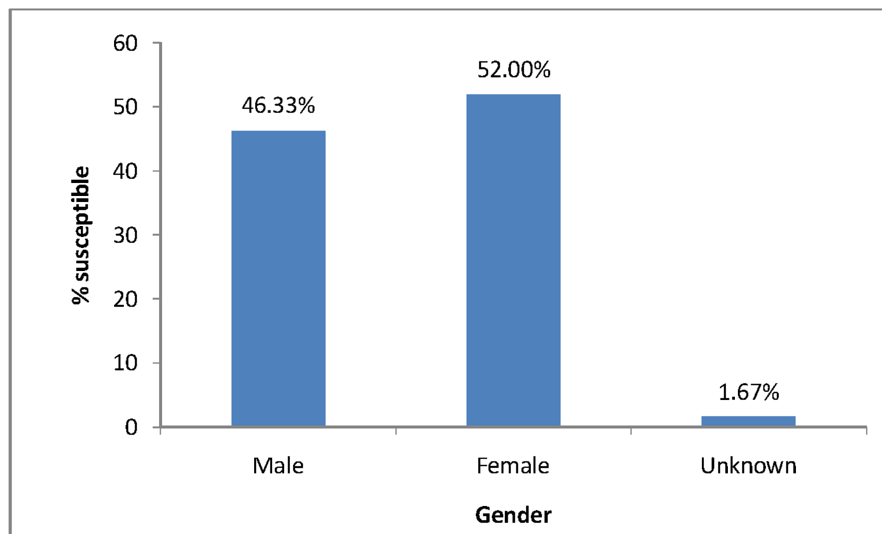
A total of 300 non-selected and non-duplicated isolates were obtained in this study. These organisms were isolated from males and females; children and adults; in-patients and out-patients. Isolates from patients were obtained from blood, urine, sputum, wound, pus, ear and aspirates.

Table 4. 1. 1 Distribution of isolates used in the study

	Isolates	Number	Percent (%)
	Klebsiella pneumoniae	142	47.33
	Escherichia coli	151	50.33
	Other *Klebsiella spp.*	7	2.33
Total		300	100.00

139 of the 300 isolates were from males while 156 were from females. Five patients who had either *E. coli* or *Klebsiella spp.* isolated from their samples did not indicate their gender. (Figure 4. 1. 1)

Figure 4. 1. 1 Gender distribution of patients from whom isolates were obtained



The ages of the individuals who had *E. coli* or *Klebsiella spp.* isolated from their samples ranged from a day old to 91 years. 30 individuals from whom these isolates were obtained did not indicate their ages. (Table 4. 1. 2)

Table 4. 1. 2 Age distribution of individuals who had *E. coli* or *Klebsiella spp.* isolated from their sample

Age Groups	Number	Percent (%)
0-10	88	29.33
11-20	18	6.00
21-30	33	11.00
31-40	26	8.67
41-50	25	8.33
51-60	24	8.00
61-70	20	6.67
71-80	25	8.33
81-90	10	3.33
91-100	1	0.33
Unknown	30	10.00
Total	300	100.00

42.00% of *E. coli* and *Klebsiella spp.* were isolated from urine, 24.67% from blood, 16.67% from wound, 11.67% from sputum, 2.67% from aspirates, 1.33% from pus and 1.00% from ear. There was no significant association between a specimen and the isolation of a particular organism as confirmed by $p = 0.085$. (Table 4. 1. 3)

Table 4. 1. 3 Distribution of the sample source of isolates

Specimen	Isolate			Total
	Klebsiella pneumoniae	*Escherichia coli*	Other *Klebsiella spp.*	
Blood	36	37	1	74
Urine	49	73	4	126
Sputum	27	7	1	35
Wound	22	27	1	50
Ear	1	2	0	3
Pus	3	1	0	4
Aspirate	4	4	0	8
	142	151	7	300

A total of 174 isolates were obtained from in-patients' samples. Of these, 82 were *E. coli*, 87 were *K. pneumoniae* and 5 were other *Klebsiella spp.* 67 isolates were also obtained from out-patients of which, 39 were *E. coli*, 26 were *K. pneumoniae* and 2 were other *Klebsiella spp.* The location of 59 of the patients from whom isolates were obtained was unknown. (Table 4. 1. 4)

Table 4. 1. 4 Distribution of the source of isolates (location of patient)

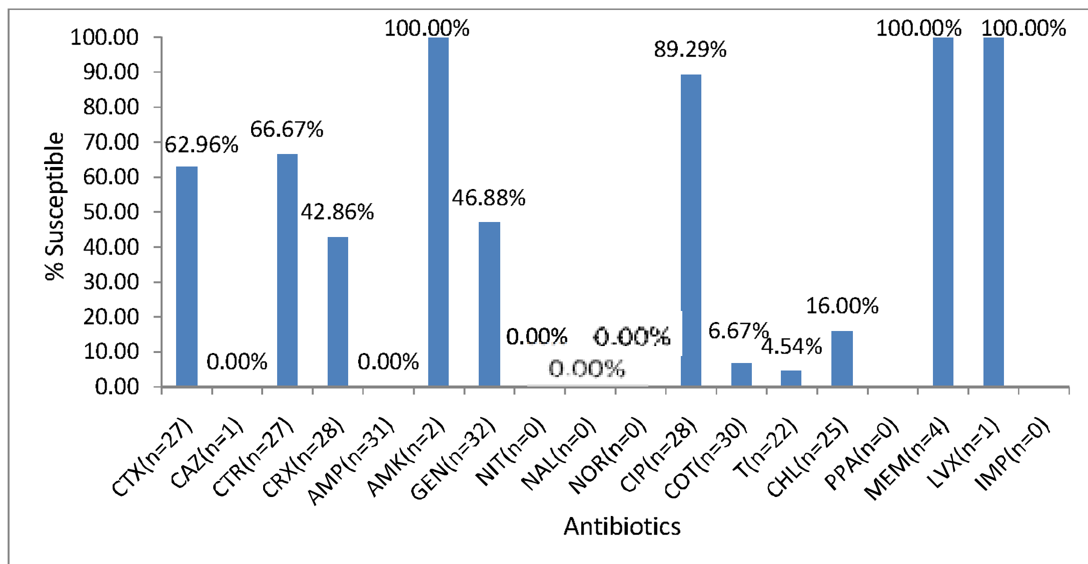
Isolate	In-Patient	Out-Patient	Total
Klebsiella pneumoniae	87	26	113
Escherichia coli	82	39	121
Other *Klebsiella spp.*	5	2	7
	174	67	241

4. 2 Antimicrobial susceptibility testing

AST was done on all the isolates. The antibiotic discs used were cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CTR), cefuroxime (CXM), ampicillin (AMP), amikacin (AMK), gentamicin (GEN), nitrofurantoin (NIT), nalidixic acid (NAL), norfloxacin (NOR), ciprofloxacin (CIP), cotrimoxazole (COT), tetracycline (TET), chloramphenicol (CHL), pipemidic acid (PPA), meropenem (MEM), levofloxacin (LVX) and imipenem (IMP). All the antibiotics above were used at least on one isolate. "n" represents the number of times an antibiotic disc was used.

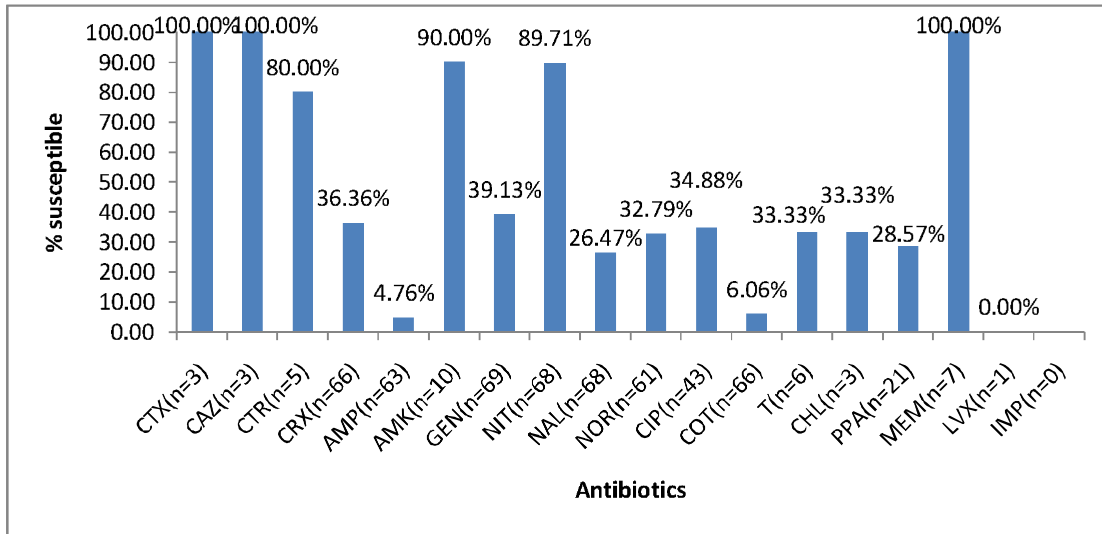
Figure 4. 2. 1 shows the AST of *E. coli* isolated from blood. 80- 100% of these isolates were susceptible to amikacin, ciprofloxacin and meropenem and 60- 70% susceptible to cefotaxime, ceftriaxone and norfloxacin. Poor susceptibilities were shown by cotrimoxazole (6.67%), chloramphenicol (16.00%) and tetracycline (4.54%). All the isolates were resistant to ampicillin and cefuroxime.

