

**PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF
EXTENDED-SPECTRUM BETA-LACTAMASES IN *KLEBSIELLA
PNEUMONIAE* AND *ESCHERICHIA COLI*
ISOLATES IN ACCRA, GHANA**

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**This thesis is submitted to the University of Ghana, Legon
in partial fulfillment of the requirement for the award of
PhD Microbiology Degree.**

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June, 2015

DECLARATION

This is to certify that this thesis is the result of research undertaken by Henry Kwadwo Hackman towards the award of Doctor of Philosophy Degree Microbiology in the Department of Microbiology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana and that the thesis has not been presented to this University or elsewhere for the award of any other degree and to the best of my knowledge, it contains no material previously published by another person, except where due acknowledgement has been made in the text.



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ABSTRACT

Extended-spectrum beta-lactamases (ESBLs) are plasmid-mediated enzymes capable of hydrolysing beta-lactams except carbapenems and cephamycins but are inhibited by beta-lactamase inhibitors. Most of these ESBL plasmids also carry genes conferring resistance to several non-beta-lactam antimicrobials. Hence, ESBL-producing isolates limit therapeutic options, contribute to treatment failure, increase morbidity and mortality, prolong hospitalization and increase cost of healthcare. There is no published work on the genetic characterization of ESBL producing strains, the characteristic antimicrobial resistance profile of CTX-M and TEM ESBL producers and the occurrence of AmpC beta-lactamases among ESBL and non-ESBL phenotypes in Accra. This work determined the phenotypic and molecular characterization of ESBLs in *K. pneumoniae* and *E. coli* isolates and their antimicrobial resistance profile in Accra. Four hundred (400) *K. pneumoniae* and *E. coli* non-duplicate isolates were collected at Korle Bu Teaching Hospital and Advent Clinical Laboratories. The species identification, ESBL detection, MIC and antimicrobial susceptibility testing were concurrently determined using Vitek 2 Compact System. The Combined Disc Synergy Method (CDM) was used to confirm ESBL-producing strains. The isolates were screened for AmpC beta-lactamase phenotypes using disc synergy testing. The genotypes of the ESBL-coding genes were determined by PCR using already published primers. The results showed that 202 (50.5%) of the bacterial isolates were ESBL-producers with high co-resistance to beta-lactams, beta-lactam/beta-lactamase inhibitors and non-beta-lactams. The sensitivity (98.5%), specificity (98.9%), positive predictive value (99%) and negative predictive value (98.5%) of Vitek 2 Compact system confirmed it as a rapid and reliable system for accurate detection of ESBL strains in

Accra. The findings of this current study showed a low rate of AmpC beta-lactamase phenotypes which might not to interfere with the detection of ESBL producers. There were significant differences ($p < 0.05$) between the resistance of ESBL producers and non-ESBL producers to beta-lactams, beta-lactam/beta-lactamase inhibitors and non-beta-lactams. Of a 100 randomly selected ESBL producers based on the MIC of cefotaxime, CTX-M (90%) and CTX-M-1 group (78%) were the dominant ESBL genes, 2% were positive for CTX-M-9 group ESBL genes and 25% had TEM genes. None of the ESBL producers possessed SHV genes. CTX-M-type ESBLs are more efficient in hydrolysing cefotaxime with typical cefotaxime MIC of $\geq 64 \mu\text{g/ml}$. TEM-type ESBL producers appeared to be more efficient in hydrolysing ceftazidime than CTX-M-types ESBL producers and hydrolysed both ceftazidime and cefotaxime. The CTX-M-type and TEM-type ESBLs showed co-resistances to beta-lactams, beta-lactam/beta-lactamase inhibitors and non-beta-lactams and hydrolysed cefepime with less efficiency. Imipenem and amikacin were the drugs of choice for managing CTX-M and TEM-type ESBL producers. It is vital to routinely detect ESBL-phenotypes and implement appropriate antimicrobial stewardship programs in health facilities. Further studies into the sequencing of ESBL genes is recommended to determine specific ESBL gene present in a strain.

DEDICATION

This thesis is dedicated to Mrs Mavis Ama Hackman, the crown of my life for your unparalleled love, understanding, emotional, financial and prayer support.



ACKNOWLEDGEMENTS

The LORD is my strength and my song; he has become my salvation. He is my God and I will praise him. I am indebted to my principal supervisor Professor Kingsley Twum-Danso for his guidance, patience and professionalism. My co-supervisor Dr. Charles A. Brown deserves commendation for his personal support and encouragement especially during the molecular studies. I benefited from the technical support provided by the staff at the Molecular Biology Laboratory Unit of the Department of Medical Laboratory Technology, comprising Mrs Gloria Amegashi and Mr Christian Adabor Badu under the guidance of the head of department, Mr Harry Asmah. I appreciate the data statistical analysis support from Mr. David Adjei of the Department of Medical Laboratory Technology. I acknowledge my two beautiful daughters, Henrietta Afua Afoa Hackman and Mavis Yaa Kwabea Hackman for enduring my long hours of studies. The prayer support from my caring mother, Madam Veronica Yeboah, brothers, in-laws, friends, pastors, presbyters and church members is much appreciated. Thank you. I deeply acknowledge the immense encouragement from my dear sisters Ms Mabel Nartey and Ms Emelia Ofori. You helped me financially to publish papers from this thesis in peer reviewed journals. I am grateful to the management of Central Laboratory, Korle Bu Teaching Hospital, Advent Clinical Laboratory and College of Health Sciences for their logistical and financial support. I am eternally grateful to senior members, administrative and technical staff of Department of Microbiology, School of Biomedical and Allied Health Sciences and everyone who in diverse ways contributed to the successful completion of the work.

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ABBREVIATIONS

AES	Advanced Expert System
AMC	Amoxicillin/Clavulanic Acid
AMK	Amikacin
AMP	Ampicillin
AST	Antimicrobial Susceptibility Testing
ATCC	American Typed Culture Collection
BES	Brazilian extended-spectrum Beta-lactamases
CAZ	Ceftazidime
CDM	Combined Disc Synergy Method
CFP	Cefepime
CFZ	Cefazolin
CIP	Ciprofloxacin
CLSI	Clinical Laboratory and Standards Institute
COX	Cefoxitin
CTX	Cefotaxime
CTX-M	Cefotaximase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ESBL	Extended-spectrum beta-lactamase
GEN	Gentamicin
HPA	Health Protection Agency
ID	Identification
IMP	Imipenem
MIC	Minimum Inhibitory Concentration

NIT	Nitrofurantoin
NOR	Norfloxacin
OXA	Oxacillinases
PBP	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
PER	<i>Pseudomonas</i> Extended Resistances
PIP	Piperacillin
PMABL	Plasmid-mediated AmpC beta-lactamase
PTZ	Piperacillin/Tazobactam
SHV-	Sulfhydryl variable active sites
TEM	Temoniera
TET	Tetracycline
TSX	Trimethoprim/Sulphamethoxazole
µg	microgram
µl	micro litre
VEB	Vietnamese Extended-spectrum β-lactamase

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Bacteria like other living organisms have evolved mechanisms to survive stressful conditions. This may be an inherent or acquired trait that can be transferred vertically to the progeny of the parent bacterium during replication or horizontally to other bacteria through the process of conjugation, transduction and transformation. One of these mechanisms is the enzymatic inactivation of antimicrobials due to the production of extended-spectrum beta-lactamases (ESBLs). Extended-spectrum beta-lactamases (ESBLs) are plasmid-mediated enzymes that are capable of hydrolysing beta-lactams except carbapenems and cephamycins. They are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. ESBLs are produced by Gram-negative bacilli with *Klebsiella pneumoniae* and *Escherichia coli* being the predominant ESBL-producing isolates (Paterson & Bonomo, 2005). ESBLs are grouped into three main types: SHV, TEM or CTX-M. Other less common ESBL types include OXA, PER, VEB 1 and BES 1. There are several subtypes of each of the ESBL types. More than 300 different ESBL variants are known (Paterson & Bonomo, 2005).

SHV-2 ESBL was the first to be described in Germany in 1983 as an isolate of *Klebsiella ozaenae* (Kliebe *et al.*, 1985). Studies of the SHV-2 gene indicated that it emanated from a point mutation in the SHV-1 gene which changed glycine to serine at position 238. SHV-1 does not encode an ESBL (Kliebe *et al.*, 1985). According to Paterson and colleagues (2003), SHV-2 is found worldwide and it is the predominant ESBL type in Europe and the United States. More than 60 SHV derivatives are known, however, the common ones are SHV-5 and SHV-12 (Paterson *et al.*, 2003). TEM-3, the first TEM-type ESBL isolated, arose from point mutations in the TEM-2 gene which

changed glutamic acid to lysine at position 104 and glycine to serine at position 238 (Sougakoff *et al.*, 1988). Although TEM-type beta-lactamases are most often found in *E. coli* and *K. pneumoniae*, they are also found in other species of Gram-negative bacteria with increasing frequency. Over 140 TEM-type enzymes have been described (Bradford, 2001).

CTX-M ESBLs were first reported in the late 1980s and have spread rapidly in the last decade to become the most common ESBL type in many countries (Bonnet, 2004). These ESBLs were designated so because they characteristically display a higher level of resistance to cefotaxime than ceftazidime (Bonnet, 2004). CTX-M enzymes can be sub-classified by amino acid sequence similarities. A phylogenic study revealed five major groups of acquired CTX-M enzymes (Bonnet, 2004). The CTX-M-1 group (Group I) includes eleven plasmid-mediated enzymes (CTX-M-1, CTX-M-3, CTX-M-10, CTX-M-12, CTX-M-15, CTX-M-22, CTX-M-23, CTX-M-28, CTX-M-29, CTX-M-30 and CTX-M-68). The CTX-M-2 group (Group II) includes six plasmid-mediated CTX-M enzymes (CTX-M-2, CTX-M-4, CTX-M-5, CTX-M-6, CTX-M-7 and CTX-M-20). The CTX-M-8 group (Group III) includes one plasmid-mediated member. The CTX-M-9 group (Group IV) includes nine plasmid-mediated enzymes (CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-16, CTX-M-17, CTX-M-19, CTX-M-21, CTX-M-24 and CTX-M-27). The CTX-M-25 group (Group V) includes the CTX-M-25 and CTX-M-26 enzymes (Bonnet, 2004).

Often the ESBL derivative differs by only one amino acid from the original enzyme (Bonnet, 2004), but the difference is often significant. The known risk factors for infection with ESBL producing bacteria include recent surgery, instrumentation, prolonged hospital stay, nosocomial transmission of ESBL-producing organisms via the hands of hospital staff and antibiotic exposure, especially to extended-spectrum beta-

lactam antibiotics (Paterson & Bonomo, 2005). The extended-spectrum antibiotics usage drives a selective pressure for emergence of ESBL-producing phenotypes. The resultant resistant plasmids can then be transferred horizontally to other bacteria (Paterson & Bonomo, 2005). Because ESBL enzymes are plasmid mediated, the genes encoding these enzymes are easily transferable among different bacteria. Most of these plasmids not only contain DNA encoding ESBL but also carry genes conferring resistance to several non-beta-lactam antibiotics (Todar, 2008). Consequently, most ESBL isolates are not only resistant to beta-lactams but also to other classes of antimicrobials including aminoglycosides, fluoroquinolones, tetracyclines, nitrofurans (e.g. nitrofurantoin) and trimethoprim/sulphamethoxazole (Todar, 2008). Treatment of these multiple drug-resistant organisms has proven to be a therapeutic challenge (Todar, 2008). ESBL-producing isolates pose serious public health, financial and logistics challenges because physicians are restricted in the choice of available antibiotics for effective treatment of ESBL infections.

The increased prevalence of ESBL-producing bacteria implies an urgent need for laboratory diagnostic methods that will accurately and rapidly identify the presence of these enzymes in clinical isolates. The need for laboratory detection of ESBLs came about because some ESBL-producing organisms which appeared susceptible to cephalosporins *in vitro* using conventional breakpoints were ineffective clinically. A failure to detect ESBLs followed by subsequent treatment with oxyimino-cephalosporins is associated with a higher risk of treatment failure (Paterson *et al.*, 2001). Other reports also indicate higher mortality rates (Kim *et al.*, 2002). The Clinical and Laboratories Standard Institute (CLSI) recommends a two-step phenotypic approach in detecting ESBL producers (CLSI, 2006). This involves screening for reduced susceptibility to more than one of the indicator antimicrobials (cefotaxime,

ceftazidime, cefpodoxime, ceftriaxone and aztreonam). After the ESBL screening test, the CLSI recommends the use of cefotaxime (30µg) or ceftazidime discs (30µg) with clavulanate (10µg) for phenotypic confirmation of the presence of ESBLs in *Klebsiella* species and *Escherichia coli*. The CLSI recommends that the disc tests should be performed with a confluent growth on Mueller-Hinton agar. A difference of ≥ 5 mm between the zone diameters of either of the cephalosporin discs and their respective cephalosporin/clavulanate disc is taken to be phenotypic confirmation of ESBL production (CLSI, 2006). The combined disc synergy method is accepted as a reference method for confirming ESBL-producing organism according to CLSI (CLSI, 2006).

ESBL phenotypic confirmatory testing can also be performed by broth micro-dilution 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). ESBL phenotype is confirmed when there is a threefold serial dilution reduction in MICs in the presence of clavulanate (Queenan *et al.*, 2004). Automated phenotypic methods of detecting ESBL producers have been developed to ensure rapid and accurate detection of ESBL producers. These include the use of MicroScan panels, BD Automated Microbiology System and Vitek 2 ESBL cards (Paterson & Bonomo, 2005). The Vitek 2 System (bioMérieux, France) is a rapid automated microbiological system used for bacteria and yeast identification and antimicrobial susceptibility testing (AST). It analyses MIC patterns and detects bacterial resistance mechanisms for most organisms tested using its advanced expert system. Vitek 2 ESBL test is a tool for rapid detection of a positive or negative ESBL producing strain which is based on simultaneous assessment of the inhibitory effects of cefotaxime, ceftazidime and cefepime, alone and in the presence of clavulanic acid (Teresa *et al.*, 2006).

Several molecular methods are available for research and epidemiological studies, but they may not be appropriate for routine detection of ESBL production in clinical settings (Pitout *et al.*, 2004). The easiest and most common molecular method used is the polymerase chain reaction (PCR) with oligonucleotide primers that are specific for a beta-lactamase gene. The PCR amplification of CTX-M-specific products without sequencing usually provides sufficient evidence that a CTX-M gene is responsible for the ESBL phenotype. This is unlike TEM and SHV types of ESBLs which PCR will not discriminate among different variants of TEM or SHV (Bradford, 2001). Sequencing is essential to discriminate between the non-ESBL parent enzymes (e.g., TEM 1, TEM 2, or SHV 1) and different variants of TEM or SHV ESBLs (e.g., TEM 3, SHV2, SHV 12 etc.). Nucleotide sequencing helps in the determination of the specific beta-lactamase gene present in a strain (Bradford, 2001).

AmpC beta-lactamases may interfere with ESBL detection. AmpC beta-lactamases cause resistance to penicillins, cephalosporins, aztreonam and cephamycins but resist inhibition by beta-lactamase inhibitors (Jacoby, 2009). Organisms producing enough AmpC beta-lactamase will typically give a positive ESBL screening test but fail the ESBL confirmatory test involving increased sensitivity with clavulanic acid (Thomson, 2001). Resistance due to plasmid-mediated AmpC enzymes is less common than extended-spectrum beta-lactamase production in most parts of the world but may be both harder to detect (Jacoby, 2009). The genetic determinants for AmpC beta-lactamases are commonly found on the chromosomes of *Enterobacter* and *Citrobacter*, but have now spread to other organisms, including *E. coli* and *Klebsiella* through plasmids (Heffernan *et al.*, 2007). AmpC beta-lactamase-producing organisms are resistant to cefoxitin. Cefoxitin resistance can also be caused by decreased levels of outer membrane porins and carbapenemase in both *K. pneumoniae* and *E. coli*.

Nevertheless, cefoxitin resistance is said to be a discriminative parameter for the detection of AmpC-producing strains (Peter-Getzlaff *et al.*, 2011). Furthermore, AmpC beta-lactamases are known to be inhibited by boronic acid and cloxacillin (Jacoby, 2009). AmpC beta-lactamases are detected by disc synergy testing (DST) using cefotaxime or ceftazidime with or without boronic acid (Jacoby, 2009). Yagi and colleagues (2005) found that a ≥ 5 mm increase of the zone of inhibition around a ceftazidime or cefotaxime disc when 300 μ g 3-aminophenylboronic acid was added, reliably detected all AmpC varieties.

Fluoroquinolones may be regarded as the treatment of choice for complicated urinary tract infections due to ESBL-producing strains (Paterson & Bonomo, 2005). Observational clinical studies have assessed the relative merits of fluoroquinolones and carbapenems for serious infections due to ESBL-producing organisms. Two of these studies found that carbapenems were superior to fluoroquinolones (Endimiani *et al.*, 2004), whereas the other found that they were equivalent in effectiveness (Kang *et al.*, 2004). Carbapenems are recommended as the drugs of choice for serious infections with ESBL-producing organisms (Paterson & Bonomo, 2005).

1.2 PROBLEM STATEMENT

- i. Extended-spectrum beta-lactamase-producing organisms have emerged as a major contributing factor to nosocomial infections.
- ii. There is no routine laboratory detection of ESBL-producing isolates in most health facilities in Ghana.
- iii. ESBL-producing organisms may produce AmpC beta-lactamases together with ESBL. AmpC beta-lactamases are associated with multi-drug resistance and also aggravate the difficulty in laboratory detection of ESBL isolates.

- iv. ESBL-producing organisms appear susceptible to cephalosporins *in vitro* but are ineffective *in vivo*. Most of the ESBL plasmids not only contain DNA encoding ESBL but also carry genes conferring resistance to several non-beta-lactam antibiotics. Therefore ESBL-producing isolates limit therapeutic options, contribute to treatment failure, increase morbidity and mortality, prolong hospitalization and increase cost of health care.
- v. Due to the high treatment failure associated with ESBL-producing organisms worldwide, it is imperative to ascertain their antimicrobial resistance profile and select the appropriate drug for managing ESBL infections in Accra.
- vi. There is no published work on the genetic characterization of
- vii. ESBL-producing strains and their characteristic antimicrobial susceptibility profile based on their minimum inhibitory concentrations in Accra.
- viii. There is no published work on the occurrence of AmpC beta-lactamases in Accra.

1.3 JUSTIFICATION AND RELEVANCE OF THE STUDY

ESBL-producing organisms are prevalent in all continents including Africa. However, clinical microbiology laboratories make no effort to detect ESBL-producing organisms routinely or are ineffective at doing so (Paterson & Bonomo, 2005). In a 1998 survey of 369 American clinical microbiology laboratories, only 32% (117 of 369) reported performing tests to detect ESBL production in the *Enterobacteriaceae*. A subsequent survey of laboratory personnel at 193 hospitals showed that only 98 (51%) correctly reported a test organism as an ESBL producer (Paterson & Bonomo, 2005). Currently, there are no systems to routinely detect ESBL-producing isolates in most clinical laboratories in Ghana. ESBL-producing isolates also producing AmpC beta-lactamases

which makes ESBL detection very difficult. This may lead to treatment failure and increased morbidity and mortality in patients infected by ESBL-producing bacteria. It is imperative to routinely detect ESBL-producing bacteria and possible AmpC beta-lactamases-producing phenotypes. This work will therefore assess the occurrence of ESBL-producing isolates in Accra and assess the possibility of *E. coli* and *K. pneumoniae* also produce AmpC beta-lactamases. Furthermore, it will also compare the detection of ESBL phenotypes using Vitek 2 system with combined disc synergy method by determining the sensitivity and specificity of Vitek 2 ESBL test. Accurate and rapid phenotypic diagnosis of ESBL-producing infection in patients will ensure quality healthcare and help to monitor and control the prevalence of ESBL-producing bacteria. This would be the first time that Vitek 2 system would be used in Ghana on a large scientific scale and may be recommended to the Ministry of Health for routine use by laboratories in Ghana when the outcome proves successful.

CTX-M-type, TEM-type and SHV-type ESBLs are the main types of ESBL produced by bacteria. There is a high prevalence of CTX-M-type ESBLs in North America, South America, Western Europe, China, India and Africa. Some researchers are speculating that CTX-M-type ESBLs are now the most frequent ESBL type worldwide as compared to SHV-type and TEM-type ESBLs (Paterson & Bonomo, 2005). CTX-M-15 and CTX-M-14 seem to be the most widespread globally, while many of the other CTX-M ESBLs tend to be more limited in their distribution (Heffernan *et al.*, 2007). The specific ESBL-producing organisms have different genetic characteristics which mark their identification at the molecular level. The various ESBL encoding genes in bacteria may reflect characteristic differences in relation to antimicrobial resistance expression and response to therapy. Genetic characterization of ESBL-producing organisms is also essential for epidemiological use.

The rate of antibiotic resistance globally to beta-lactams and other antibiotic classes is becoming alarming. This rate is enhanced by the transfer of genetic material between related and unrelated strains via transposons, integrons and transmissible plasmids. The antimicrobials used in this current study included penicillins, cephalosporins, carbapenems, cephamycins, aminoglycosides, fluoroquinolones, tetracyclines, nitrofurans, beta-lactam/beta-lactamase inhibitors and trimethoprim/sulphamethoxazole. This is because most of the plasmid-mediated ESBL producers carry genes conferring resistance to several beta-lactams and non-beta-lactams (Paterson & Bonomo). More so, these groups of antimicrobials are commonly available and used in Ghana (Newman *et al.*, 2006). Reports of ESBL-associated drug resistance in Kumasi (Adu-Sarkodie, 2010) and Nigeria (Aibinu *et al.*, 2003) underscore the importance of monitoring the antimicrobial resistance profile of ESBL-producing organisms in Accra. The outcome of this work will update the clinical community on the drug of choice for managing ESBL infections in Accra, Ghana, and help to reduce treatment failure, morbidity and mortality, shorten hospitalization and reduce cost of health care. This work will analyse the antimicrobial resistance profile among ESBL-producing strains with CTX-M and TEM-type ESBL genes.

1.4 HYPOTHESES

This work hypothesized the following:

- i. CTX-M-type and CTX-M-1group ESBL producers are more prevalent in Accra as compared to TEM-type and SHV-type ESBL producers.
- ii. There are increasing levels of resistance of CTX-type ESBL producers to non-beta-lactams and beta-lactam/beta-lactamase inhibitor combinations

- iii. There are low levels of AmpC beta-lactamase producers among ESBL and a non-ESBL-producing isolates in Accra.

1.5 AIM

This work determined the phenotypic and molecular characterization of ESBLs in *K. pneumoniae* and *E. coli* isolates and their antimicrobial resistance profile in Accra.

1.6 SPECIFIC OBJECTIVES

1. To determine the reliability of Vitek 2 System in the detection of ESBL-producing phenotypes in comparison with combined disc synergy method (CDM) in Accra.
2. To determine the resistance profile of ESBL and non-ESBL- producing isolates to beta-lactams, beta-lactam/beta-lactamase inhibitor combinations and non-beta-lactams.
3. To investigate the occurrence of AmpC beta-lactamase-producing phenotypes among ESBL and non-ESBL producers.
4. To characterize CTX-M, CTX-M-1 group, CTX-M-9 group, TEM and SHV ESBL-producing *K. pneumoniae* and *E. coli* isolates using polymerase chain reaction.
6. To determine the characteristic resistance profile of CTX-M-type and TEM-type ESBL producers to beta-lactams, beta-lactam/beta-lactamase inhibitor combinations and non-beta-lactams based on their minimum inhibitory concentrations (MICs).

CHAPTER TWO

LITERATURE REVIEW

2.1 THE *ENTEROBACTERIACEAE* FAMILY AS ESBL PRODUCERS

The family *Enterobacteriaceae* is a large heterogeneous group of Gram-negative rods that are normal intestinal flora of humans and animals. They are also found in soil and water. They are facultative anaerobes while some are motile and others non-motile. They ferment glucose with the production of acid and reduce nitrates to nitrites but do not produce oxidase or liquefy alginate (Zinsser & Joklik, 1992). They are catalase-positive and have a G + C DNA ratio of 39-59% (Brooks *et al.*, 2004).

Their complex bilayer is composed of murein, lipoprotein, phospholipid, protein and lipopolysaccharides arranged in layers. The murein-lipoprotein layer constitutes approximately 20% of the total bilayer and is responsible for cellular rigidity (Zinsser & Joklik, 1992). The remaining portion of the bilayer forms the lipid bilayer and its major constituent is lipopolysaccharides (LPS) which determine its endotoxic activity. The genera of the *Enterobacteriaceae* include *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Morganella*, *Citrobacter*, *Salmonella*, *Shigella* and *Yersinia*.

Infections caused by organisms other than *Salmonella*, *Shigella*, *Yersinia* and other pathogenic *E. coli* are considered as opportunistic. However, *Salmonella*, *Shigella* and *Yersinia* are regarded as true enteric pathogens and their isolation from the intestine of an individual implies either a diseased or carrier state (Brooks *et al.*, 2004). The *Enterobacteriaceae* such as *E. coli* and *K. pneumoniae* may cause infections such as urinary tract infection, pneumonia, bacteremia, thrombophlebitis, cholecystitis, upper respiratory tract infection, wound infection, osteomyelitis and meningitis. Sepsis and

septic shock may follow entry of organisms into the blood from a focal source (Umeh & Berkowitz, 2009).

Most genera of the *Enterobacteriaceae* are known to be producers of ESBLs. The levels of ESBL producers among nosocomial *Enterobacteriaceae* isolates have risen substantially in several countries (Falagas & Karageorgopoulos, 2009). Additionally some other members of the *Enterobacteriaceae* such as *Enterobacter cloacae*, *E. aerogenes*, *Citrobacter freundii*, *Serratia marscesens*, *Morganella morganii*, *Aeromonas* species, *Providencia* species and *Hafnia alvei* also produce inducible chromosomal AmpC beta-lactamases. These are resistant to cephalosporins, penicillins, aztreonam and cephamycins. Others such as *Klebsiella* species, *Salmonella* species, *C. freundii*, *E. aerogenes*, *P. mirabilis* and *E. coli* also produce plasmid-mediated AmpC beta-lactamases which are associated with multi-drug resistance (Thomson, 2010).

2.2 BETA-LACTAM ANTIMICROBIALS

Beta-lactam drugs are characterized by the presence of beta-lactam ring. Beta-lactam antimicrobials have been classified into penicillins, cephalosporins, monobactams, carbapenems and cephamycins (Brooks *et al.*, 2004). They irreversibly inhibit enzymes involved in the final steps of cell wall synthesis. Beta-lactam drugs are bound by penicillin-binding-proteins (PBPs) and competitively inhibit their enzymatic activity. This disrupts the cell wall biosynthesis leading to cell lysis. They are therefore bactericidal against a variety of bacteria. Beta-lactam drugs are more active during the log phase of bacterial cell growth than during the stationary phase. Resistance is due to synthesis of beta-lactamases, decreased affinity to PBPs or decreased uptake.

2.2.1 Penicillins

The penicillins share a common basic structure. The difference among the various penicillin derivatives is due to modification of the side chains. Penicillins have a five-member ring and are substituted in only one place. Natural penicillins are produced by the mold *Penicillium chrysogenum* (Levinson, 2004). They act by inhibiting transpeptidases, the enzymes that catalyse the final cross-linking step in the synthesis of peptidoglycan. Apart from inhibiting penicillin-binding proteins, penicillin treated cells activate murein hydrolases which degrade the peptidoglycan of cell walls.

Benzylpenicillin (penicillin G) is one of the most widely used antibiotics. It has four disadvantages, three of which have been successfully overcome by chemical modification of the side chain (Levinson, 2004). The acid hydrolysis of benzylpenicillin in the stomach has been addressed by the production of penicillin V. The limited effectiveness of penicillin G against Gram-negative rods has been overcome by a series of chemical changes in the side chain resulting in the synthesis of broad-spectrum penicillins such as ampicillin and amoxicillin. However, these drugs are inactivated by bacterial beta-lactamases including those produced by *E. coli* and *K. pneumoniae* (Levinson, 2004). Ticarcillin and piperacillin have greater activity against *Pseudomonas* species and other Gram-negative bacteria, though they are inactivated by many beta-lactamase-producing organisms. The inactivation of penicillins by beta-lactamases has led to the syntheses of penicillinase-resistant penicillins such as methicillin, oxacillin and dicloxacillin as well as combinations such as ticarcillin with clavulanic acid, piperacillin with tazobactam and amoxicillin with clavulanic acid (Levinson, 2004).