Figure 4. 2. 1 Antimicrobial susceptibility profile of all *E. coli* isolated from blood at KATH



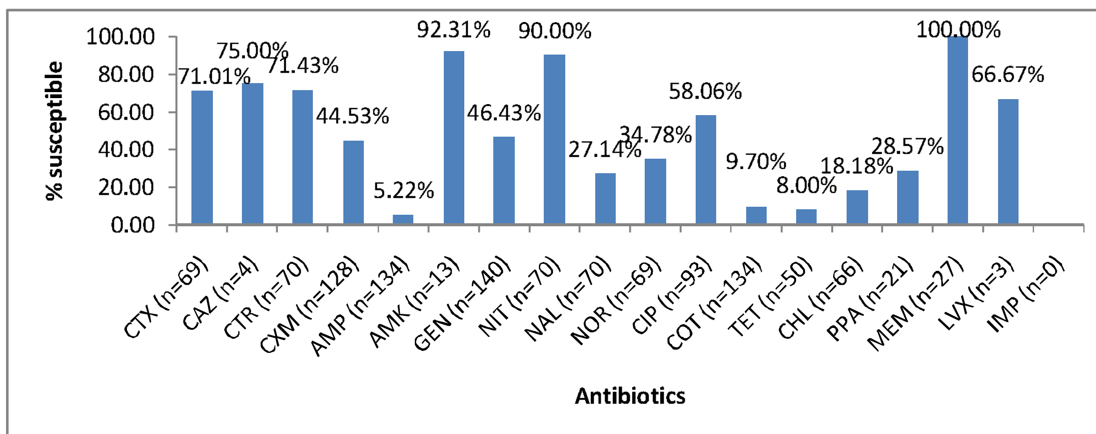
Less than 40.00% of all *E. coli* isolated from urine were susceptible to cefuroxime (36.36%), ciprofloxacin (34.88%), norfloxacin (32.79%), nalidixic acid (26.47%) and cotrimoxazole (6.06%).nitrofurantoin (89.71%), amikacin (90.00%) and meropenem (100.00%) were the most effective drugs against *E. coli* isolated from urine at KATH.

Figure 4. 2. 2 Antimicrobial susceptibility profile of all *E. coli* isolated from urine at KATH



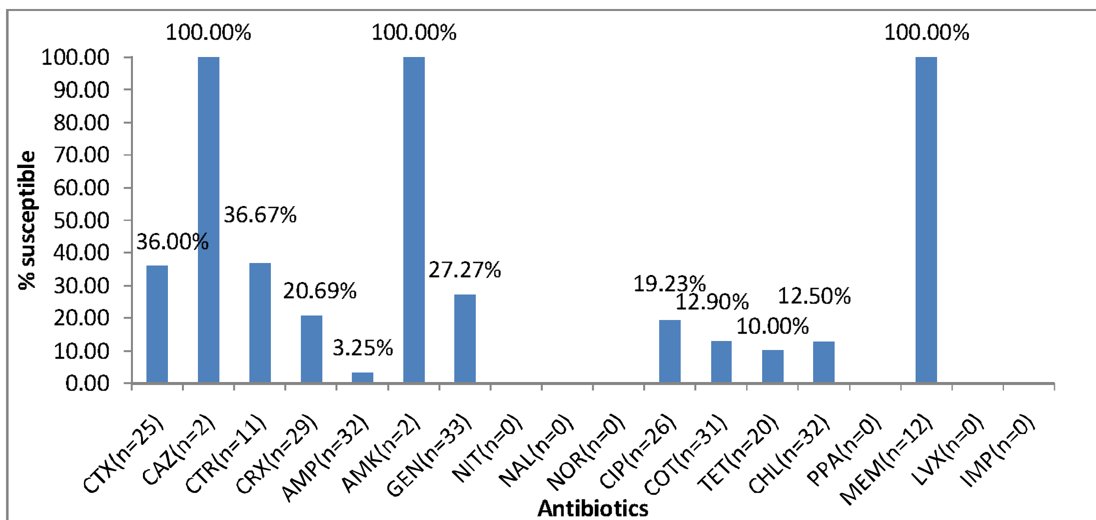
Summary of *E. coli* susceptibility to all agents tested in this study is shown in Figure 4. 2. 3.

Figure 4. 2. 3 Antimicrobial susceptibility profile of all *E. coli* isolated at KATH



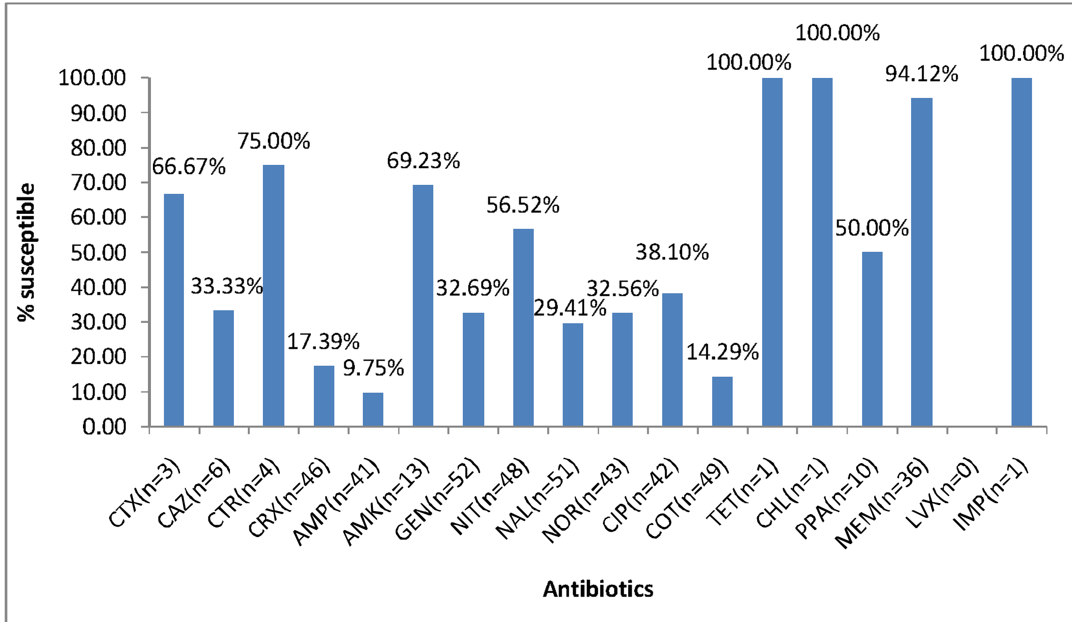
All the *Klebsiella spp.* isolated from blood were susceptible to ceftazidime, amikacin and meropenem but showed high resistance to remaining antimicrobial agents.

Figure 4. 2. 4 Antimicrobial susceptibility profile of *Klebsiella spp.* isolated from blood



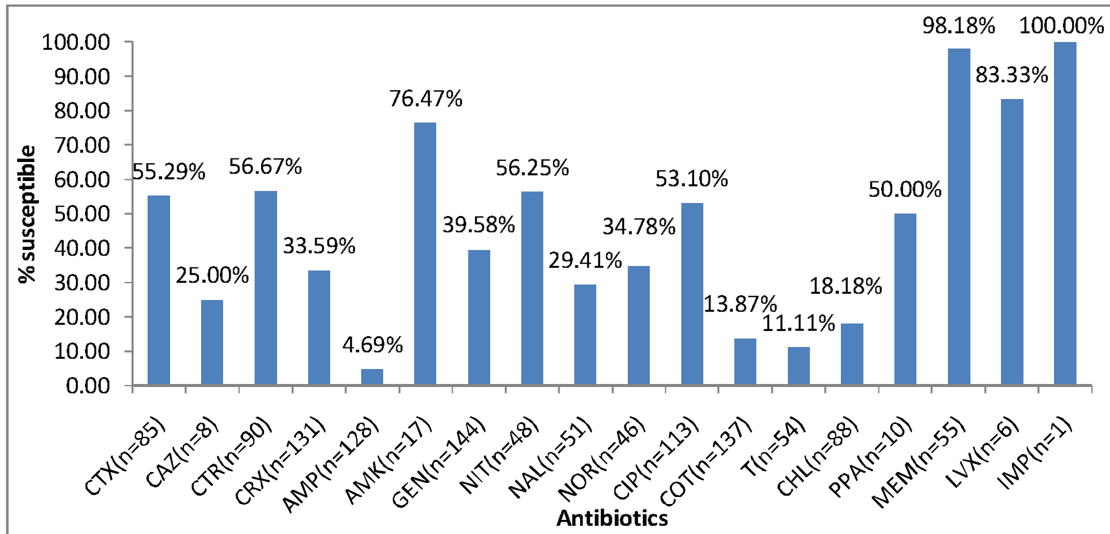
80-100% of all *Klebsiella spp.* isolated from urine were susceptible to meropenem. Less than 40% of all these isolates were susceptible to cefuroxime, ampicillin, gentamicin, nalidixic acid, norfloxacin, ciprofloxacin and cotrimoxazole.