2.2.2 Cephalosporins

Most cephalosporins are products of the fungus *Acremonium cephalosporium*. The cephalosporins differ from the penicillins in that they have six-member ring adjacent to the beta-lactam ring and are substituted in two places on the 7-aminocephalosporanic acid nucleus. Cephalosporins are effective against a broad range of organisms and are generally tolerated and produce fewer hypersensitivity reactions than penicillins (Levinson, 2004). Their chemical structure makes them resistant to some beta-lactamases. Like penicillins, cephalosporins have been chemically modified to produce a family of related antibiotics. They are grouped as first- (cefazolin), second- (cefuroxime), third- (cefotaxime, ceftazidime, cefpodoxime, ceftriaxone) and fourth- (cefepime) generation cephalosporins. First generation cephalosporins are active primarily against Gram-positive cocci. The later generations are generally more effective against Gram-negative bacteria (Levinson, 2004).

2.2.3 Monobactams

Structurally, monobactams are slightly different from other beta-lactam drugs in that their beta-lactam ring is without an adjacent sulphur-containing ring structure; that is, they are monocyclic. Aztreonam, the most therapeutically useful monobactams has excellent activity against many Gram-negative rods, such as members of the family *Enterobacteriaceae* and *Pseudomonas* but are inactive against Gram-positive and anaerobic bacteria. It is useful in patients with penicillin allergy (Levinson, 2004).

2.2.4 Carbapenems

Carbapenems are structurally different from penicillins and cephalosporins. Carbapenems have the widest spectrum of activity of the beta-lactam drugs. They have

effective bactericidal activity against many Gram-positive, Gram-negative and anaerobic bacteria. They are also not inactivated by most beta-lactamases. Examples include imipenem, meropenem and ertapenem (Levinson, 2004)

2.2.5 Cephamycins

Cephamycins are naturally occurring antibiotics having a 7-methoxy group and possessing marked resistance to the action of beta-lactamases from Gram-positive and Gram-negative organisms. They are active against aerobic and anaerobic bacteria. These include cefmetazole, cefotetan and ceftiofur. ESBL-producing organisms such as *E. coli* and *K. pneumoniae* are susceptible to cephamycins *in vitro*. However, they become resistant to cephamycins when produced together with plasmid-mediated AmpC beta-lactamases (Jacoby, 2009).

2.2.6 Non-Beta-lactam Antimicrobials

There are several non-beta-lactam drugs that are becoming ineffective against ESBL-producing isolates due to other resistant genes carried on the plasmids of ESBL-producing organisms. These classes of antibiotics include aminoglycosides, fluoroquinolones, tetracyclines, nitrofurans, and trimethoprim/sulphamethoxazole (Paterson & Bonomo, 2005).

Aminoglycosides are bactericidal antibacterial agent that inhibit protein synthesis by binding to the 30s ribosomal subunit and cause a misreading of the genetic code. They exhibit bactericidal activity against Gram negative aerobes but generally not against anaerobic bacteria. Due to its nephrotoxicity and ototoxicity, aminoglycoside use has been clinically limited to severe infections. Examples include gentamicin, amikacin, tobramycin, kanamycin and streptomycin (Levison, 2012).

Fluoroquinolones are broad-spectrum antimicrobial agents that are used to treat serious infections and other hospital-acquired infection in which resistance to other antibacterial classes is suspected. They exert bactericidal activity by inhibition of nucleic acid synthesis. Fluoroquinolones bind to the DNA gyrase and interrupt a process that leads to the negative supercoiling of bacterial DNA which renders the bacteria unable to multiply and survive. Examples include ciprofloxacin, norfloxacin and levofloxacin (Hooper, 2001).

Tetracyclines are bacteriostatic antimicrobial agents that inhibit protein synthesis by binding to receptors on the 30S ribosomal subunit of the bacteria, preventing attachment of aminoacyl-tRNA to the RNA-ribosome complex. This prevents the addition of amino acids to the elongating peptide chain, preventing synthesis of proteins. They are broad spectrum that exhibit activity against a wide range of Gram-positive, Gram-negative bacteria, atypical organisms such as chlamydiae, mycoplasmas, rickettsiae and protozoan parasites. They have a role in reducing the duration and severity of chlorella. However their use in treating infections of the urinary tract, respiratory tract and intestines is limited due to antimicrobial resistance. Examples include tetracycline, doxycycline and minocycline (Paterson & Bonomo, 2005).

Nitrofurantoin is an antimicrobial agent that is indicated for the treatment of uncomplicated urinary tract infections. Nitrofurantoin works by attacking bacterial DNA, ribosomal proteins, respiration, pyruvate metabolism and other macromolecules within the cell (Paterson & Bonomo, 2005).

Trimethoprim/sulphamethoxazole or co-trimoxazole is an antibiotic used in the treatment of a variety of bacterial, fungal, and protozoal infections. It consists of one part trimethoprim to five parts sulphamethoxazole. Co-trimoxazole is generally

considered bactericidal, although its components are individually bacteriostatic. It inhibits the synthesis and metabolism of folate (Falagas *et al.*, 2008).

2.2.7 Beta-lactam/Beta-lactamase Inhibitor Combinations Antimicrobials

Clavulanate, sulbactam and tazobactam are beta-lactamase inhibitors with limited intrinsic antibacterial activity but inhibit the activity of a number of plasmid-mediated beta-lactamases. They generally do not inhibit chromosomally mediated beta-lactamases. Combination of these agents with amoxicillin, piperacillin, ticarcillin and ampicillin results in antibiotics with an enhanced spectrum of activity against many plasmid-mediated beta-lactamases (Bush & Johnson, 2000).

2.3 RESISTANCE MECHANISMS OF ANTIMICROBIALS

One of the major public health challenges confronting clinicians, microbiologists, drug development experts and public health specialists is the prevalence of antibiotic resistance in most known bacterial pathogens. Antibiotic resistance in bacteria may be an inherent character of the organism that renders it naturally non-susceptible to specific antibiotics. Other antibiotic resistances are acquired by means of mutation of DNA of the bacteria or acquisition of resistance-conferring DNA from another source (Barker, 1999). The genes for drug resistance may be located on the bacterial chromosome, plasmid or transposons. Once the resistance genes have developed, they can be vertically transferred to the progeny of the parent bacterium during replication. The resistant genome can also be horizontally transferred to bacteria of the same species or even different species through the process of conjugation, transduction and transformation (Linton, 1977). The problem of antimicrobial resistance is compounded

by the principles of natural selection. In the selective environment of the antibiotic, the wild sensitive bacterium is killed and the resistant mutants survive and flourish.

A number of mechanisms have evolved in bacteria which confer antibiotic resistance on them. One of these mechanisms renders the antibiotic inactive through physical removal from the cellular membrane through efflux pumps. An alternative approach is to alter the target site so that the bacterium is not recognized by the antibiotic or alter their metabolic pathway. The most common mode of resistant mechanism is enzymatic inactivation of the antibiotic (Todar, 2008). Most Gram-negative bacteria produce beta-lactamases that inactivate beta-lactams.

2.4 CLASSIFICATION OF BETA-LACTAMASES

Beta-lactamase production remains the most important contributing factor to beta-lactam resistance in Gram-negative pathogens (Livermore, 2003). Beta-lactamases constitute a heterogeneous group of enzymes, produced by almost all Gram-negative bacteria. They hydrolyse the beta-lactam ring of penicillins, cephalosporins, monobactams and carbapenems destroying their antibiotic activity.

Several classification schemes of the beta-lactamases have been proposed according to their hydrolytic spectrum, susceptibility to inhibitors, genetic location (either plasmid or chromosomes) and amino-acid sequence. Ambler introduced the four molecular classes of beta-lactamases (Ambler, 1980). It was based on the amino-acid sequence designated A to D. Classes A, C and D act by serine-based mechanisms and class B or metallo-beta-lactamases are the zinc-dependent enzymes. Bush and colleagues described the functional classification scheme of beta-lactamases (Bush *et al.*, 1995). The molecular classification correlates with the functional classification.

Bush and colleagues classified beta-lactamases under four groups according to their substrate and inhibitor profiles. Group 1 are cephalosporinases that are not well inhibited by clavulanic acid, belonging to the molecular class C. They are referred to as the AmpC beta-lactamases (Jacoby, 2009). AmpC beta-lactamases are cephalosporinases produced by some Gram-negatives and a few other organisms. Many Gram-negative bacteria produce chromosomally mediated AmpC beta-lactamases which, when hyperproduced, may cause resistance to penicillins, cephalosporins, aztreonam and cephamycins (Jacoby, 2009). *E. cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*, *Providencia* species, *Morganella morganii*, *Hafnia alvei*, *Aeromonas* species and *P. aeruginosa* isolates have the potential to produce inducible AmpC beta-lactamases by mutating to develop resistance during therapy with beta-lactam antibiotics other than the carbapenems and fourth-generation cephalosporins (Thomson, 2010). Plasmid mediated AmpC beta-lactamases have been detected in some isolates of *Klebsiella* species, *E. coli*, *Salmonella* species, *C. freundii*, *E. aerogenes* and *P. mirabilis* (Jacoby, 2009).

Group 2 are the penicillinases, cephalosporinases and broad-spectrum beta-lactamases that are generally inhibited by active site-directed beta-lactamase inhibitors corresponding to the molecular classes A and D reflecting the original TEM and SHV genes (Paterson & Bonomo, 2005). However, because of the increasing number of TEM- and SHV-derived beta-lactamases, they were divided into two subclasses, 2a and 2b. The 2a subgroup contains only penicillinases. The 2b are broad-spectrum beta-lactamases, which are capable of inactivating penicillins and cephalosporins at the same rate. Furthermore, new subgroups were segregated from subgroup 2b. The subgroup 2be represents the extended-spectrum beta-lactamases (ESBLs), which are capable of inactivating third-generation cephalosporins (ceftazidime, cefotaxime and cefpodoxime)

as well as monobactams (aztreonam). The subgroup 2br enzymes have reduced binding to clavulanic acid and sulbactam. They are also called inhibitor-resistant TEM-derivative enzymes. However, they are commonly susceptible to tazobactam, except where an amino acid replacement exists at position met69. The subgroup 2c which forms the carbenicillinase was segregated from group 2 because these enzymes inactivate carbenicillin more than benzylpenicillin, with some effect on cloxacillin. The subgroup 2d enzymes inactivate cloxacillin more than benzylpenicillin, with some activity against carbenicillin. These enzymes are poorly inhibited by clavulanic acid, and some of them are ESBLs. Subgroup 2e enzymes are cephalosporinases that can also hydrolyse monobactams and they are inhibited by clavulanic acid. The subgroup 2f enzymes are serine-based carbapenemases, in contrast to the zinc-based carbapenemases (Paterson & Bonomo, 2005).

Group 3 are the zinc based or metallo beta-lactamases, corresponding to the molecular class B, which are the only enzymes acting by the metal ion zinc. Metallo beta-lactamases are able to hydrolyse penicillins, cephalosporins and carbapenems. The emergence and diversity of carbapenemase-producing strains is extremely problematic. Metallo-beta-lactamase enzymes hydrolyse virtually all beta-lactams except aztreonam. Carbapenems are the only reliably active antibiotics against many multi-resistant Gram-negative pathogens, particularly those with extended-spectrum beta-lactamases (ESBLs) and AmpC enzymes (Pitout & Laupland, 2008). The clue to the presence of a carbapenemase is the increased minimum inhibitory concentration values or resistance of the enterobacteria to imipenem, meropenem and ertapenem (Nordmann *et al.*, 2009).

Group 4 are penicillinases that are not inhibited by clavulanic acid and they do not yet have a corresponding molecular class.

2.5 EXTENDED-SPECTRUM BETA-LACTAMASE

The emergence of beta-lactamases in *Enterobacteriaceae* following the introduction of ampicillin and other beta-lactams in the early 1960s was partly due to the spread of plasmid-encoded beta-lactamase strains (Fisher *et al.*, 2005). The first plasmid-mediated beta-lactamase, TEM-1, was isolated from *Escherichia coli* in a girl named Temoniera in 1965. Within a few years, it was widespread among the family *Enterobacteriaceae* (Datta & Kontomichalou, 1965). Plasmid encoded beta-lactamases of SHV-1 and OXA-1 also became established and spread rapidly. TEM-1, TEM-2, SHV-1 and OXA-1 beta-lactamases confer resistance to anti-Gram -negative penicillins and first-generation cephalosporins. Further mutations in TEM-1, TEM-2, SHV-1 and OXA-1 beta-lactamases have extended their spectrum of activity to even second-generation cephalosporins. TEM-1, TEM-2, SHV-1 and OXA-1 enzymes constitute the parent beta-lactamases from which most ESBLs have evolved (Jacoby & Munoz-Price, 2005).

When third-generation cephalosporins were introduced into medical practice in the 1980s, it was acclaimed as a major breakthrough in the fight against beta-lactamase-mediated bacterial resistance to antibiotics (Paterson & Bonomo, 2005). These cephalosporins had been developed in response to the increased prevalence of beta-lactamases in certain organisms such as *E. coli*, *K. pneumoniae*, *Haemophilus influenzae* and *Neisseria gonorrhoeae*. Not only were the third-generation cephalosporins effective against most beta-lactamase-producing organisms but they had the major advantage of lessened nephrotoxic effects compared to aminoglycosides and polymyxins. Nevertheless, resistance to these expanded-spectrum beta-lactam antibiotics due to beta-lactamases emerged quickly.

The first of these enzymes capable of hydrolysing the third generation cephalosporins, SHV-2, was found in a single strain of *Klebsiella ozaenae* isolated in

Germany (Kliebe *et al.*, 1985). The gene encoding the beta-lactamase showed a mutation of a single nucleotide compared to the gene encoding SHV-1. Other beta-lactamases were soon discovered which were closely related to TEM-1 and TEM-2, but which had the ability to confer resistance to the extended-spectrum cephalosporins (Brun-Buisson *et al.*, 1987). Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended-spectrum beta-lactamases.

There are two schools of thought concerning the functional evolution of beta-lactamases into ESBLs. The first indicates that the selective pressure and overuse of extended-spectrum cephalosporins had induced point mutations in the active sites of TEM-1, TEM-2, SHV-1, and OXA parent beta-lactamases (Jacoby and Medeiros, 1991; Perez *et al.*, 2007). The ESBLs represented the first example in which beta-lactamase-mediated resistance to beta-lactam antibiotics resulted from fundamental changes in the substrate spectra of the enzymes.

The second school of thought shows that the functional model of ESBLs suggests an inheritance of principal characteristics from some chromosomal ancestral ESBLs rather than the products of mutational events in beta-lactamase enzymes. Once selected, the ESBL variant may spread by clonal dissemination of the ESBL-producing strain to multiple patients or the horizontal transmission of the ESBL-containing plasmid among unrelated strains (Palucha *et al.*, 1999).

With the exception of OXA-type enzymes (which are class D enzymes), ESBLs belong to Ambler's molecular class A (Ambler, 1980). Class A enzymes are characterized by an active-site serine, a molecular mass of approximately 29,000 Da, and the preferential hydrolysis of penicillins (Medeiros *et al.*, 1988). The molecular classification scheme does not sufficiently differentiate the many different types of class

A enzymes. Bush, Jacoby and Medeiros use the biochemical properties of the enzyme plus the molecular structure and nucleotide sequence of the genes to place beta-lactamases into functional groups (Bush *et al.*, 1995). Using this functional classification scheme, ESBLs are defined as beta-lactamases capable of hydrolysing oxyimino-cephalosporins that are inhibited by clavulanic acid and are placed into functional group 2be (Bush *et al.*, 1995).

The ESBLs derived from TEM-1, TEM-2, or SHV-1 are differentiated from their progenitors by one amino acid. This leads to a dramatic change in the enzymatic activity of the ESBLs, such that they can now hydrolyse the third-generation cephalosporins or aztreonam. Some enzymes generally regarded as ESBLs (for example, TEM-7 and TEM-12) do not really meet the hydrolysis criteria above. However, they hydrolyse ceftazidime more than the parent TEM-1 and TEM-2 enzymes, leading to increased MICs of ceftazidime for organisms bearing such beta-lactamases (Bush *et al.*, 1995). Consequently, these TEM beta-lactamases are included in group 2 be and are widely considered as ESBLs (Bush *et al.*, 1995).

2.5.1 Evolution and Diversity of Extended-Spectrum Beta-lactamase Genes

Extended-spectrum beta-lactamases are a rapidly evolving group of beta-lactamases. The common types include SHV, TEM and CTX-M. Others are OXA, PER and VEB-1 (Paterson & Bonomo, 2005). Typically, they are derivatives of genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these beta-lactamases. It has been observed that the same organism may harbour both CTX-M-type and other type of ESBLs or CTX-M-type ESBLs and AmpC-type beta-lactamases, which may change the antibiotic resistance phenotype (Yamasaki *et al.*, 2003).

2.5.1.1 TEM-Type ESBL

The TEM-type ESBLs are derivatives of TEM-1 and TEM-2. Over 100 TEM-type beta-lactamases have been described, majority of which are ESBLs. Their isoelectric points range from 5.2 to 6.5 (Paterson & Bonomo, 2005). Some TEM derivatives have been found which have reduced affinity for beta-lactamase inhibitors. TEM-type enzymes which are less susceptible to the effects of beta-lactamase inhibitors have negligible hydrolytic activity against the extended-spectrum cephalosporins and are not considered ESBLs (Paterson & Bonomo, 2005).

The genesis of TEM-type ESBL is traced from a plasmid-mediated beta-lactamase found in *Klebsiella pneumoniae* isolates in France in 1984. The enzyme, now termed TEM-3, differed from TEM-2 by two amino acid substitutions (Sougakoff *et al.*, 1988). The strain is said to have come from a neonatal unit which had been stricken by an outbreak of *Klebsiella oxytoca* producing TEM-1. Ceftazidime was used to treat these infected patients, but subsequent isolates of *Klebsiella pneumoniae* from the same unit harboured the TEM-type ESBL (Du Bois *et al.*, 1995). This is a typical example of the emergence of ESBLs as a response to the selective pressure induced by extended-spectrum cephalosporins.

2.5.1.2 SHV-Type ESBL

A beta-lactamase produced by *Klebsiella ozaenae* which efficiently hydrolysed cefotaxime and to a lesser extent ceftazidime was isolated in Germany in 1983 (Knothe *et al.*, 1983). After sequencing, the beta-lactamase was shown to differ from SHV-1, by replacement of glycine by serine at the 238 position. This mutation accounts for the extended-spectrum properties of this beta-lactamase, designated SHV-2. Within 15

years of the discovery of this enzyme, organisms harbouring SHV-2 were found in every inhabited continent (Paterson *et al.*, 2003), implying that selective pressure from third-generation cephalosporins in the first decade of their use was responsible. SHV-ESBLs occur predominantly in *Klebsiella* species and *Escherichia coli* and less commonly in other *Enterobacteriaceae* (Jacoby & Munoz-Price, 2005). Outbreaks of SHV-producing *Pseudomonas aeruginosa* (Poirel *et al.*, 2004) and *Acinetobacter* species (Huang *et al.*, 2004) have also been reported.

Unlike the TEM-type beta-lactamases, there are relatively few derivatives of SHV-1. The majority of SHV variants possessing an ESBL phenotype are characterized by the substitution of a serine for glycine at position 238 (Bradford, 2001). A number of variants related to SHV-5 also have a substitution of lysine for glutamate at position 240. The serine residue at position 238 is critical for the efficient hydrolysis of ceftazidime and the lysine residue at position 240 is critical for the efficient hydrolysis of cefotaxime (Huletsky *et al.*, 1993). The majority of SHV-type derivatives possess the ESBL phenotype. However, one variant, SHV-10, is reported to have clavulanic acid-resistant phenotype. This enzyme appears to be derived from SHV-5 and contains one additional amino acid substitution of glycine for serine 130 (Prinarakis *et al.*, 1997).

2.5.1.3 CTX-M-Type ESBL

CTX-M-type ESBL was named to reflect the potent hydrolytic activity of these beta-lactamases against cefotaxime. *E. coli* is most often responsible for producing CTX-M beta-lactamases and seems to be a true community ESBL pathogen (Pitout *et al.*, 2005). Organisms producing CTX-M-type beta-lactamases typically have cefotaxime MICs in the resistant range (>64 µg/ml) (Paterson & Bonomo, 2005). CTX-M-type ESBLs may actually hydrolyse ceftazidime and confer resistance to this cephalosporin as reported

by Sturenburg and colleagues (2004). However, the ceftazidime MICs are usually in the apparently susceptible range (2 to 8 µg/ml). CTX-M-type beta-lactamases are known to hydrolyse cefepime with high efficiency (Tzouveleakis *et al.*, 2000). It has been suggested that the serine residue, which is present in all of the CTX-M enzymes, plays an important role in the extended-spectrum activity of the CTX-M-type beta-lactamases (Tzouveleakis *et al.*, 2000).

According to Bush and others (1993), CTX-M-type ESBL producers exhibit 10-fold greater inhibitory activity to tazobactam than clavulanic acid in contrast to other types of ESBLs.

2.5.1.3.1 Factors underlying the rapid dissemination of the increasing prevalence of CTX-M ESBLs

The CTX-M enzymes are replacing SHV and TEM enzymes as the prevalent type of ESBLs in urinary tract, bloodstream and intra-abdominal infections (Falagas & Karageorgopoulos, 2009).

The prevalence of CTX-M-type ESBLs is rapidly expanding and they have now been detected in every continent (Paterson & Bonomo, 2005). CTX-M is the predominant ESBLs across Europe (Pitout *et al.*, 2007^a). Reports from Spain have shown that CTX-M-producing *E. coli* are an important cause of community-onset bloodstream infections (Rodriguez-Bano *et al.*, 2006). Ben Ami and colleagues (2006) from Tel-Aviv, Israel, investigated patients with Gram-negative bacteraemia admitted to their hospital and found that 14% were caused by ESBL-producing organisms of which CTX-M-producing *E. coli* were in the majority. These bacteria also showed co-resistance to co-trimoxazole (64%), gentamicin (61%), and ciprofloxacin (64%). Other studies in Europe also showed that strains producing CTX-M enzymes were

substantially more resistant to ciprofloxacin than strains lacking CTX-M genes (Pitout *et al.*, 2007^a).

CTX-M-producing ESBLs have also become the most prevalent type of ESBLs described in Canada and South American countries (Canton & Coque, 2006; Pitout *et al.*, 2007^a). In a study at Calgary Laboratory Services in Canada, Pitout and colleagues examined 175 clinical ESBL-producing strains and observed that 14% were positive for CTX-M-1 genes, 54% were positive for CTX-M-14 genes, and the remaining 32% were negative for CTX-M genes.

Initially, SHV-2, SHV-5, and SHV-12 ESBL-types dominated in most molecular studies of ESBL in Asia (Lee *et al.*, 2003). However, the occurrence of CTX-M-type ESBLs in India (Karim *et al.*, 2001), China (Chanawong *et al.*, 2002), Japan (Ma *et al.*, 2002), Korea (Pai *et al.*, 2001) and Taiwan (Yu *et al.*, 2002) established that CTX-M-type ESBL may be the dominant ESBL types in Asia.

CTX-M enzymes are increasing in Africa. Paterson and colleagues (2003) reported that 11.7% ESBL-producing organisms among a collection of 455 isolates of *K. pneumoniae* in South Africa were positive for CTX-M-type-ESBL. Blomberg and colleagues (2005) have reported increased levels of CTX-M-type ESBL in Tanzania in *Escherichia coli* strains. In a study in Southwest Nigeria, 30 selected multidrug-resistant *Klebsiella pneumoniae* strains isolated from patients with community-acquired urinary tract infections were characterized. All the isolates produced at least one type of ESBL, with 57% producing CTX-M enzymes (Olysegun *et al.*, 2006).

There are several factors underlying the rapid spread of CTX-M genes. The prevalence of CTX-M encoding chromosomes in *Klyuvera* species in the environment represents a major reservoir for the acquisition of CTX-M ESBLs by community pathogens (Bonnet, 2004). Bonnet further stated that CTX-M is easily transferred to

other genetic sites due to the fact that encoding genes usually harbour insertion sequence ISEcp1. Moreover, CTX-M-encoding plasmids are often transmissible by conjugation with high transfer frequencies of 10^{-7} to 10^{-2} per donor cell (Bonnet, 2004). The potential for CTX-M-type ESBLs to spread beyond the hospital environment serves to heighten public health concerns (Bonnet, 2004).

2.5.2 Geographical Occurrence and Distribution of ESBL-Producers

2.5.2.1 Europe

ESBL-producing organisms were first detected in Europe. Although the initial reports were from Germany (Knothe *et al.*, 1983) and England (Du Bois *et al.*, 1995), the vast majority of reports in the first decade after the discovery of ESBLs were from France (Philippon *et al.*, 1989). By the early 1990s, 25% to 35% of hospital-acquired *K. pneumoniae* infections in France were ESBL producing (Marty & Jarlier, 1998). However, outbreaks of infection with ESBL-producing organisms have been reported from virtually every European country. A large study from more than 100 European intensive care units found that the prevalence of ESBLs in *Klebsiella* species ranged from as low as 3% in Sweden to as high as 34% in Portugal (Hanberger *et al.*, 1999). In Turkey, a survey of *Klebsiella* species from intensive care units from eight hospitals showed that 58% of 193 isolate harboured ESBLs (Gunseren *et al.*, 1999).

2.5.2.2 Asia

In some parts of Asia, the percentage of ESBL production in *E. coli* and *K. pneumoniae* varies from 4.8% in Korea (Pai *et al.*, 2001) to 8.5% in Taiwan (Yan *et al.*, 2000) and up to 12% in Hong Kong (Ho *et al.*, 2005). In a survey of 196 institutions across Japan, <0.1% of *E. coli* and 0.3% of *K. pneumoniae* strains were ESBL producers (Yagi *et al.*,

2000). In a major teaching hospital in Beijing, 27% of *Escherichia coli* and *Klebsiella pneumoniae* blood culture isolates collected from 1997-1999 produced ESBL (Du *et al.*, 2002).

2.5.2.3 North America

ESBL-producing organisms were first reported in the United in 1988 (Jacoby *et al.*, 1988). In 1989, significant infections with TEM-10-producing *Klebsiella pneumoniae* were noted in Chicago by Quinn and colleagues (1989). TEM-10-producing organisms have been responsible for several unrelated outbreaks of ESBL-producing organisms in the United States for a number of years (Bradford *et al.*, 1994). Outbreaks with SHV-type ESBLs (Jacoby, 1997^a) and CTX-M-type ESBLs have also been described in the United States and Canada (Moland *et al.*, 2003) (Pitout *et al.*, 2004).

2.5.2.4 South and Central America

ESBLs have been found in 30 to 60% of klebsiellae from intensive care units in Brazil, Colombia and Venezuela (Otman *et al.*, 2002). In 1988 and 1989, isolates of *Klebsiella pneumoniae* from Chile and Argentina were reported as harbouring SHV-2 and SHV-5 ESBLs (Casellas & Goldberg, 1989). Organisms with CTX-M-type ESBL have spread throughout many parts of South America (Radice *et al.*, 2002) and Brazil (Bonnet *et al.*, 2000).

2.5.2.5 Africa

Several outbreaks of infections with ESBL-producing *Klebsiella* have been observed in South Africa (Shipton *et al.*, 2001). It has been reported that 36.1% of *K. pneumoniae* isolates collected in a single South African hospital in 1998 and 1999 were ESBL

producers (Bell *et al.*, 2002). Other works in North Africa have documented ESBL prevalence of 38.5% in Egypt (Bouchillon *et al.*, 2004) and characterized the presence of TEM-3 ESBL-producing *Salmonella typhimurium* for the first time in Morocco (Aitmhand *et al.*, 2002). In Tanzania, Blomberg and colleagues (2005) reported that 15% of 126 enterobacteria isolates studied were ESBL-producing *E. coli* and *Klebsiella* species. They also discovered the presence of CTX-M-15 ESBL producing organism for the first time in Africa.