Figure 4. 2. 5 Antimicrobial susceptibility profile of *Klebsiella spp.* Isolated from urine



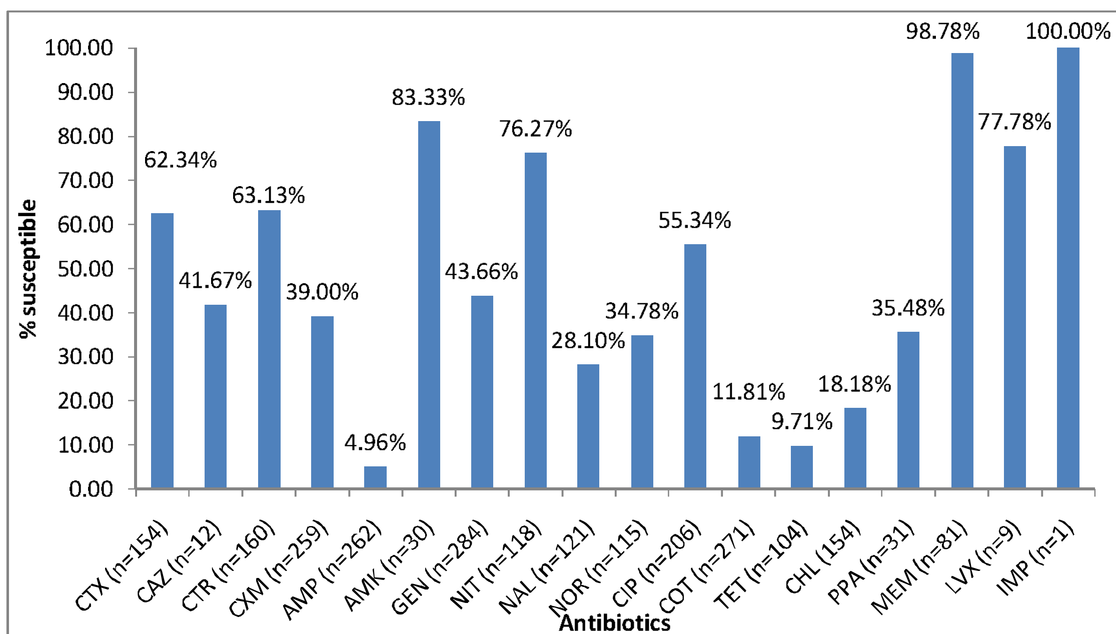
Klebsiella spp. generally showed high resistance to ampicillin, tetracycline, cotrimoxazole, chloramphenicol, ceftazidime, nalidixic acid, cefuroxime, norfloxacin and gentamicin. The most effective antimicrobials against *Klebsiella spp.* were imipenem (100.00%), meropenem (100.00%), levofloxacin (83.33%) and amikacin (76.47%).

Figure 4. 2. 6 Antimicrobial susceptibility profile of all *Klebsiella spp.* isolated from KATH



Susceptibility of both *E. coli* and *Klebsiella spp.* to meropenem (98.78%) and amikacin (83.33%) was very high whilst susceptibility to ampicillin (4.96%), tetracycline (9.71%), cotrimoxazole (11.81%) and chloramphenicol (18.18%) was very low.

Figure 4. 2. 7 Antimicrobial susceptibility profile of *E. coli* and *Klebsiella spp.* at KATH



4.3 ESBL producing *E. coli* and *Klebsiella spp.*

Out of the total number of *E. coli* and *Klebsiella spp.* isolated, 55.63% (79) of *K. pneumoniae*, 44.37% (67) of *E. coli* and 42.86% (3) of the other *Klebsiella spp.* were ESBL-producers. (Table 4. 3. 1)

Table 4. 3. 1. Distribution of ESBL- and Non ESBL-producers

Isolates	ESBL-producer n(%)	Non ESBL-producer n(%)	Total number (n)
Klebsiella pneumoniae	79 (55.63%)	63 (44.37%)	142 (100.00%)
Escherichia coli	67 (44.37%)	84 (55.63%)	151 (100.00%)
Other *Klebsiella spp.*	3 (42.86%)	4 (57.24%)	7 (100.00%)
	149	151	300

69 out of the 139 isolates from males and 78 out of the 156 isolates from females were ESBL-producers. Of the total ESBL-producers isolated 46.94% were from males and 53.06% from females. There was no significant association between ESBL production in isolates and gender (Odds Ratio (OR) =0.99, Confidence Interval (CI) =0.62-1.56, p= 0.97). Two individuals who had ESBL-producers isolated from their samples did not indicate their gender (Table 4. 3. 2).

Table 4. 3. 2 Distribution of ESBL-producers among males and females

Gender	*Klebsiella pneumoniae* n (%)	*Escherichia coli* n (%)	Other *Klebsiella spp.* n (%)	Total number (n)
Male	38 (48.10%)	29 (43.28%)	2 (66.67%)	69
Female	41 (51.90%)	36 (53.73%)	1 (33.33%)	78
Unknown	0 (0.00%)	2 (2.99%)	0 (0.00%)	2
	79 (100.00%)	67 (100.00%)	3 (100.00%)	149

Of all the ESBL-producers isolated, urine accounted for 48.32% (72), blood for 28.06% (42), sputum for 8.72% (13), wound for 12.08% (18), ear 0.00% (0), pus for 0.67% (1), and aspirates for 1.34% (2). *P* value = 0.871. (Table 4. 4. 3)

Table 4. 3. 3 Distribution of the source of ESBL-producers

Specimen	*Klebsiella pneumoniae*	*Escherichia coli*	Other *Klebsiella spp.*	Total (n)
Blood	22 (27.85%)	20 (29.85%)	1 (33.33%)	43
Urine	35 (44.30%)	35 (52.23%)	2 (66.67%)	72
Sputum	9 (11.39%)	4 (5.97%)	0 (0.00%)	13
Wound	10 (12.66%)	8 (11.94%)	0 (0.00%)	18
Pus	1 (1.27%)	0 (0.00%)	0 (0.00%)	1
Aspirates	2 (2.53%)	0 (0.00%)	0 (0.00%)	2
	79 (100.00%)	67 (100.00%)	3 (100.00%)	149

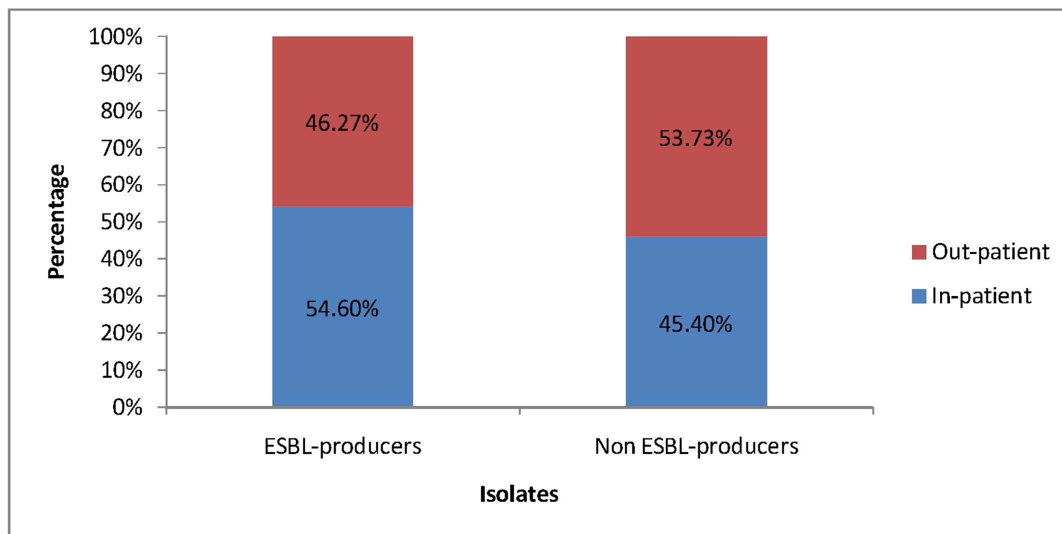
132 out of the 149 individuals from whom ESBL-producers were isolated from their sample indicated their age. Of these 37.88% (50) were between 0-10 years, 4.55% (6) between 11-20 years, 12.88% (17) between 21-30 years, 7.58% (10) between 31-40 years, 9.09% (12) between 41-50 years, 7.58% (10) between 51-60 years, 5.30% (7) between 61-70 years, 8.33% (11) between 71-80 years, 6.06% (8) between 81-90 years and 0.76% (1) between 91-100 years. There was however no significant association between age and the isolation of an ESBL-producer in this study (p value=0.192). (Table 4. 3. 4)

Table 4. 3. 4 Distribution of ESBL-producers among the ages

Age Groups (years)	*Klebsiella pneumoniae*	*Escherichia coli*	Other *Klebsiella spp.*	Total (n)
0-10	30	19	1	50
11-20	5	1	0	6
21-30	5	12	0	17
31-40	4	6	0	10
41-50	6	5	1	12
51-60	6	4	0	10
61-70	1	6	0	7
71-80	6	5	0	11
81-90	6	2	0	8
91-100	1	0	0	1
	70	60	2	132

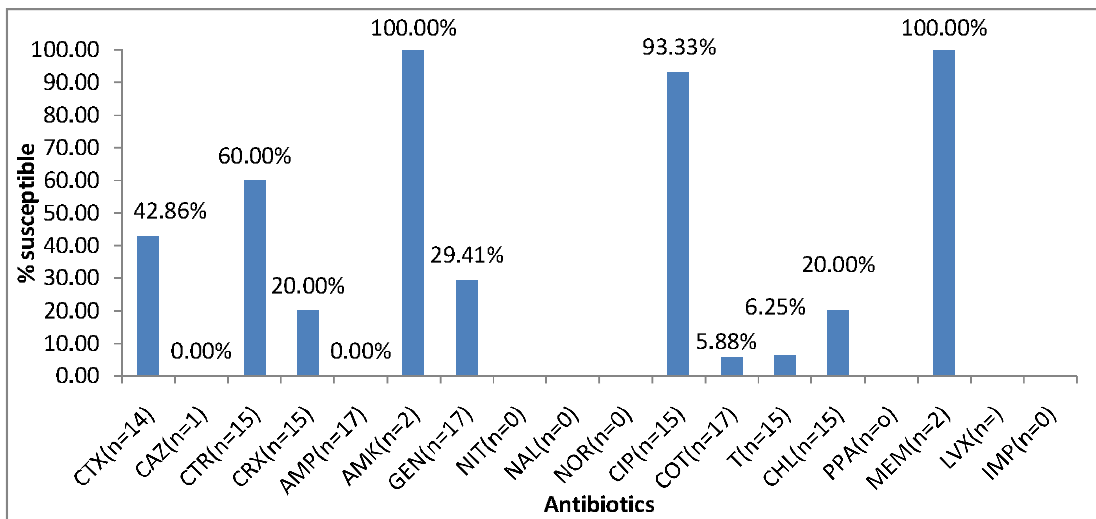
Of the 174 organisms isolated from in-patients, 95 (54.60%) were ESBL-producers whilst 31(46.27%) out of 67 from out-patients were ESBL-producers. ESBL-producers were more common among in-patients (54.60%) than out-patients (46.27%), although the difference was not statistically significant (OR = 1.40, 95% CI = 0.79-2.46, p= 0.30). (Figure 4. 3. 1)

Figure 4. 3. 1 ESBL-producers from in-patients and out-patients



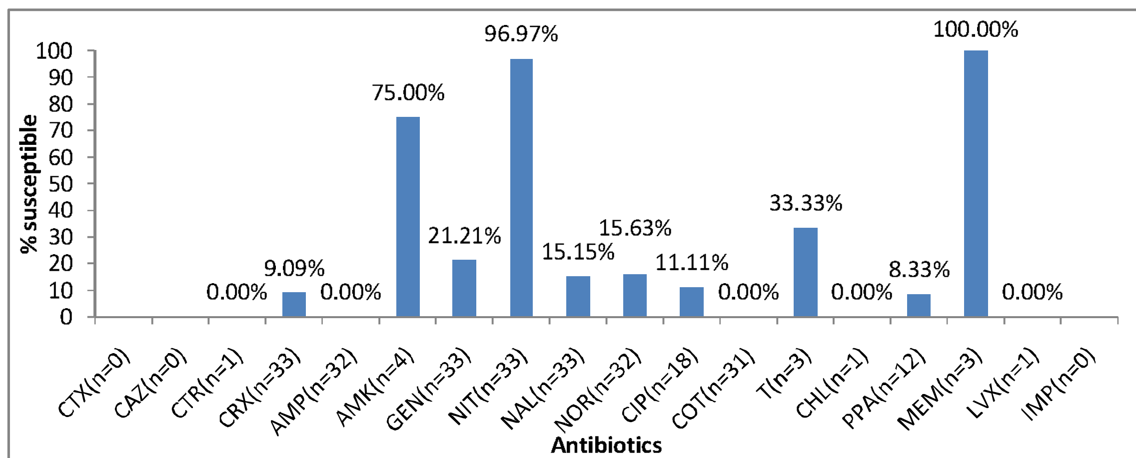
90-100% of ESBL-producing *E. coli* isolated from blood were susceptible to amikacin, ciprofloxacin and meropenem. 40-60% were susceptible to cefotaxime and ceftriaxone. The remaining antimicrobials were generally ineffective *in vitro*.

Figure 4. 3. 2 Antimicrobial susceptibility profile of ESBL producing *E. coli* isolated from blood



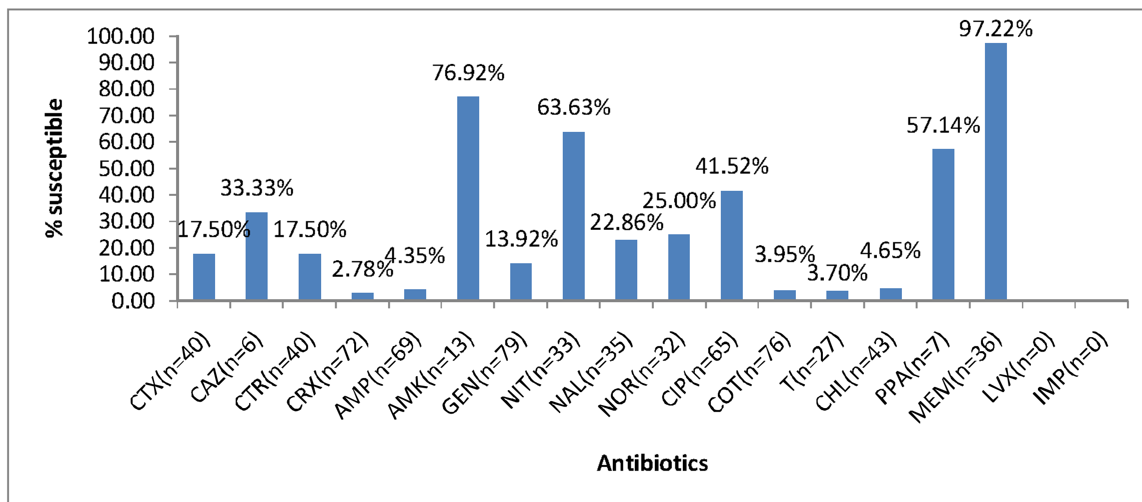
Only amikacin, nitrofurantoin and meropenem were effective *in vitro* against ESBL-producing *E. coli* isolated from urine.

Figure 4. 3. 3 Antimicrobial susceptibility profile of ESBL producing *E. coli* isolated from urine



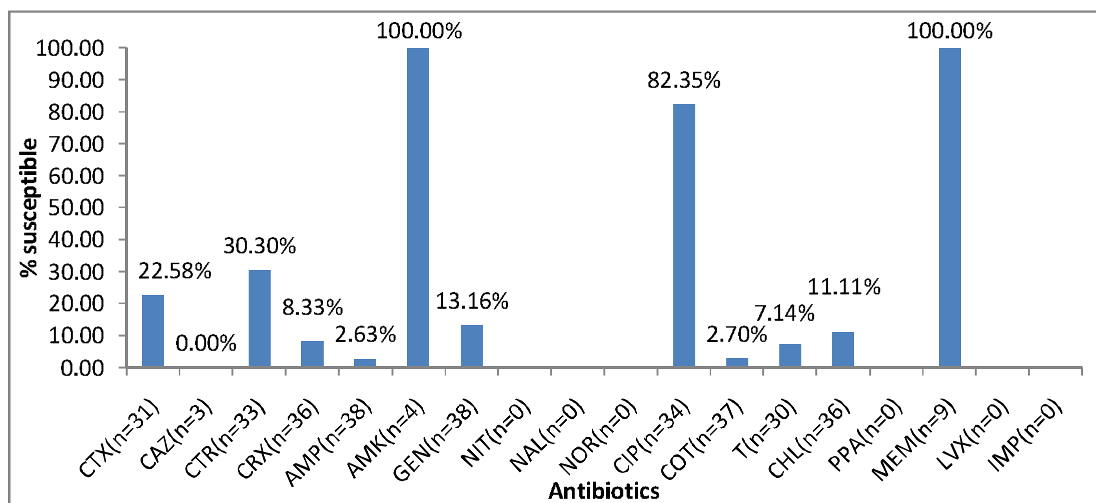
Again only amikacin, nitrofurantoin, and meropenem were effective *in vitro* against ESBL-producing *Klebsiella spp.* isolated from urine.

Figure 4. 3. 4 Antimicrobial susceptibility profile of all ESBL producing *Klebsiella spp.*



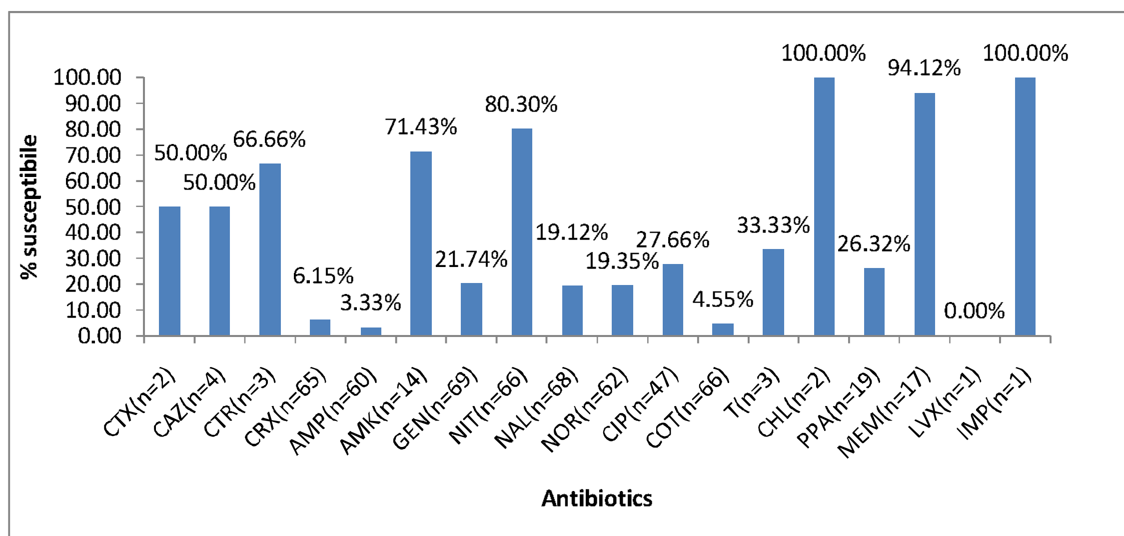
Ciprofloxacin, amikacin and meropenem were the most effective drug *in vitro* against ESBL-producers isolated from blood.