Kesah and Odugbemi (2002) reported more than 40% ESBL production among *Enterobacteriaceae* isolates in Lagos, Nigeria. This was collaborated by Aibinu and colleagues in 2003 by reporting 42% *Enterobacter*-producing ESBL organisms in clinical isolates from Lagos, Nigeria. In 2006, Olysegun and others (2006) also observed 50% ESBL production rate in *K. pneumoniae* isolates studied from Northwestern Nigeria.

In Ghana, Adu-Sarkodie (2010) reported that ESBL has been isolated from 50.3% *Klebsiella* and 49.7% *E. coli* in Komfo Anokye Teaching Hospital, Kumasi. Obeng-Nkrumah *et al.*, (2013) reported that 49.3% of 300 enterobacteria isolates studied in Korle Bu Teaching Hospital were ESBL-producers.

2.5.3 Medical Significance of Detecting ESBL Production in Clinical Laboratories

Organisms that produce ESBLs are considered to be an important reason for treatment failure with beta-lactams for Gram negative bacterial infections and have serious consequences for infection control as well as epidemiologic implications. It is therefore imperative for clinical microbiology laboratories to be able to detect and report ESBL-producing organisms. ESBL-producing organisms appear susceptible to cephalosporins *in vitro* when conventional breakpoints are used but are ineffective during treatment.

Patterson and colleagues (2001) performed a multinational study on patients treated with cephalosporins (cefotaxime, ceftazidime, ceftriaxone and cefepime) which had showed high *in-vitro* activity against ESBL-producing *K. pneumoniae*. Treatment failure was detected in 54% of the patients when MICs of the cephalosporins used were within susceptible ranges. LaBombardi and colleagues (2006) also reported that, *in vitro* susceptibilities rates of ESBL-producing strains to cephalosporins, especially cefepime was as high as 80% but recorded 100% treatment failure rates.

Therefore, CLSI recommends that any organism that is confirmed for ESBL production according to CLSI criteria be reported as resistant to all penicillins, cephalosporins and aztreonam, regardless of the *in vitro* susceptibility test result (CLSI, 2007).

Although reports show an increasing prevalence of ESBLs worldwide, the problem is underestimated in sub-Saharan Africa due to the limited laboratory detection and reporting infrastructure (Bush, 1996). The epidemiologic implications of failure to detect ESBL-producing organism cannot be over emphasized. Furthermore, the detection of ESBL-producing organisms is important for infection control. Monitoring ESBL prevalence is necessary in estimating the magnitude of the ESBL problem, which contribute to the adoption of good antibiotic therapeutic policy and appropriate infection control policy (Falagas & Karageorgopoulos, 2009). Studies have reported control of outbreaks due to ESBL-producing enterobacteria as a result of careful monitoring of local prevalence data (Lucet *et al.*, 1999).

Current breakpoints for cephalosporin sensitivity set for clinical efficacy are unable to detect early ESBL-producing. The presence of these enzymes does not always increase MICs of oxyimino cephalosporins and monobactams to levels indicative of

resistance defined by the CLSI. There is therefore the need to detect the ESBL-producing organisms rather than to rely on conventional *in vitro* susceptibility testing.

2.5.4 Methods of Detecting ESBL Resistance Mechanism

The methods for detection of ESBLs can be broadly divided into two groups: phenotypic and molecular (genotypic) methods. The phenotypic methods use techniques, which detect the ability of the ESBL enzymes to hydrolyse different cephalosporins. The genotypic methods use molecular techniques to detect the gene responsible for the production of the ESBL. Clinical diagnostic laboratories use mostly phenotypic methods because these tests are easy to perform, cost effective and have been incorporated in most automated susceptibility systems, making them widely accessible. However, phenotypic methods are not able to distinguish between the specific enzymes responsible for ESBL production. Several research or reference laboratories use genotypic methods for the identification of the specific gene responsible for the production of the ESBL, which would be missed by phenotypic methods (Woodford & Sundsfjord, 2005). Furthermore, molecular assays also have the potential to be performed directly on clinical specimens without culturing the bacteria, with subsequent reduction of detection time (Tenover, 2007). The clinical diagnostic microbiology laboratory plays an important part in the detection and reporting of ESBL-producing bacteria, but many laboratories may not be equipped with the best phenotypic methods for detecting them (Thomson, 2001). The consequence has been several treatment failures in patients who received inappropriate antibiotics for ESBL-producing organisms.

The US Clinical and Laboratory Standards Institute (2006) and the UK Health Protection Agency (HPA) (2009) have published guidelines for ESBL detection in

Enterobacteriaceae specifically for *E. coli*, *Klebsiella* species and *Proteus* species. The HPA guidelines (2009) also include other species, such as *Salmonella* species. These guidelines are based on the principle that most ESBLs hydrolyse third-generation cephalosporins although they are inhibited by clavulanate and recommend initial screening with 8 mg/l (CLSI) or 1 mg/l (HPA) of cefpodoxime, 1 mg/l each of cefotaxime, ceftazidime, ceftriaxone or aztreonam. This is to be followed by confirmatory tests (including the E-test ESBL strips) with both cefotaxime and ceftazidime in combination with clavulanate at a concentration of 4 µg/ml. Automated systems that use similar detection principles have proved to be popular in clinical laboratories, especially those in North America and certain European countries (Luzzaro *et al.*, 2006). The phenotypic detection of ESBLs in bacteria other than *E. coli*, *Klebsiella* species and *Proteus* species remains problematic and controversial (Thomson, 2001). This is because the effect of clavulanate inhibition of ESBLs is often masked by other types of beta-lactamases, such as AmpC enzymes in species of *Enterobacter* and *Citrobacter*. Other ESBL detection methods includes the modifications of double-disc method with cefepime (Pitout *et al.*, 2003) (Tzelepi *et al.*, 2000), chromogenic agar (Glupczynski *et al.*, 2007), three-dimensional methods (Ho *et al.*, 2005) and micro-dilution methods that use clavulanate with different beta-lactams (including fourth-generation cephalosporins, such as cefepime) (Thomson *et al.*, 1999).

The increased prevalence of *Enterobacteriaceae* producing ESBLs suggests a need for laboratory testing methods that will accurately identify the presence of these enzymes in clinical isolates. The CLSI breakpoints for susceptibility of members of the *Enterobacteriaceae* to extended-spectrum cephalosporins and aztreonam were developed in the early 1980s. During that period the clinical success rate of cephalosporin treatment for organisms with cephalosporin MICs of 8 µg/ml was >95%

(Paterson *et al.*, 2001). Unfortunately these breakpoints were developed probably prior to the advent of ESBLs and therefore must be revised. It is therefore recommended by the CLSI that clinical microbiology laboratories perform specialized tests for detection of ESBLs. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has revised the cephalosporin breakpoints and similar action is under way by the CLSI for better prediction of clinical outcome by antibiotic MIC values (Kahlmeter, 2008). It is still controversial whether this revision might allow clinical laboratories to dispense with ESBL detection (Paterson & Bonomo 2005; Kahlmeter, 2008).

2.5.5 Phenotypic Screening Test for ESBL Production

The CLSI has proposed disc diffusion methods for screening for ESBL production by *Klebsiella* species, *E. coli* and *Proteus mirabilis*. Laboratories using disc diffusion methods for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters with a high level of suspicion for ESBL production (CLSI, 2006). Cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone is used for the screening of ESBL production. However, the use of more than one of these agents for screening improves the sensitivity of detection. It is recommended that isolates with a zone diameter of 22mm should undergo phenotypic confirmatory tests for ESBL production except for cefpodoxime, because the cefpodoxime (10µg) screening test using a zone diameter of 22 mm lacks specificity when used to screen *Escherichia coli* isolates for ESBL production (Tenover *et al.*, 2003). Therefore, the CLSI now recommends a change in the cefpodoxime screening breakpoint to 17 mm (CLSI, 2006). However, a more clinically useful screening test for cefpodoxime is to use a cefpodoxime MIC of 8µg/ml as a trigger to perform phenotypic confirmatory tests for

ESBL production (CLSI, 2006). When the screening test for the other cephalosporin indicates MIC of 2µg/ml, ESBL phenotypic confirmatory test must be performed.

2.5.6 Phenotypic Confirmatory Test for ESBL Production

If any of the zone diameters indicate suspicion for ESBL production, phenotypic confirmatory tests is used to ascertain the diagnosis. The CLSI advocates the use of cefotaxime (30µg) or ceftazidime discs (30µg) with clavulanate (10µg) for phenotypic confirmation of the presence of ESBLs (CLSI, 2006) in *Klebsiella* species and *Escherichia coli*. The combined disc synergy method is a reference method of detecting ESBL-producing isolates according to CLSI. The CLSI recommends that the disc tests should be performed using Mueller-Hinton agar. A difference of ≥ 5 mm between the zone diameters of either of the cephalosporin discs and their respective cephalosporin/clavulanate disc is taken to be phenotypic confirmation of ESBL production (CLSI, 2006). Phenotypic confirmatory testing can also be performed by broth micro-dilution 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) (Queenan *et al.*, 2004). Phenotypic confirmation is considered as a three-fold-serial-dilution decrease in MIC of either cephalosporin in the presence of clavulanic acid compared to its MIC when tested alone.

According to CLSI guidelines, isolates which phenotypically produce ESBLs should be reported as resistant to all cephalosporins and other beta-lactams regardless of their conventional MIC except the cephamycins and carbapenems.

2.5.7 Etest for Detection of ESBL-producers

The Etest ESBL (AB biodisc, Solna, Sweden) is a plastic drug-impregnated strip with one side containing a concentration gradient of ceftazidime (0.5-32 µg/ml) and the other side containing a concentration gradient of ceftazidime (0.064 - 4 µg/ml) plus a constant concentration of clavulanate (4µg/ml). Other strips are impregnated with cefotaxime/clavulanate or cefepime/clavulanate. These strips are useful for both screening and phenotypic confirmation of ESBL production. The reported sensitivity of the Etest method as a phenotypic confirmatory test for ESBLs is 87 to 100% and the specificity is 95 to 100% (Cormican *et al.*, 2006). The result of Etest ESBL is interpreted as positive when a two-fold decrease in the MIC value of the tested drug is observed in the presence of clavulanate (Leverstein-van *et al.*, 2002).

2.5.8 Phenotypic Automated Test for ESBL Production

Some automated systems such as MicroScan Panels (Sacramento, California, USA), BD Phoenix Automated Microbiology System (Becton Dickinson, Sparks, MD, USA) and Vitek 2 System (bioMérieux, France) are available for detecting ESBL producing phenotypes. In this work however, Vitek 2 ESBL test was the phenotypic automated ESBL test method used for detecting ESBL production.

2.5.8.1 MicroScan Panels ESBL Test

MicroScan panels (Sacramento, California, USA) contain combinations of ceftazidime or cefotaxime and beta-lactamase inhibitors. They have been shown to be reliable in studies of large numbers of ESBL-producing isolates according to a work done by Komatsu and colleagues in 2003.

2.5.8.2 The BD Phoenix ESBL Test

The BD Phoenix Automated ESBL test (Becton Dickinson, Sparks, MD, USA) uses cefpodoxime, ceftazidime, ceftriaxone and cefotaxime, with or without clavulanic acid to detect the production of ESBLs. Results are usually available within 6 hours. The BD Phoenix ESBL method detected ESBL production in over 90% of strains genotypically confirmed to produce ESBLs. The method correctly detected ESBL production by *Enterobacter*, *Proteus* and *Citrobacter* species in addition to *Klebsiella* species and *E. coli* (Sanguinetti *et al.*, 2003).

2.5.8.3 The Vitek 2 ESBL Test

The Vitek 2 System (bioMérieux, France) is a rapid automated microbiological system used for bacteria identification, antimicrobial susceptibility testing (AST), resistance mechanism detection and epidemiologic trending. The Vitek 2 system performs antimicrobial susceptibility testing (AST) based on kinetic analysis of growth data. The Advanced Expert System (AES) is a critical component of VITEK 2 technology. It analyses MIC patterns and detects bacterial resistance mechanisms and phenotypes for most organisms tested. The Advanced Expert System (AES) analyses the AST data using >100,000 references, >2,000 described phenotypes, >20,000 MIC distributions, >100 resistance mechanisms detected and >99 organisms (bioMérieux, 2009). Barry and colleagues (2003) have concluded that VITEK 2 performed susceptibility tests accurately and the AES detected and interpreted resistance mechanisms appropriately.

The VITEK 2 ESBL test (bioMérieux, France) is a phenotypic confirmatory tool for rapid detection of a positive ESBL-producing strain. Its principle is based on simultaneous assessment of the inhibitory effects of cefotaxime, ceftazidime and cefepime, alone and in the presence of clavulanic acid (Teresa *et al.*, 2006). Robin and

colleagues (2008) examined 94 ESBL-positives and 57 ESBL-negative non-duplicate isolates of *Enterobacteriaceae*. They indicated that for *E. coli* and *K. pneumoniae*, the sensitivity and specificity of CSLI phenotypic confirmatory test and Vitek 2 ESBL test was 91.8% and 100% respectively. They concluded that the Vitek-2 ESBL test seemed to be an efficient method for routine detection of ESBL-producing isolates of *Enterobacteriaceae*, including isolates producing AmpC-type enzymes. The reliability of the test to detect ESBLs in species other than *K. pneumoniae*, *K. oxytoca* and *E. coli* is unknown (Midolo *et al.*, 2002). Other reports on the reliability of Vitek 2 ESBL test to rapidly detect ESBL-producing isolates in comparison against molecular methods indicated that the Vitek 2 ESBL test has sensitivity of 98.1% with 99.3% positive predictive value and a specificity of 99.7% with a negative predictive value of 99.3% (Teresa *et al.*, 2006). According to Umeh and Berkowitz (2009), Vitek ESBL test has sensitivity of at least 99.5% and specificity of 100%. It is therefore a reliable ESBL single-test alternative to the manual disc confirmatory synergy test and polymerase chain reaction. False-negative results have been observed in *K. pneumoniae* isolates producing both an ESBL and an AmpC-type beta-lactamase (Tzouveleakis *et al.*, 1999). However, studies by Rhodes *et al.*, 2014 found high error margins in the use of the Vitek 2 for testing cefepime.