Figure 4. 3. 5 Antimicrobial susceptibility profile of 43 ESBL-producers isolated from blood



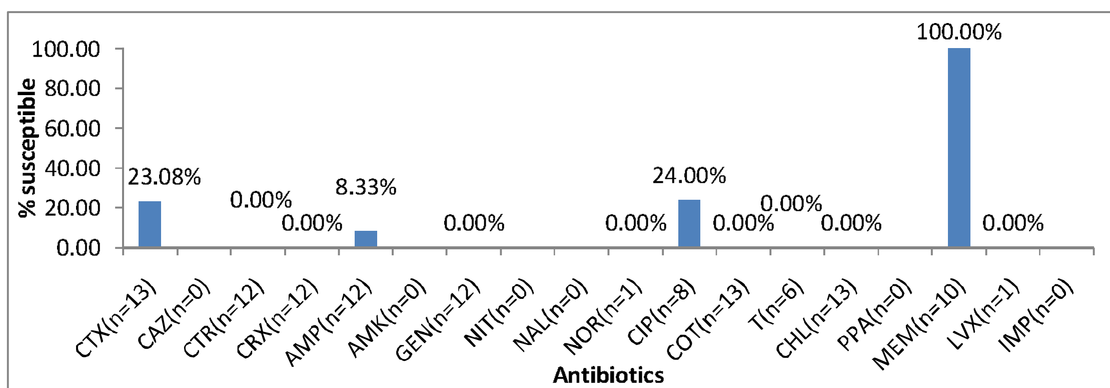
70-100% of ESBL-producers isolated from urine were susceptible to amikacin, nitrofurantoin, chloramphenicol, meropenem and imipenem whilst 50-60% of them were to cefotaxime, ceftazidime and ceftriaxone. High level of resistance was observed amongst cefuroxime, ampicillin, gentamicin, nalidixic acid, norfloxacin, ciprofloxacin, cotrimoxazole, tetracycline, chloramphenicol, pipemidic acid and levofloxacin.

Figure 4. 3. 6 Antimicrobial susceptibility profile of 72 ESBL-producers isolated from urine



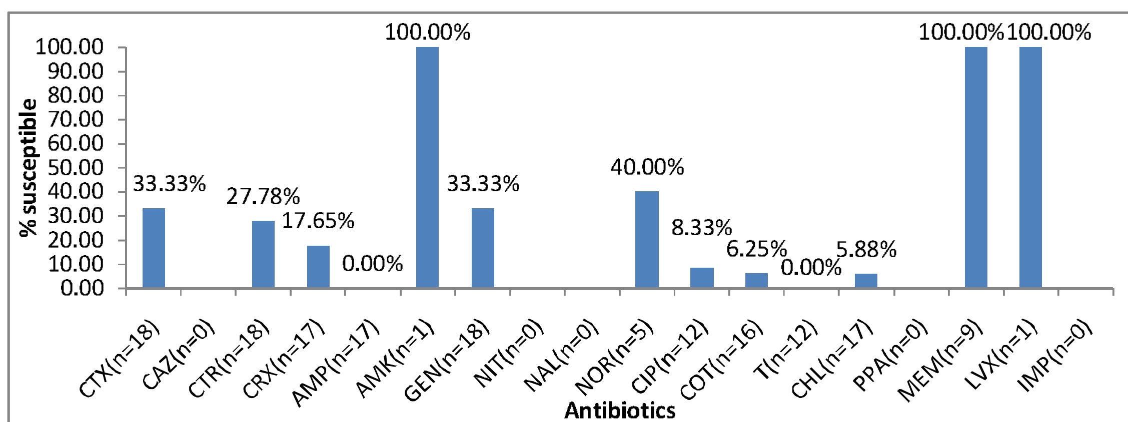
All the ESBL-producers isolated from sputum were generally resistant to all the antimicrobial agents with the exception of meropenem.

Figure 4. 3.7 Antimicrobial susceptibility profile of 13 ESBL-producers isolated from sputum



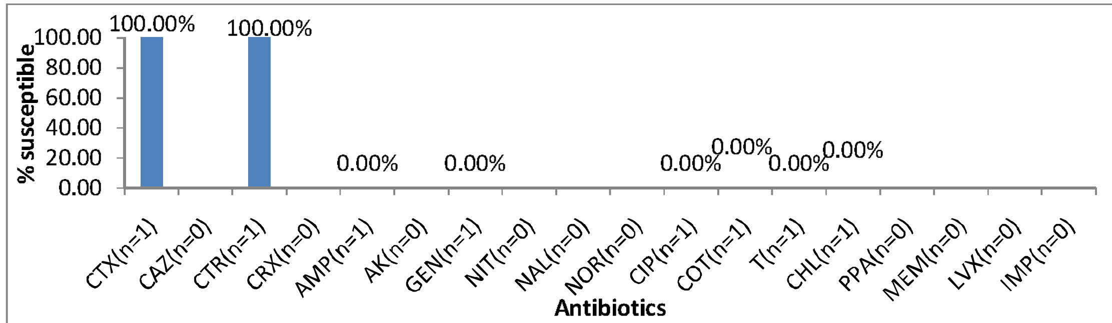
Amikacin, meropenem and levofloxacin were effective *in vitro* against all the ESBL-producers isolated from wound. The remaining antimicrobials were generally ineffective *in vitro*.

Figure 4. 3. 8 Antimicrobial susceptibility profile of 18 ESBL-producers isolated from wound



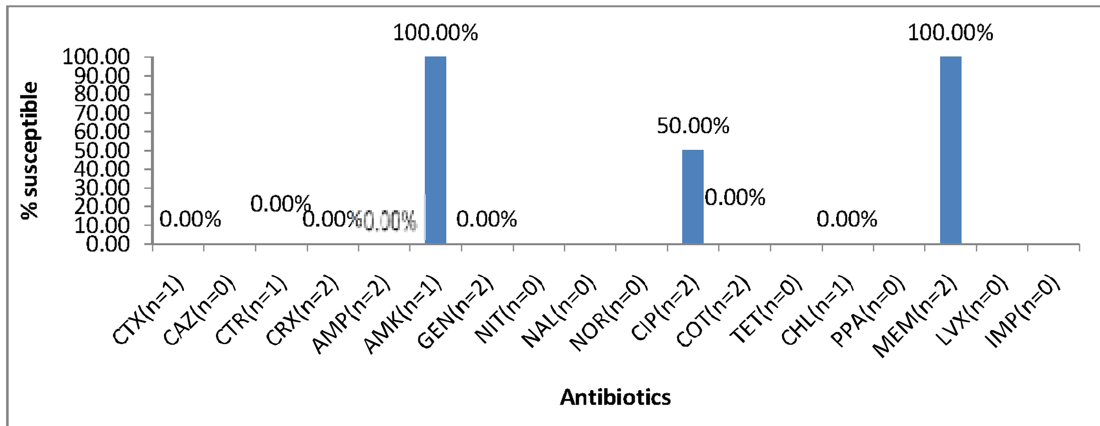
The ESBL-producer isolated from pus was suceptible to only cefotaxime and ceftriaxone *in vitro*.

Figure 4.3.9 Antimicrobial susceptibility profile of an ESBL-Producer isolated from pus



Amikacin and meropenem were effective *in vitro* against the ESBL-producers isolated from aspirates. One out of two ESBL-producers were susceptible to ciprofloxacin. The remaining antimicrobials were not effective *in vitro.*

Figure 4. 3. 10 Antimicrobial susceptibility profile of two ESBL-producers isolated from aspirates



On the average 23.21% of ESBL-producers were susceptible to the cephalosporins (CTX, CAZ, CTR and CXM), and 3.08% to ampicillin.

Figure 4. 3. 11 ESBL-producers which were 'susceptible' to cephalosporins

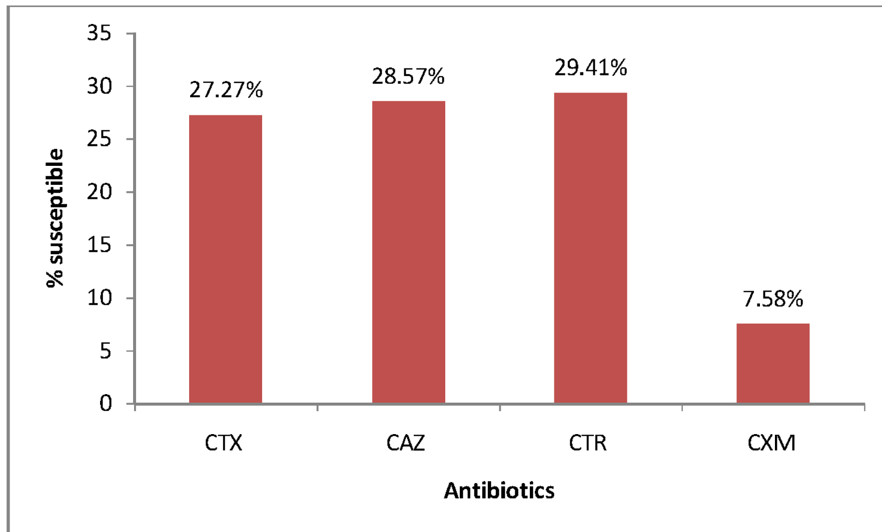


Table 4. 3. 5 Susceptibility profile of all isolates, non ESBL-producers and ESBL-producers

Antimicrobial agents	Sensitive (%)	Resistant (%)			Total number
	All isolates	All isolates	Non ESBL-producers	ESBL-producers	
CTX	62.34	37.66	11.36	72.72	154
CAZ	41.67	58.33	40.00	71.43	12
CTR	63.13	36.88	11.96	70.58	160
CXM	39.00	61.00	28.35	92.42	259
AMP	4.96	95.04	93.18	96.92	262
AMK	83.33	16.67	10.00	20.00	30
GEN	43.66	56.34	31.94	81.43	284
NIT	76.27	23.73	28.86	19.70	118
NAL	28.10	71.90	60.38	80.88	121
NOR	34.78	65.22	44.68	79.41	115
CIP	55.34	44.66	32.35	56.73	206
COT	11.81	88.19	80.15	96.30	271
TET	9.71	90.29	86.27	94.23	103
CHL	18.18	81.82	75.00	90.00	154
PPA	35.48	64.52	50.00	73.68	31
MEM	98.78	1.22	0.00	2.12	82
LVX	77.78	22.23	0.00	66.67	9
IMP	100.00	0.00		0.00	1

Table 4. 3. 6 Predictors of ESBL production

Antimicrobial agents	Odds ratio	95% confidence interval	p Value	Total number (n)
CTX	**20.8**	**8.87-48.79**	**0.001**	**154**
CAZ	3.75	0.33-42.47	0.636	12
CTR	**17.67**	**7.8-40.04**	**0.001**	**160**
CRX	**30.84**	**14.55-65.38**	**0.001**	**259**
AMP	2.3	0.69-7.68	0.268	262
AMK	2.25	0.22-23.32	0.865	30
GEN	**9.34**	**5.38-16.21**	**0.001**	**284**
NIT	0.61	0.26-1.42	0.348	118
NAL	**2.78**	**1.23-6.29**	**0.023**	**121**
NOR	**4.78**	**2.1-10.87**	**0.001**	**115**
CIP	**2.74**	**1.55-4.84**	**0.001**	**206**
COT	**6.44**	**2.40-17.29**	**0.001**	**271**
TET	2.6	0.63-10.67	0.305	103
CHL	**3**	**1.19-7.56**	**0.029**	**154**
PPA	2.8	0.61-12.86	0.346	31

CHAPTER FIVE

5.0 Discussion

5. 1. Antimicrobial susceptibility of *E. coli* and *Klebsiella spp.*

5. 1. 1 Antimicrobial susceptibility of the isolates to ampicillin, tetracycline, cotrimoxazole and chloramphenicol

For several years in Ghana, a lot of microorganisms have been reported to have high antimicrobial resistance to ampicillin, tetracycline, cotrimoxazole and chloramphenicol (Newman *et al*, 2006) and this assertion was confirmed in this study. Very high resistance levels were observed amongst *E. coli* and *Klebsiella spp.* to ampicillin (95.04%), tetracycline (90.29%), cotrimoxazole (88.19%) and chloramphenicol (81.82%). These drugs, with the exception of cotrimoxazole, are therefore no longer administered at KATH. Cotrimoxazole however is given as prophylaxis to HIV/AIDS patients.

The high resistance levels observed are due to the fact that these drugs have been abused over a long period of time in Ghana (Newman *et al.* 2006). This is because they are easily accessible even without prescription and are relatively inexpensive (Newman *et al.* 2006). The inability of the Ministry of Health and hospital authorities to enforce the drug policy on the use of antibiotics in Ghana (Newman *et al.* 2006) has also not helped matters.

5. 1. 2 Antimicrobial susceptibility of isolates to cephalosporins

There has been a marked increase in the prevalence of drug resistance amongst *E. coli* and *Klebsiella spp.* to cephalosporins namely, ceftriaxone (6.00-10.00% to 36.87%), cefotaxime

(20.00% to 37.66%) and cefuroxime (27.00% to 61.00%) between 2004 and 2008 (KATH records, 2004-2008). The high level of resistance observed among isolates studied from blood to cefuroxime, cefotaxime and ceftriaxone and those from urine to cefuroxime was mainly due to ESBL-producers that are capable of hydrolyzing extended spectrum cephalosporins, making them ineffective. Other factors responsible for the increased resistance may include insufficient control of drug prescription, leading to poor prescribing habits (Newman *et al.,* 2006), lack of competent personnel to perform cultures and AST (Newman *et al.,* 2006), and lack of facilities to perform cultures and AST in most health facilities in Ghana (Newman *et al.,* 2006).

5. 1. 3 Antimicrobial susceptibility of isolates to the quinolones

Brooks *et al.* (2004) reported that ciprofloxacin and norfloxacin have high antimicrobial activity against *Enterobacteriaceae*, including those resistant to the third generation cephalosporins. This was however not the case in this study. There has been a dramatic increase in resistance to ciprofloxacin (6.00-10.00% to 44.66%) and norfloxacin (20.00% to 65.22%) by *E. coli* and *Klebsiella spp.* over a period of just four years (KATH records, 2002-2006). A high level of resistance was observed particularly amongst isolates from urine (63.53%). A possible reason for this high incidence of ciprofloxacin-resistant urine isolates may have been because ciprofloxacin has been highly abused. Reasons for this abuse may include the fact that ciprofloxacin is inexpensive and has low toxicity (Brooks *et al.*, 2004). Many people take ciprofloxacin on their own or on the advice of their local pharmacists whenever they suspect they have typhoid fever, gonorrhoea or UTI. This behavior allows for the selection of ciprofloxacin-resistant strains in the urinary tract, which may later on cause infections.

Another reason for such high resistance could have been due to the fact that some of these isolates were obtained from patients with recurrent UTIs and indwelling urinary catheters. These conditions also allow for the selection of ciprofloxacin-resistant strains since ciprofloxacin might have been used repeatedly to treat the recurrent UTI.

On the contrary, ciprofloxacin was very effective against isolates from blood stream infections.

Levofloxacin, a relatively new fluoroquinolone on the Ghanaian market, was generally effective *in vitro* but Paterson and Bonomo (2005) suggested in their work that these new drugs are unlikely to provide any significant benefits over ciprofloxacin.

Nalidixic acid, a quinolone, was also reported to be effective against urine isolate because of its antiseptic property (Brooks *et al.*, 2004). However results obtained from this study show that nalidixic acid was generally ineffective. More than 70.00% of the urine isolates were resistant to it. The reasons for this observation are that nalidixic acid has been abused over a very long period of time in Ghana because it is easily accessible and inexpensive (Newman *et al.* 2006).

5. 1. 4 Antimicrobial susceptibility of isolates to the aminoglycosides

This study showed that only 42.96% of all the *E. coli* and *Klebsiella spp.* isolated were susceptible to gentamicin. Gentamicin is used in serious infections caused by Gram negative bacteria which were resistant to other drugs (Brooks *et al.*, 2004). The high level of resistance observed may be due to of the emergence of ESBL-producers. Plasmids which contain genes encoding for ESBLs also harbor genes which encode for resistance to many aminoglycosides (Paterson *et al.*, 2000).

Other causes of resistance may be attributed to the poor prescribing habits of doctors and the abuse of gentamicin by the general public (Newman *et al.*, 2006).

Again, the use of creams and solutions containing gentamicin sulfate, in the treatment of infected burns or skin lesions may have also resulted in the selection of gentamicin-resistant organisms (Brooks *et al.*, 2004).

Amikacin, unlike gentamicin, was very effective *in vitro* against both *E. coli* and *Klebsiella spp.* From the study, 83.33% of all the isolates were susceptible to the action of amikacin. This is because amikacin is generally more effective than gentamicin and it is also resistant to many of the enzymes that inactivate gentamicin (Brooks *et al.*, 2004). Again amikacin is a relatively new drug in Ghana (Newman *et al.*, 2006) and it is not often prescribed for use or abused because it is a parenteral drug.

5. 1. 5 Antimicrobial susceptibility of isolates to nitrofuran

Nitrofurantoin, a nitrofuran, was active against many urine isolates, especially *E. coli* (89.71%) because of its urine antiseptic property. It was however less active against *Klebsiella spp.* (56.25%). This confirms earlier reports by Melzer and Petersen (2007). The reason for nitrofurantoin maintaining its efficacy may be because it has been off the Ghanaian market since the introduction of ciprofloxacin into the country. If an antibiotic is re-introduced, after a long period of time, resistance of microorganisms to the said antibiotic falls (Holliman, 1999).

Just like ciprofloxacin, nitrofurantoin is an inexpensive drug.

Melzer and Petersen (2007) also suggested that nitrofurantoin may fail to eradicate upper urinary tract infections. This is because nitrofurantoin fails to produce significant levels in tissues but

effectively lowers bacteria count in the lower urinary tract (Brooks *et al.*, 2004). Another possible problem in administering nitrofurantoin is that it is contraindicated in renal impairment (Melzer and Petersen, 2007) so it should not be given in that condition.

5. 1. 6 Antimicrobial susceptibility of isolates to the carbapenems

Meropenem, a second-line drug, was tested against 82 isolates which were resistant to all or most of the first-line drugs. It was the most effective antimicrobial against both *E. coli* and *Klebsiella spp. in vitro.* Meropenem, like amikacin, is new in Ghana and it is used only in serious infections when all other antimicrobials have failed. Meropenem, unlike most drugs, is not abused because it is extremely expensive and also a parenteral drug.

5. 2. 1 ESBL production amongst *E. coli and Klebsiella spp.*

Several studies have shown that ESBL- production amongst *Enterobacteriaceae* is a worldwide problem (Kader *et al*, 2006) and its prevalence varies from country to country. The prevalence levels of ESBL-producers obtained in this study (44.37% *E. coli,* 55.67% *Klebsiella pneumoniae* and 42.86% other *Klebsiella spp.*) are higher than those reported for *E. coli* and *K. pneumoniae* in most parts of the world including, USA (Bradford, 2001), India (Babypadmini and Appalaraju, 2004), China (Ling *et al.*, 2006) and Nigeria (Aibinu *et al*, 2003). The high prevalence of ESBL producing *K. pneumoniae* at KATH was however comparable to the situation in Turkey which was 57.1% (Taşh and Bahar, 2005).