2.5.9 Molecular Detection of ESBL

The common and easiest molecular method used is the PCR amplification of the TEM and SHV and CTX-M genes with specific oligonucleotide primers, followed by sequencing. Sequencing is essential to discriminate between the non-ESBLs (TEM 1, TEM 2, or SHV1) and ESBL-producing isolates (TEM 3, SHV2 etc). Other molecular methods that do not use sequencing have been developed to characterize ESBLs. These

include PCR with RFLPs (Arlet *et al.*, 1995), PCR with single-strand conformational polymorphism (M'Zali *et al.*, 1996), ligase chain reaction (Kim & Lee, 2000), restriction site insertion PCR (Chanawong *et al.*, 2001) and real-time PCR (Randegger & Hachler, 2001). However, the increasing number of additional subtypes within each ESBL family has placed strict limitations on some molecular techniques with regard to their ability to cover the whole range of variants with different point mutations. PCR amplification followed by nucleotide sequencing (TEM and SHV genes) is used for the identification of specific point mutation of ESBL genes (Fluit *et al.*, 2001).

2.5.10 Extended-Spectrum Beta-lactamase-associated Antimicrobial Resistance

Beta-lactamases remain one of the major causes of drug resistance in Gram negative organisms. The dominant resistance mechanism involves extended-spectrum beta-lactamases, though the effects of AmpC beta-lactamases and carbapenemases are very significant.

ESBL-producing organisms are increasingly becoming multi-drug resistant as compared to non-ESBL-producing bacteria. Apart from their expected resistance to beta-lactams (except carbapenems and cephamycins), ESBL-producing isolates are also becoming resistant to aminoglycosides, chloramphenicol, fluoroquinolones, trimethoprim/sulphamethoxazole and tetracyclines. Several studies in many parts of the world have underscored this observation. A study by Aibinu and colleagues (2003) in hospitals in Lagos reported significant co-resistance of 75%, 89%, 63% and 100% to ciprofloxacin, streptomycin, amikacin and gentamicin and trimethoprim/sulphamethoxazole respectively. In contrast, Olysegun and others (2006) did not report any antibiotic co-resistance genes associated with ESBLs on plasmids of ESBL-producing *Klebsiella pneumoniae* in Northwestern Nigeria (Olysegun *et al.*, 2006). This

may reflect diagnostic challenges of detecting ESBLs in ESBL-associated antibiotic resistance prevalence. In South Africa, Bell and co-workers (2002) have demonstrated resistance in ESBL-producing *Enterobacter cloacae* to gentamicin, ciprofloxacin and co-trimoxazole to be 65%, 60% and 20% respectively. A study by Adu-Sarkodie (2010) in Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana reported high resistant rates of ESBL-producing *Escherichia coli* and *Klebsiella* species to non-beta-lactam. He indicated that the rate of resistance of ciprofloxacin, norfloxacin, gentamicin, chloramphenicol, tetracycline, co-trimoxazole, amikacin, nitrofurantoin, meropenem and imipenem were 56.73%, 79.41%, 81.43%, 90.00%, 94.23%, 96.30% 20.00%, 19.7%, 2.12% and 0.00% respectively. A study by Obeng-Nkrumah *et al.*, (2013) in Korle Bu Teaching Hospital (KBTH), Accra, recorded the following co-resistance prevalence of ESBL producers to non-beta-lactams: co-trimoxazole (70%), gentamicin (70%), tetracycline (85.9%), chloramphenicol (80.5%), ciprofloxacin (29.1%), amikacin (29.2%) and meropenem (0%).

Other surveys in Europe and North America also illustrate a high rate of co-resistance among ESBL-producing organisms to non-beta-lactam antimicrobial agents. In one such survey by the Health Protection Agency (HPA) across Britain in over 42 medical centres, Woodford and others (2004) reported that, among all the *Klebsiella* and *Escherichia coli* isolates studied, over 70% of the ESBL-producers were resistant to ciprofloxacin (MICs >8mg/l) and trimethoprim (MICs >2mg/l) and resistance to aminoglycosides and beta-lactamase-inhibitor combinations ranged from 50% to 65%. Pitout and others (2007) worked on *Escherichia coli* and *Klebsiella pneumoniae* isolates in Canada and indicated 66% co-resistance to ciprofloxacin, co-trimoxazole and amikacin despite the low ESBL prevalence ($\leq 5\%$) in this region.

2.5.11 Treatment Responses of ESBL-Producing Organisms

The third-generation cephalosporins are poor choices for the treatment of serious infections due to ESBL-producing organisms. Treatment failures are high when MICs of the cephalosporins are elevated (for example, 4 or 8 µg/ml) but still within the susceptible range (Paterson *et al.*, 2001). In common with other extended-spectrum cephalosporins, MICs for cefepime rise substantially when the inoculum of infecting organisms is high (Thomson & Moland, 2001). However, published clinical experience with the use of cefepime for the treatment of ESBL-producing organisms has been quite limited. In a randomized trial of cefepime versus imipenem for nosocomial pneumonia, clinical response for infections with ESBL-producing organisms was 100% in patients treated with imipenem but only 69% in patients treated with cefepime (Yuan *et al.*, 1998). Yu and colleagues (2002) have observed that cefepime resistance may be more frequent in strains which produce the CTX-M-type ESBLs.

It is reported that the main cause of mortality by ESBL-producing bacteria is inappropriate initial antimicrobial therapy (Oteo *et al.*, 2010). Treatment of severe ESBL-producing infections includes the use of carbapenems, amikacin, tigecycline and beta-lactam/beta-lactamase inhibitor combinations, though tigecycline and beta-lactam/beta-lactamase inhibitor combinations usage lack enough clinical evidence (Oteo *et al.*, 2010). For urinary tract infections, fosfomycin and nitrofurantoin could be useful (Oteo *et al.*, 2010).

Beta-lactam/beta-lactamase inhibitor combinations have increasing MICs as inoculum rises (Thomson & Moland, 2001). Published clinical experience with beta-lactam/beta-lactamase inhibitor combinations in the treatment of serious infections due to ESBL producers is limited to just a few patients (Paterson & Bonomo, 2005).

Fluoroquinolones may be regarded as the treatment of choice for complicated urinary tract infections due to ESBL-producing organisms (Paterson & Bonomo, 2005). Unfortunately, increasing *in vitro* resistance of ESBL producers to fluoroquinolones will limit its role in the future. Observational clinical studies have assessed the relative merits of fluoroquinolones and carbapenems for serious infections due to ESBL-producing organisms. Two of these studies found that carbapenems were superior to quinolones (Endimiani *et al.*, 2004), whereas one of the studies found that they were equivalent in effectiveness (Kang *et al.*, 2004). Carbapenems are the recommended drugs of choice for treating serious infections with ESBL-producing organisms. This is due to the *in vitro* susceptibility of ESBL-producing organisms to carbapenems and increasingly extensive clinical experience (Paterson & Bonomo, 2005). There is a vast clinical use of imipenem as compared to meropenem to treat ESBL-producing bacteria, but MICs are slightly lower for meropenem (Paterson & Bonomo, 2005). Ertapenem shares the good *in vitro* activity of the other carbapenems. The fact that ertapenem can be used once daily makes it potentially useful in serious infections with ESBL producers (Jacoby *et al.*, 1997^b).

There are few published reports of the use of cephamycins in the treatment of ESBL producers. In one of these reports, selection of porin resistant mutants occurred during therapy, resulting in cefoxitin resistance and relapse of infection. In addition, combined cephamycin and carbapenem resistance in *Klebsiella pneumoniae* has been observed in the setting of widespread cephamycin use in response to an outbreak of infection with ESBL-producing organisms (Paterson & Bonomo, 2005).

The available therapeutic options for the treatment of ESBL-associated infections are limited by drug resistance conferred by the ESBLs, along with the

frequently observed co-resistance to various antibiotic classes, including fluoroquinolones, aminoglycosides, tetracycline and trimethoprim /sulphamethoxazole.

Relevant clinical data regarding the effectiveness of different regimens for ESBL-associated infections are limited. Although certain cephalosporins may appear active *in vitro*, associated clinical outcomes are often suboptimal. Beta-lactam/beta-lactamase inhibitor combinations may be of value, but clinical experience is limited. Carbapenems are regarded as the agents of choice and may be more effective than fluoroquinolones for serious infections. The antimicrobial activity of tigecycline against ESBL-producing bacteria merits further evaluation (Falagas & Karageorgopoulos, 2009).

2.6 AMPC BETA-LACTAMASE AND THEIR TREATMENT RESPONSES

AmpC beta-lactamases hydrolyse penicillins, cephalosporins, monobactams and cephamycins and resist inhibition by clavulanate, sulbactam and tazobactam. Many Gram-negative bacilli produce a chromosomally mediated AmpC which, when hyper-produced, may cause resistance to penicillins, aztreonam, cephamycins, and cephalosporins. Plasmid-mediated AmpC beta-lactamases (PMABLs) have been detected in some isolates of *Klebsiella* species, *Salmonella* species, *C. freundii*, *E. aerogenes*, *P. mirabilis* and *E. coli* and are typically associated with multidrug resistance (Thomson, 2010). Accurate prevalence data on AmpC beta-lactamases are scarce due to lack of testing, but they appear to be less common than ESBLs (Thomson, 2010).

Resistance due to plasmid-mediated AmpC enzymes is less common than extended-spectrum beta-lactamase production in most parts of the world (Jacoby, 2009). Organisms with AmpC genes are often resistant to multiple antibiotics, making the

selection of an effective antibiotic difficult. Beta-lactam/beta-lactamase inhibitor combinations and most cephalosporins and penicillins are not effective because of *in vitro* resistance and the potential for AmpC induction or selection of high-enzyme-level mutants (Jacoby, 2009). Studies show that many AmpC-producing organisms are susceptible to cefepime with a conventional inoculum. This may be due to the fact that cefepime is a poor inducer of AmpC beta-lactamase and rapidly penetrates through the outer cell membrane (Jacoby, 2009). Carbapenem therapy has usually been successful. Reduced imipenem susceptibility has been reported in porin-deficient clinical isolates of *K. pneumoniae*. Fluoroquinolone therapy may be used especially for treating non-life-threatening infections such as urinary tract infection, if the isolate is susceptible. Reports indicate that tigecycline has good activity *in vitro* against 88% of AmpC-hyper-producing isolates of *E. coli*, *Enterobacter*, *Klebsiella* and *Citrobacter* species from the United Kingdom. However, few *P. aeruginosa* isolates and only 22% of nosocomial *Acinetobacter* isolates were susceptible to tigecycline (Jacoby, 2009).

2.6.1 Detection of AmpC Beta-lactamase

Detecting AmpC activity remains problematic in organisms that also produce ESBLs (Russel *et al.*, 2007). Boronic acid is known to inhibit the activity of AmpC beta-lactamases and several studies have reported its usefulness in the detection of organisms producing both AmpC beta-lactamase and ESBL. AmpC beta-lactamases are detected by disc synergy testing (DST) using cefotaxime or ceftazidime with or without boronic acid (Jacoby, 2009).

Yagi and colleagues (2005) found that a difference of ≥ 5 -mm in the zone of inhibition around a ceftazidime or cefotaxime disc and when 300 μ g 3-aminophenylboronic acid was added, reliably detected all AmpC varieties. The 3-

aminophenylboronic acid together with cefotaxime or ceftazidime has also been reported to potentiate the sensitivity and specificity of the combined disc method when applied to organisms with chromosomal or plasmid-mediated AmpC production (Song *et al.*, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS AND REAGENTS

The materials, reagents and equipment used for this present study are listed in appendix I.

3.2 STUDY SITES

Five hundred (500) lactose fermenting bacterial isolates were collected from the Central Laboratory of the Korle Bu Teaching Hospital (KBTH) and Advent Clinical Laboratories; both in the Accra metropolis. KBTH is the leading national referral centre in Ghana.

Advent Clinical Laboratory located in Dzorwulu in Accra is a private clinical laboratory with the state-of-the-art clinical diagnostic equipment. It serves as a preferred diagnostic centre for some clinics in the Accra metropolis.

3.3 RESEARCH ETHICS

The research protocol was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School (protocol identification number: MS-Et/M.9 – P.4.14/2010-11).

3.4 BACTERIAL ISOLATES

Klebsiella pneumoniae and *E. coli* isolates were used for bacteriological analysis. Over 90% of the isolates were from urine sources.

3.5 SAMPLE SIZE

A total of 400 *K. pneumoniae* and *E. coli* isolates were identified and used in this present study. This sample size was calculated based on the expected prevalence and using appropriate levels of precision at 95% confidence level, according to the formula below:

$$\begin{aligned}
 N &= \frac{[Z]^2 [P, Q]}{E^2} \\
 &= \frac{[1.96]^2 [0.5] [1-0.5]}{[0.05]^2} \\
 &= 384
 \end{aligned}$$

where

N = minimum sample size

P = maximum expected prevalence rate: 50%

Q = 100 – P: 50

E = margin of sampling error tolerated: 5%

Z = the centile of the standard normal distribution: 1.96 (confidence interval of 95%)

3.6 INCLUSION CRITERIA

Non-duplicate pure cultures of *K. pneumoniae* and *E. coli* were used in the work.

3.7 EXCLUSION CRITERIA

All isolates not confirmed as *K. pneumoniae* and *E. coli* and all duplicate cultures were excluded. A total of 100 isolates were excluded from the work since they were not identified as *K. pneumoniae* and *E. coli*.

3.8 STUDY DESIGN

Four hundred (500) lactose fermenting isolates were collected at the Central Laboratory of Korle Bu Teaching Hospital and Advent Clinical Laboratories and stored in glycerol broth in Eppendorf tubes and frozen (-20°C). After thawing, the 500 isolates were initially sub-cultured on blood agar and then onto MacConkey agar. Four hundred (400) lactose fermenters were definitively identified as *K. pneumoniae* and *E. coli* using Vitek 2 system and concurrently analysed for ESBL-producing phenotypes, minimum inhibitory concentration of selected 17 antimicrobials and antimicrobial susceptibility testing using Vitek 2 System (appendix V) at Advent Clinical Laboratories, Accra. The isolates were also confirmed for ESBL-producing strains using the combined disc synergy method. The 400 bacterial isolates were screened for the phenotypic production of AmpC beta-lactamase using disc synergy method. The ESBL encoding genes were amplified and characterized using polymerase chain reaction (PCR) with already published primers and reaction conditions. After electrophoresis of the PCR products, the bands on the agarose gel were visualized by ultraviolet trans-illumination and photographed using gel documentation system. The results were collated and analysed statistically.