Bradford (2001) reported that high volumes and indiscriminate use of extended-spectrum cephalosporins in hospitals was a common feature associated with hospitals with high incidence of ESBL-producers. This situation is no different at KATH. Some clinicians prescribe

ceftriaxone and cefuroxime to patients suspected to have conditions such as septicaemia, meningitis, typhoid fever, UTIs and STIs but do not obtain samples for AST as required, to ascertain whether these drugs are effective against the organisms causing the infections or not. Again ESBL testing is not performed at KATH. These situations have allowed for the development and spread of ESBL-producers.

Strict adherence to infection control practices, such as the regular disinfection of hands and equipment, which resulted in decreased incidence of ESBL-producing organisms in Western Europe (Paterson and Bonomo, 2005), has not been employed at KATH, resulting in the spread of ESBL producing organisms within the hospital.

The transfer of patients between wards, together with the fact that health workers do not comply strictly with infection control measures when moving patients within the hospitals may have also facilitated the spread of ESBL-producers. Bradford (2001) reported that these conditions contribute to the spread of ESBL-producers.

ESBL-producers may have spread through colonized hospital equipment such as thermometers or bronchoscopes, and contaminated hands of health-care-givers as was reported elsewhere (Paterson and Bonomo, 2005).

With the exception of the ear specimens (*n*= 3), ESBL-producing organisms were isolated from all sites of the body from which samples were obtained namely, blood, urine, sputum, wound, pus and aspirates. More than 50% of the isolates from blood (58.11%) and urine (57.14%) were ESBL-producers with blood accounting for the highest incidence of ESBL-producers. This observation is disturbing because of the serious nature of blood stream infections. Patients who

were treated with any of the cephalosporins may not have benefited from its use as a result of treatment failures.

In contrast to findings in earlier reports (Melzer and Petersen, 2007), hospitalization was not a risk factor associated with infection caused by ESBL-producers in this study. This is because ESBL producing *E. coli* or *Klebsiella spp.* were wide spread among both in-patients and out-patients. This observation therefore confirms the assertion by Pitout *et al.* (2005) that ESBL-producers are indeed as much a problem in the communities as in the hospitals.

ESBL-producers may have spread through communities, especially those with poor hygienic and sanitation conditions, through faecal contamination of soil and water, since most patients with ESBL- producers may have had their gastrointestinal tracts colonized for a long period of time by these organisms as was reported by Louis (2001) and Paterson and Bonomo (2005).

Cockroaches were reported to be vectors of ESBL-producers in a study conducted by D'Agata *et al.* (1998). They observed that, ESBL-producing *K. pneumoniae* isolated from cockroaches were indistinguishable from those infecting patients. It is therefore possible that cockroaches and other insects, which are common in many homes in Ghana, could have contributed to the spread of ESBL-producers in the community by contaminating water and food.

5. 2. 2 Antimicrobial susceptibility of ESBL-producers isolated from blood

In vitro susceptibility studies of ESBL- producing *E. coli* and *Klebsiella spp.* isolated from blood showed that meropenem (a carbapenem) and amikacin were the most effective (100.00%) antimicrobials against the ESBL-producers. These antimicrobials however were tested only nine and four times respectively because they are second-line antibiotics. Ciprofloxacin, a

fluoroquinolone, was also effective against 82.35% of the ESBL-producers and may be a viable antimicrobial therapy against ESBL-producers which cause bacteraemia. Kang *et al.* (2004) have reported that the outcome of ciprofloxacin treatment in bloodstream infections was similar to that of carbapenems. Ciprofloxacin is thus recommended for the treatment of bloodstream infections whenever an organism is ciprofloxacin susceptible. The carbapenems on the other hand should be used to treat only serious or life threatening infections in order to minimize cases of carbapenem resistance though rare (Paterson and Bonomo, 2005). Worryingly, one ESBL producing *Klebsiella pneumoniae* was resistant to meropenem. This is of great concern given that Klebsiellae have a high propensity to host plasmids (Paterson and Bonomo, 2005). Appropriate prevention and infection control measures must be put in place to prevent the spread of these strains within the hospital and the community.

5. 2. 3 Antimicrobial susceptibility of ESBL-producers isolated from urine

In contrast to ESBL-producers isolated from blood, those isolated from urine were generally resistant to the fluoroquinolones, including ciprofloxacin. Only 27.7% of the ESBL-producers were susceptible to ciprofloxacin, a recommended drug for the treatment of UTIs caused by ESBL-producers (CGMIEB, 2001 and Paterson and Bonomo, 2005). Gould (2008) reported that quinolone resistance in *Enterobacteriaceae* in many hospitals is high and this may be due to the increasing use of ciprofloxacin in the community in many countries. In Ghana ciprofloxacin is recommended for the treatment of many infections including typhoid fever, acute cystitis, acute prostatitis, gonorrhoea and osteomylitis (STG, 2004). Again many individuals abuse ciprofloxacin whenever they experience fever, especially when the symptoms of malaria persist after taking anti-malaria drugs. They then take ciprofloxacin on the assumption that they are

suffering from typhoid fever. This situation allows for the selection of ciprofloxacin-resistant strains.

Paterson and Bonomo (2005) reported that the increasing *in vitro* resistance of ESBL-producers against the quinolones will limit the role of these antibiotics in the future. This assertion is true for Ghana as resistance to ciprofloxacin has been rising over the years (Newman *et al.* 2006). Nitrofurantoin, which is recommended for use in areas with high prevalence of ESBL-producers (Melzer and Petersen, 2007) and amikacin were effective *in vitro* in this study. 77.94% and 71.43% of the ESBL-producers were susceptible to nitrofurantoin and amikacin respectively. Nitrofurantoin should therefore be considered as the drug of choice whenever an ESBL-producer isolated from urine is susceptible to it.

5. 2. 4 Antimicrobial susceptibility of ESBL-producers isolated from sputum, wound and aspirates

Meropenem seemed to be the only effective antimicrobial *in vitro* against ESBL producing organisms isolated from sputum, wound and aspirates. However carbapenems are not easily accessible because they are too expensive for the average Ghanaian to purchase and they are also not covered by the National Health Insurance Scheme in Ghana. Their use for such infections may also not be appropriate.

5. 2. 5 ESBL-producers which were 'susceptible' to cephalosporins

It is worth noting that, without specific ESBL testing, ESBL-producing organisms may be reported as susceptible to cephalosporins, penicillins and monobactams. The ESBL-producer isolated from pus was susceptible to only cephalosporins, i.e. cefotaxime and ceftriaxone. This

isolate was thus reported to be susceptible to the cephalosporins and so no second-line susceptibility testing was done. This is against the CLSI standards which state that all ESBL-producing strains should be reported as resistant to all penicillins, cephalosporins and aztreonam (CLSI, 2007). The patient may have been given one of the cephalosporins which could have resulted in treatment failure. The high level of false sensitives (23.21%) recorded especially for the cephalosporins is alarming as some patients may have been given a cephalosporin or any of the antimicrobial agents which are rendered ineffective in the presence of ESBL, resulting in treatment failure and prolonged hospital stay. Lautenbach *et al.* (2001) reported that infections caused by ESBL producing *E. coli* and *Klebsiella spp.* were associated with significantly longer duration of hospital stay and consequently greater hospital charges.

5. 2. 6 Impact of ESBL-producers

The results of the antibiogram suggest that *E. coli* and *Klebsiella spp.* are becoming more resistant to the cephalosporins (48.48%), aminoglycosides (36.51%), quinolones (51.00%) and tetracyclines (90.29%) as compared with data obtained in 2006 (KATH Records, 2006). This pattern may be due to the emergence of ESBL producing *E. coli* and *Klebsiella spp.* Table 4.1 clearly shows that the presence of ESBL producing organisms accounts for the high level of resistance to the antibiotics. It was however observed that resistance to nitrofurantoin by *E. coli* and *Klebsiella spp.* though low, was higher in the non ESBL-producers than it was in ESBL-producers buttressing the fact that nitrofurantoin is very active against ESBL-producers.

5. 2. 7 Predictors of ESBL-producers at KATH

The high odds ratio for an organism reported as resistant to cefotaxime (OR=20.80), ceftriaxone (OR=17.67), cefuroxime (OR=30.84), gentamicin (OR=9.34), nalidixic acid (OR=2.27), norfloxacin (OR=4.78), ciprofloxacin (OR=2.74) and cotrimoxazole (OR=6.44) being an ESBL-producer shows that there is a close association between the high levels of resistance observed at KATH and the production of ESBL by *E. coli* and *Klebsiella spp.* It also confirms reports that ESBL production confers resistance to several of the commonly used antimicrobials such as gentamicin (Ling *et al.*, 2006), cotrimoxazole, and fluoroquinolones (Babypadmini and Appalaraju, 2004 and Pitout *et al.*, 2005). Chloramphenicol, a phenicol, was also co-resistant with cephalosporins in the presence of an ESBL producing *E. coli* or *Klebsiella spp.* (OR= 3.00, 95% CI 1.19-7.56, $p = 0.029$).

Any *E. coli* or *Klebsiella spp.* isolated at KATH which is resistant to cefotaxime, ceftriaxone, cefuroxime, gentamicin, nalidixic acid, norfloxacin, ciprofloxacin, cotrimoxazole and chloramphenicol should be regarded as a potential ESBL-producer, and hence it should undergo ESBL testing. This is because there was a close association between ESBL production and resistance to any of the above mentioned antimicrobial agents by these organisms.