3.9 PHENOTYPIC ANALYSIS OF BACTERIAL ISOLATES

Of the 500 lactose fermenting isolates stored in glycerol broth, 400 were identified as *K. pneumoniae* and *E. coli*. ESBL phenotypes were confirmed and antimicrobial susceptibility test were performed. Bacterial isolates were screened for AmpC beta-lactamase producing organisms.

3.9.1 Culturing of the Bacterial Isolates

The isolates were sub-cultured on blood and MacConkey agar and incubated at 35°C for 24 hours.

3.9.2 Operation of the Vitek 2 System

The Vitek 2 System (bioMérieux, France) is a rapid semi-automated microbiological system used for bacteria identification, minimum inhibitory concentration determination, antimicrobial susceptibility testing (AST), resistance mechanism detection and epidemiologic trending and reporting. The VITEK 2 ESBL test is a phenotypic confirmatory tool for rapid detection of ESBL-producing organism.

During its operation (Appendix II), sterile test tubes used to prepare inoculums were filled with 3ml of 0.45% saline water and placed in a cassette. The identification (ID) test tube was used to prepare inoculum from the pure colonies and mixed thoroughly using a vortex until a suspension of 0.5 – 0.63 McFarland (according to manufacturer's instruction) was formed. The McFarland opacity was determined using Densichek (bioMérieux, France). From the ID test tube, 45µl of the inoculum was pipetted into the AST test tube and mixed thoroughly. The Gram negative (GN) ID test cards and AST test cards were inserted in the respective test tubes and loaded into the Vitek instrument. While in the Vitek instrument, the cards were automatically filled,

sealed and incubated in the Vitek 2 system incubator until results (organism species, ESBL phenotype, antibiotic MIC and interpretation) were generated by the expert advanced system (AES) of the Vitek 2 system

3.9.2.1 Identification of Bacterial Isolates

The isolates were identified based on their gram reaction and 60 biochemical reaction characteristics in the ID test cards wells using the Vitek 2 system.

3.9.2.2 Detection of ESBL Phenotypes using Vitek 2 ESBL Test

The detection of a positive or negative ESBL producing strains was based on simultaneous assessment of the inhibitory effects of cefotaxime, ceftazidime and cefepime, alone and in the presence of clavulanic acid in accordance with the CLSI (2006).

3.9.2.3 Antimicrobial Susceptibility Testing using Vitek 2 System

The Vitek 2 system was used to determine the MICs of the selected antimicrobial agents by the micro-dilution method. The antimicrobial agents in each AST card included ampicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefazolin, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, tetracycline, nitrofurantoin and trimethoprim/sulphamethoxazole. The MIC at which 50% of the ESBL-phenotypes were susceptible or resistant to a particular antimicrobial agent (MIC_{50}) for all the selected 17 antimicrobial agents were determined. The MIC at which 90% of the ESBL-phenotypes were susceptible or resistant to a particular antimicrobial agent (MIC_{90}) for all the 17 antimicrobial agents were also determined.

The Vitek 2 system (bioMérieux, France) performs antimicrobial susceptibility testing (AST) based on kinetic analysis of growth data. The therapeutic significance of the MIC of the antimicrobials was determined using the Vitek 2 Compact system based on CLSI-2008 breakpoints. At the end of the incubation cycle, MIC values and their interpretations (susceptible, resistant and intermediate) were generated for each antibiotic on the card. All ESBL producers were considered resistant to penicillins and cephalosporins irrespective of their MICs according to CLSI (2006).

3.9.3 Detection of ESBL Phenotype using ESBL Screening and Combined Disc Synergy Method

MAST IDTM ESβL Detection Discs (Mast Group, UK) was used to screen and confirm the ESBL phenotypes. The MAST IDTM ESβL Detection Discs comprises cefpodoxime 30µg discs, cefpodoxime 30µg + clavulanic acid 10µg discs; ceftazidime 30µg discs, ceftazidime 30µg + clavulanic acid 10µg discs and cefotaxime 30µg discs, cefotaxime 30µg + clavulanic acid 10µg discs.

Using a pure culture of the test organism, a suspension was prepared in distilled water equivalent in density to a McFarland 0.5 opacity standard and mixed with a rotator. A sterile swab of the suspension was spread uniformly across the surface of Mueller-Hinton agar plate. With a sterile forceps, one of each MAST IDTM ESβL Detection Discs was placed onto the inoculated medium ensuring that they were evenly spaced. The plates were incubated aerobically at 35-37°C for 18 – 20 hours. The diameters of any zones of inhibition were measured and recorded (Appendix IV).

The zone of inhibition for the cefpodoxime, ceftazidime and cefotaxime alone and with clavulanic acid combination discs was compared. An increase in zone diameter

of ≥ 5 mm in the presence of clavulanic acid for any or all of the sets of MAST ID™ ESβL Detection Discs indicated the presence of ESBL in the test organism.

3.9.4 Detection of AmpC Beta-lactamase-producing Phenotypes

AmpC beta-lactamases producing phenotypes were detected by disc synergy testing (DST) using cefotaxime or ceftazidime with or without boronic acid. Using a pure culture of the test organism, a suspension in distilled water equivalent in density to a McFarland 0.5 opacity standard was prepared. A sterile swab of the suspension was spread uniformly across the surface of a Mueller-Hinton agar plate. Cefotaxime or ceftazidime with or without boronic acid was placed onto the inoculated medium with a sterile forceps ensuring that they were evenly spaced. The plates were incubated aerobically at 35-37°C for 18 – 20 hours. The diameter of any zones of inhibition was measured and recorded. An increase of ≥ 5 mm in the diameter of the zones of inhibition for the ceftazidime and cefotaxime alone and in combination of boronic acid disc indicated the presence of AmpC beta-lactamases in the test organism.

3.10 MOLECULAR ANALYSIS OF ESBL-CODING GENES

Of the 202 ESBL phenotypes, 100 were randomly selected for molecular analysis based on the MICs of cefotaxime. The molecular investigations of the ESBL-coding genes included extraction of DNA template from the phenotypic ESBL-producing bacterial isolates, preparation of the PCR reaction mixture using appropriate primers and published reaction conditions, standard PCR reaction in a BIOER GenePro thermocycler (Bioer Technology, China), agarose gel electrophoresis of PCR products, bands visualization by ultraviolet trans-illumination and photography using gel documentation system.

3.10.1 *Klebsiella pneumoniae* and *E. coli* Genomic DNA Extraction

DNA was extracted by a simple boiling method (Heffernan *et al.*, 2007, Garza-González *et al.*, 2011) and used to prepare the PCR reaction mixture. A loopful of bacterial colony was picked from an isolate and suspended in 100µl of double distilled H₂O in an Eppendorf tube. The bacterial suspension was incubated at 100°C in a water bath for 5 minutes and snapped cold on ice for 10 minutes. The cell lysate was then centrifuged briefly at high speed (12.000 rpm for 3 min), and the supernatant containing the genomic DNA was transferred into a fresh sterile Eppendorf tube. The extracted DNA was stored at -21°C until required for PCR.

3.10.2 PCR Detection of ESBL-coding Gene

Polymerase chain reaction of the PCR reaction mixture was carried out using BIOER GenePro thermocycler (Bioer Technology, China). Taq PCR Kit (New England Biolabs, USA) was used to prepare 25µl PCR reaction mixture for a primer set as shown in Table 3.3. The primers used were already published primers (Table 3.1) for CTX-M, TEM and SHV and their corresponding PCR conditions (Table 3.2). Using the primers for CTX-M-1group and CTX-M-9 group, PCRs were performed with isolates possessing CTX-M gene previously determined (Heffernan *et al.*, 2007). Negative (sterile distilled water) controls were included in each round of PCR.

3.10.3 Agarose Gel Electrophoresis

A buffer (1 X TAE buffer) was prepared and subsequently used to prepare 2% agarose gel. The suspension was boiled in a microwave for 2 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5µg/ml ethidium bromide (which

absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 μ l) of 10X bromophenol blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel; it is inert and does not react with the DNA) was added to 10 μ l of each PCR product and loaded into the wells. A 100bp DNA ladder (BioPioneer, USA) was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes using either a midi or a maxi gel system. The bands on the gels were visualized by ultraviolet trans-illumination (Uvitec, Canbridge, UK) with a wavelength of 312nm and photographed using gel documentation system. The band sizes were estimated by comparison with the mobility of a 100bp molecular weight DNA ladder that was ran alongside experimental samples in the gel.

Table 3.1: Primers used for the detection of ESBL genes (Heffernan *et al.*, 2007)

Primer Name	Sequence (5'-3')	Target Gene	Band Size(bp)
SHV-F	GCCGGGTTATTCTTATTTGTCCG	SHV	1007
SHV-R	ATGCCGCCGCCAGTCA		
TEM-F	GTATCCGCTCATGAGACAATA	TEM	966
TEM-R	TCTAAAGTATATATGAGTAAAC		
CTX-M-F	TTTGCGATGTGCAGTACCAGTAA	CTX-M	590
CTX-M-R	CGATATCGTTGGTGGTGCCATA		
CTX-M-1-F	CCCATGGTTAAAAAATCACTG	CTX-M-1 group	891
CTX-M-1-R	CCGTTTCCGCTATTACAAAC		
CTX-M-9-F	GTGACAAAGAGAGTGCAACGG	CTX-M-9 group	857
CTX-M-9-R	ATGATTCTCGCCGCTGAAGCC		

Table 3.2: PCR conditions used for the detection of ESBL genes (Heffernan *et al.*, 2007)

Target Gene	PCR Conditions	
SHV	Initial denaturation for 5min at 94°C	1 cycle
	94°C for 30s	
	68°C for 30s	35 cycles
	72°C for 60s	
	Final extension at 72°C for 7min	1 cycle
TEM	Initial denaturation for 5min at 94°C	1 cycle
	94°C for 60s	
	55°C for 30s	35 cycles
	72°C for 60s	
	Final extension at 72°C for 10min	1 cycle
CTX-M	Initial denaturation for 5min at 94°C	1 cycle
	94°C for 30s	
	60°C for 30s	30 cycles
	72°C for 60s	
	Final extension at 72°C for 7min	1 cycle
CTX-M-1 group	Initial denaturation for 10min at 94°C	1 cycle
	94°C for 60s	
	55°C for 60s	30 cycles
	72°C for 2mins	
	Final extension at 72°C for 5min	1 cycle
CTX-M-9 group	Initial denaturation for 10min at 94°C	1 cycle
	94°C for 30s	
	55°C for 30s	25 cycles
	72°C for 60s	
	Final extension at 72°C for 10min	1 cycle

Table 3.3: PCR reaction mixture

Reagent	Volume (μl)	Final concentration
Nuclease-free water	16.175	-
10X PCR buffer + MgCl ₂	2.5	1X
10mM DNTP mix	0.4	200 μM each
10μM forward primer	0.4	0.2μM
10μM reverse primer	0.4	0.2μM
5U/μl Taq polymerase	0.125	0.5U
Template DNA	5	(≤1 μg/reaction)
TOTAL volume	25	

3.11 QUALITY CONTROL

Escherichia coli ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative quality control strains for ESBL producing bacteria respectively (Appendix IV). The Vitek 2 System performed and interpreted the MIC of the antibiotics according to the breakpoints of CLSI.

3.12 STATISTICAL ANALYSIS OF DATA

In comparison with results of the combined disc synergy method, the sensitivity, specificity, positive predictive value and negative predictive value of Vitek 2 ESBL test was calculated based on the formulae below:

$$\text{Sensitivity} = \frac{\text{true positive ESBL} \times 100}{\text{true positive ESBL} + \text{false negative ESBL}}$$

$$\text{Specificity} = \frac{\text{true negative ESBL} \times 100}{\text{true negative ESBL} + \text{false positive ESBL}}$$

$$\text{Positive predictive value} = \frac{\text{true positive ESBL} \times 100}{\text{true positive ESBL} + \text{false positive ESBL}}$$

$$\text{Negative predictive value} = \frac{\text{true negative ESBL} \times 100}{\text{true negative ESBL} + \text{false negative ESBL}}$$

Comparisons were made between groups of organisms to determine if there was a significant difference in distribution of resistance using the chi-square test. *P* values < 0.05 were considered significant.

The MIC₅₀ (MIC at which 50% of the strains were susceptible or resistant to a particular antimicrobial agent) was determined using Microsoft Excel.

The MIC₉₀ (MIC at which 90% of the strains were susceptible or resistant to a particular antimicrobial agent) was determined using Microsoft Excel.

CHAPTER FOUR

RESULTS

4.1 BACTERIAL ISOLATES

Of the 400 bacterial isolates collected, 175 (43.7%) were *K. pneumoniae* and 225 (56.3%) were *E. coli*.

4.2 ESBL PRODUCING PHENOTYPES

The Vitek 2 System detected 202 (50.5%) out of the total 400 *K. pneumoniae* and *E. coli* isolates (Table 4.1). The combined disc synergy method (CDM) detected 203 (50.8%) of ESBL producers among the 400 total bacterial isolates. There was no significant difference ($p \geq 0.05$) between the ESBL phenotypes detected by the combined disc synergy method and the Vitek 2 ESBL test in both the *K. pneumoniae* and *E. coli* isolates.

Table 4.1 Occurrence of ESBL-producing phenotypes

ESBL Method	Detection	Number of Phenotypes (%)		
		<i>K. pneumoniae</i>	<i>E. coli</i>	All Isolates
		n=175	n=225	n=400
*CDM		130 (74.3)	73 (32.4)	203 (50.8)
Vitek 2 System		129 (73.7)	73 (32.4)	202 (50.5)
P-Value		1.000	1.000	1.000

*CDM: Combined Disc Synergy Method

Results in Table 4.1 and Table 4.2 demonstrate significant occurrence of ESBL phenotypes among the isolates.

4.3 NON-ESBL PRODUCING PHENOTYPES

The total non-ESBL producing isolates detected by the Vitek 2 System was 198 (49.5%) of the 400 bacterial isolates (Table 4.2). The combined disc synergy method detected 197 (49.3%) of non-ESBL producers among the 400 total bacterial isolates.