There is the need to train biomedical scientists in the laboratory to test for the production of ESBL among these organisms and for the health facilities to purchase equipment such as the VITEK machine that will allow for the detection of ESBL-producers.

There was no significant association ($p > 0.05$) between the production of ESBL and an isolate's resistance/sensitivity to ceftazidime, amikacin and pipemidic acid even though the odds ratios

were all greater than two. This observation may be due to the fact that these drugs are seldom used for susceptibility testing in hospitals.

Nitrofurantoin resistance cannot be used to predict the production of ESBLs because it was more effective *in vitro* against ESBL-producers than non ESBL-producers.

Again there was no association between isolates that were resistant to ampicillin and tetracycline and the production of ESBL because both ESBL- and non ESBL-producers investigated were generally resistant to these drugs. Of all the isolates studied only 4.96% and 9.71% were susceptible to the action of ampicillin and tetracycline respectively. Unlike ceftazidime, amikacin and pipemidic acid, ampicillin and tetracycline were used in susceptibility tests at least on 103 different organisms. Perhaps it is time these drugs are no longer used for AST because of their predictable resistance.

CHAPTER SIX

6. 0 Conclusion and recommendation

6. 1 Conclusion

This study has shown that there is a high prevalence of ESBL producing *E. coli* and *Klebsiella spp.* at KATH. ESBL-producing *E. coli* and *Klebsiella spp.* were generally resistant to the quinolones, aminoglycosides, tetracyclines, trimethoprim-sulfamethoxazole and phenicol but susceptible to the carbapenems. There was however no independent risk factor associated with the isolation of an ESBL producing *E. coli* or *Klebsiella spp.* in this study in terms of age, gender and location (i.e. in-/ out-patient) of a patient.

The high prevalence of ESBL-producing organisms will create significant therapeutic problems in the near future. There is therefore the need to better define strategies to reduce their spread in hospitals and the community.

6. 2 Recommendation

The following are proposed;

1. ESBL detection should be performed routinely on all blood and urine isolates.
2. Laboratory scientists should be trained to detect the production of ESBLs.
3. A modified form of the Combined Disc Method should be employed in the detection of ESBL-producers. This can be done by replacing ampicillin and tetracycline with cefpodoxime and cefpodoxime/ clavulanic acid disc in the first-line in susceptibility testing.
4. Hospitals and clinics should perform cultures and AST before prescribing antibiotics as often as practicable.
5. Ciprofloxacin should be considered as the treatment of choice in bloodstream infections whenever the isolates are susceptible to them.
6. Ciprofloxacin should not be used for the treatment of UTIs due to the high level of resistance observed.
7. Attempts to control and/ or prevent the spread of ESBL-producers should include the judicious use of all antimicrobial agents especially the carbapenems.
8. Health-care-givers should be educated to make them appreciate the clinical significance of such a high prevalence rate of ESBL-producers amongst *E. coli* and *Klebsiella spp.*
9. Retrospective studies should be done to determine the treatment outcomes of the patients from whom ESBL-producers were isolated.

10. A similar study should be conducted on other *Enterobacteriaceae* such as *Proteus mirabilis, Salmonella spp.* and *Enterobacter spp.* to determine the prevalence of ESBL-producers among them.
11. The high prevalence of ESBL-production among *E. coli* and *Klebsiella spp.* established in this study warrants genetic characterization of the isolates.

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Appendices

Appendix 1

Modified Combined Disc Method (cefpodoxime and cefpodoxime/clavulanic acid are added to the first line drugs to test for ESBL-producers)

Appendix 2

Control for antimicrobial susceptibility test (*E. coli* ATCC 25922)

Appendix 3

A picture of a non ESBL-producer as demonstrated by the CDM

Appendix 4

A picture of an ESBL-producer as demonstrated by the CDM

Appendix 5

BSAC recommended breakpoint for the cephalosporins

Antibiotic	Disc content (µg)	Interpretation of Zone diameter (m)	
		R≤	S≥
Cefotaxime	30	29	30
Ceftazidime	30	21	22
Cefpodoxime	10	25*	26*

*The BSAC zone diameter breakpoints for cefpodoxime have recently been revised to ≤19 (R) and ≥20 (S). Zone sizes of 21-25mm may deserve further investigation but are unlikely to produce ESBLs (SUESL, 2008).

Appendix 6

CLSI standards, 2007

Antimicrobial Agents	Disc content (µg)	Interpretation of Zone diameter (mm)		
		R≤	I	S≥
Cefotaxime	30µg	14	15-22	23
Ceftazidime	30µg	14	15-17	18
Cefpodoxime	10µg	17	18-20	21

Appendix 7

Gender distribution of sample source

Sample source	Gender		Total (p = 0.086)
	Male	Female	
Blood	31	40	71
Urine	51	73	124
Sputum	24	11	35
wound Swab	27	23	50
Ear Swab	2	1	3
Pus	1	3	4
Aspirates	3	5	8
	139	156	295

Appendix 8

Age distribution of sample source

Age Groups	Sample Source							Total P= 0.001
	Blood	Urine	Sputum	Wound	Ear	Pus	Aspirates	
0-10	41	30	2	8	2	1	4	88
11-20	6	6	3	1	1	0	1	18
21-30	3	22	5	3	0	0	0	33
31-40	4	12	3	7	0	0	0	26
41-50	1	8	6	8	0	0	2	25
51-60	2	7	6	7	0	1	1	24
61-70	3	11	1	4	0	1	0	20
71-80	2	14	4	5	0	0	0	25
81-90	1	6	2	1	0	0	0	10
91-100	0	1	0	0	0	0	0	1
	63	117	32	44	3	3	8	270

Appendix 9

Gender distribution amongst the age groups

Age Groups	Gender		Total $p= 0.001$
	Male	Female	
0-10	37	50	87
11-20	11	7	18
21-30	7	26	33
31-40	7	19	26
41-50	13	12	25
51-60	11	13	24
61-70	13	7	20
71-80	20	5	25
81-90	7	3	10
91-100	0	1	1
	126	143	269

Appendix 10

Gender distribution of ESBL-producers from sample source

Sample	Sex		Total $p= 0.225$
	Male	Female	
Blood	16	25	41
Urine	33	39	72
Sputum	10	3	13
Wound swab	8	10	18
Pus	1	0	1
Aspirates	1	1	2
	69	78	147

Appendix 11

Gender distribution of ESBL-producers amongst age groups

Age Groups	Gender		Total
	Male	Female	p=0.001
0-10	21	29	50
11-20	5	1	6
21-30	3	14	17
31-40	1	9	10
41-50	6	6	12
51-60	5	5	10
61-70	4	3	7
71-80	9	2	11
81-90	7	1	8
91-100	0	1	1
	61	71	132

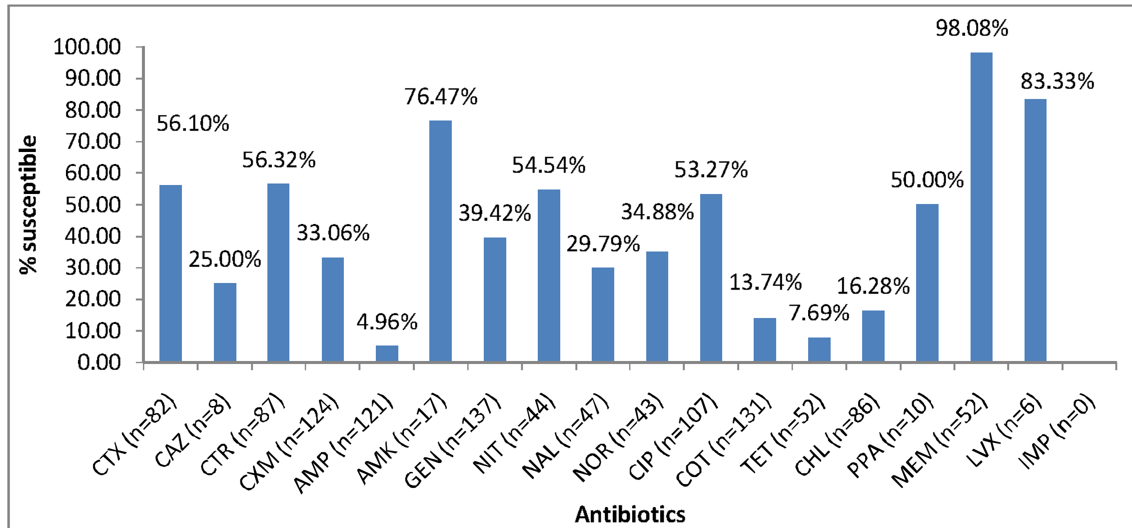
Appendix 12

Age distribution of ESBL-producers from sample source

Age Groups	Blood	Urine	Sputum	Wound swab	Aspirates	Total P=0.001
0-10	27	19	0	2	2	50
11-20	3	2	0	1	0	6
21-30	1	12	2	2	0	17
31-40	2	5	2	1	0	10
41-50	0	4	4	4	0	12
51-60	1	3	1	5	0	10
61-70	1	6	0	0	0	7
71-80	0	9	1	1	0	11
81-90	1	5	2	0	0	8
91-100	0	1	0	0	0	1
	36	66	12	16	2	132

Appendix 13

Antimicrobial susceptibility profile of 142 *Klebsiella pneumoniae* isolated at KATH



Appendix 14

Antimicrobial susceptibility profile of 7 *Klebsiella spp.* other than *K. pneumoniae* isolated at KATH