Table 4.2 Distribution of Non-ESBL-Producing Phenotypes

ESBL Method	Detection	Number of Phenotypes (%)		
		<i>K. pneumonia</i> n=175	<i>E. coli</i> n=225	All Isolates n=400
*CDM		45 (25.7)	152 (67.5)	197 (49.3)
Vitek 2 System		46 (26.3)	152 (67.5)	198 (49.5)

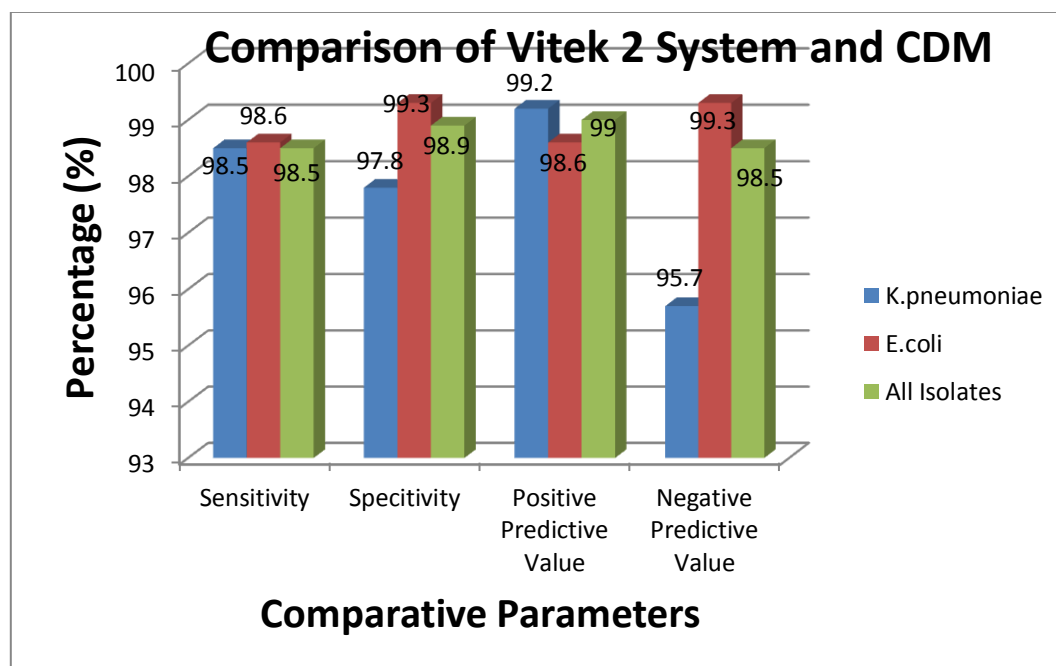
*CDM: Combined Disc Synergy Method

4.4 SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE AND NEGATIVE PREDICTIVE VALUE OF VITEK 2 SYSTEM

As indicated in table 4.3, the true positive, true negative, false positive and false negative ESBL strains among the 400 bacterial isolates as detected by Vitek 2 ESBL test were 200, 195, 2 and 3 respectively. Consequently, the sensitivity, specificity, positive predictive value and negative predictive value of Vitek 2 Compact System among the 400 bacterial isolates was 98.5%, 98.9%, 99.0% and 98.5% respectively as shown in figure 4.1.

Table 4.3 Comparison of Vitek 2 System with CDM as ESBL Detection Methods

Parameters	Number of Phenotypes (%)		
	<i>K. pneumoniae</i>	<i>E. coli</i>	All Isolates
	n=175	n=225	n=400
True Positive	128	72	200
True Negative	44	151	195
False Positive	1	1	2
False Negative	2	1	3

**Figure 4.1:** Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of Vitek 2 System

Results in Table 4.3 and figure 4.1 indicate that Vitek 2 System is concordant with combined disc synergy method in detecting ESBL phenotypes.

4.5 ANTIMICROBIAL SUSCEPTIBILITY AMONG ESBL-PRODUCING ISOLATES

Approximately eleven percent (11.4%) of the 202 ESBL producers were susceptible to amoxicillin/clavulanic acid with their MIC₅₀ and MIC₉₀ being 4µg/ml and 8µg/ml respectively (Table 4.4). Of the 202 ESBL-producers, 31.7% were susceptible to piperacillin/tazobactam with their MIC₅₀ and MIC₉₀ being 8µg/ml and 16µg/ml respectively. Approximately seventy four percent (73.8%) of the ESBL organisms were susceptible to ceftiofur with their MIC₅₀ and MIC₉₀ being ≤4µg/ml and 8µg/ml respectively. Ninety nine percent (99.0%) of the ESBL producers were susceptible to imipenem with their MIC₅₀ and MIC₉₀ being ≤1µg/ml and ≤1µg/ml respectively. Of the 202 ESBL-producers, 94.1% were susceptible to amikacin with their MIC₅₀ and MIC₉₀ being ≤2µg/ml and 4µg/ml respectively. The remaining antimicrobial agents were as shown in table 4.4.

Table 4.4 Antimicrobial Susceptibility among ESBL Producers (n=202)

Antimicrobial Agent	No. (%) of Susceptible Isolates	MIC ₅₀ (µg/ml)	MIC ₉₀
*Ampicillin	0(0.0)	0	0
Amoxicillin/Clavulanic acid	23(11.4)	4	8
*Piperacillin	0(0.0)	0	0
Piperacillin/Tazobactam	64(31.7)	8	16
*Cefazolin	0(0.0)	0	0
Cefoxitin	149(73.8)	≤4	8
*Cefotaxime	0(0.0)	0	0
*Ceftazidime	0(0.0)	0	0
*Cefepime	0(0.0)	0	0
Imipenem	200(99.0)	≤1	≤1
Amikacin	190(94.1)	≤2	4
Gentamicin	37(18.3)	≤1	2
Ciprofloxacin	37(18.3)	0.5	1
Norfloxacin	42(20.8)	2	2
Tetracycline	37(18.3)	≤1	4
Nitrofurantoin	73(36.1)	≤16	32
Trimethoprim/Sulphamethoxazole	5(2.5)	≤20	≤20

*All ESBL producers were considered resistant to penicillins and cephalosporins in accordance with CLSI recommendations.

Results in Table 4.4 indicate that imipenem and amikacin is the drug of choice for the management of ESBL-producing organisms.

A critical consideration of the MICs of the cephalosporins indicated that 2.5% of the MIC of cefazolin and cefotaxime were in the susceptible breakpoint ranges as shown in table 4.5 and 13% and 75% of the MIC of ceftazidime and cefepime were also in the susceptible breakpoint ranges as indicated in table 4.5.

Table 4.5 MIC of Cephalosporins with Susceptible Breakpoints among ESBL producers (n=202)

Cephalosporins	No (%) of MICs with Susceptible Breakpoints	MIC ₅₀	MIC ₉₀
		(µg/ml)	
Cefazolin	5 (2.5)	8	8
Cefotaxime	5 (2.5)	≤1	≤1
Ceftazidime	27(13.4)	4	4
Cefepime	152 (75.3)	2	8

Results in Table 4.5 justifies the need for routine ESBL phenotype screening in health facilities since the MIC of some cephalosporins were in the susceptible breakpoints after conventional antimicrobial susceptibility testing.

4.6 ANTIMICROBIAL RESISTANCE AMONG ESBL-PRODUCING ISOLATES

Approximately thirty two percent (31.7%) of the 202 ESBL producers were resistant to amoxicillin/clavulanic acid and 52.5% were resistant to piperacillin/tazobactam. Approximately eighteen (17.9%) of the ESBL producers showed resistance to ceftazidime. Only one percent (1.0%) of ESBL organisms were resistant to imipenem and 0.5% was resistant to amikacin. Of the 202 ESBL-producers, 82.2% were resistant to gentamicin. Approximately eighty percent (79.7%) of the ESBL producers were resistant to ciprofloxacin and 79.2 % were resistant to norfloxacin. Of the 202 ESBL-producers,

70.8 % were resistant to tetracycline and 46.5 % were resistant to nitrofurantoin. Ninety seven percent (97.0%) of ESBL-producing organisms were resistant to trimethoprim / sulphamethoxazole as indicated in figure 4.2.

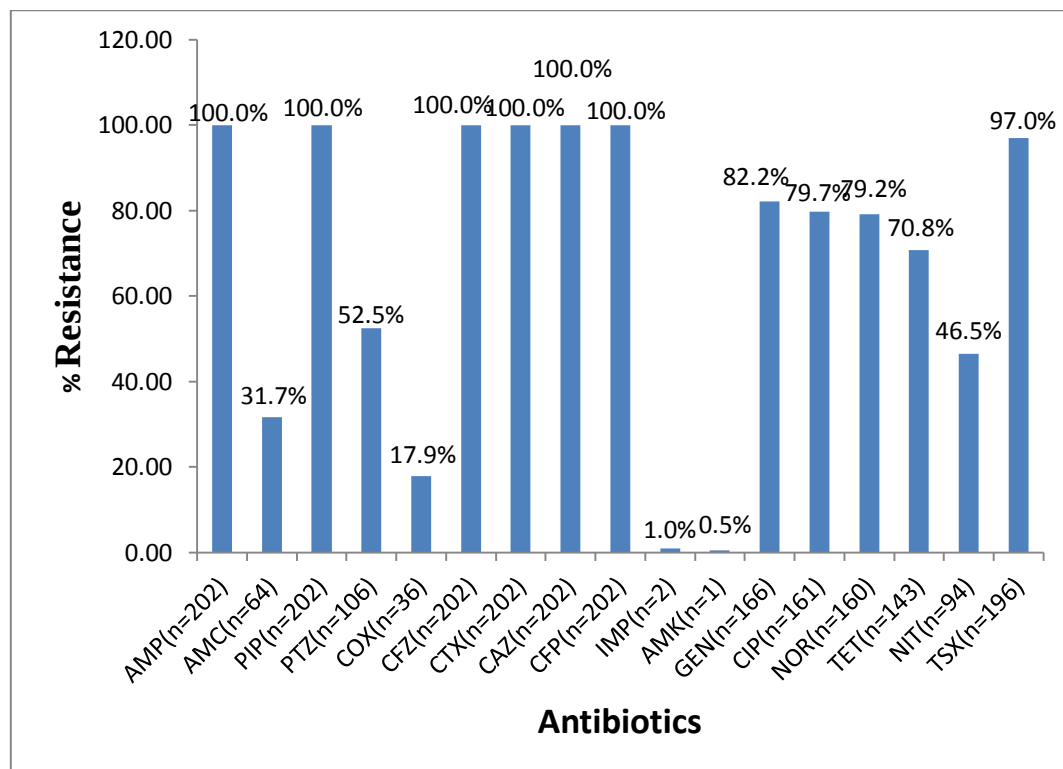


Figure 4.2: Antimicrobial Resistance among ESBL-Producers

Results in figure 4.2 indicate high resistance of ESBL-producing organisms to beta-lactams, non-beta-lactams and beta-lactam/beta-lactamase inhibitor combination.

4.7 ANTIMICROBIAL RESISTANCE AMONG NON-ESBL-PRODUCING ISOLATES

Of the 198 non-ESBL-producing organisms, 59.1%, 77.8% and 68.2% were co-resistant to piperacillin, tetracycline, and trimethoprim/sulphamethoxazole respectively. Approximately 14%, 19%, 16% and 17% of the non-ESBL producers were resistant to amoxicillin/clavulanic acid, piperacillin/tazobactam, cefazolin and gentamicin respectively. Cefotaxime, ceftazidime, cefepime, imipenem, amikacin and nitrofurantoin recorded resistance percentages of less than 5%. Of the 198 non-ESBL producers, 7.1% were resistant to ceftazidime and approximately 39% were resistance to ciprofloxacin and norfloxacin (table 4.6).

Table 4.6 Antimicrobial Resistance among Non-ESBL-Producers (n=198)

Antimicrobial Agent	No. (%) of Resistant Isolates	MIC ₅₀ (µg/ml)	MIC ₉₀
Amoxicillin/Clavulanic acid	27(13.6)	≥32	≥32
Piperacillin	117(59.1)	≥128	≥128
Piperacillin/Tazobactam	37(81.7)	≥128	≥128
Cefazolin	31(15.7)	≥64	≥64
Cefoxitin	14(7.1)	≥64	≥64
Cefotaxime	4(2.0)	≥64	≥64
Ceftazidime	4(2.0)	32	32
Cefepime	4(2.0)	32	32
Imipenem	0(0.0)	0	0
Amikacin	2(1.0)	≥64	≥64
Gentamicin	34(17.2)	≥16	≥16
Ciprofloxacin	78(39.4)	≥4	≥4
Norfloxacin	78(39.4)	≥16	≥16
Tetracycline	154(77.8)	≥16	≥16
Nitrofurantoin	6(3.0)	256	256
Trimethoprim/Sulphamethoxazole	135(68.2)	≥320	≥320

On comparing the antimicrobial resistance profile of ESBL producers and non-ESBL producers, there was a significant difference ($p < 0.05$) among all cephalosporins, some beta-lactams and non-beta-lactams as indicated in table 4.7.

Table 4.7 Comparison of Antimicrobial Resistance between ESBL-Producing and Non-ESBL-Producing Isolate

Antimicrobial Agent	ESBLs (n=202)	Non-ESBLs (n=198)	P-value
Amoxicillin/Clavulanic acid	64 (31.7)	27 (13.6)	0.001
Piperacillin	202 (100)	117 (59.1)	0.001
Piperacillin/Tazobactam	104 (52.5)	37 (18.7)	0.001
Cefazolin	202 (100)	31 (15.7)	0.001
Cefoxitin	36 (17.9)	14 (7.1)	0.001
Cefotaxime	202 (100)	4 (2.0)	0.001
Ceftazidime	202 (100)	4 (2.0)	0.001
Cefepime	202 (100)	4 (2.0)	0.001
Imipenem	2 (1.0)	0 (0.0)	0
Amikacin	1 (0.5)	2 (1.0)	0.466
Gentamicin	166 (82.2)	34 (17.2)	0.001
Ciprofloxacin	161 (79.7)	78 (39.4)	0.001
Norfloxacin	160 (79.2)	78 (39.4)	0.001
Tetracycline	143 (70.8)	154 (77.8)	0.001
Nitrofurantoin	94 (46.5)	6 (3.0)	0.001
Trimethoprim/Sulphamethoxazole	196 (97.0)	135 (68.2)	0.001

Results in Table 4.6 and Table 4.7 demonstrate significant differences between the resistance profile of ESBL-phenotypes and non-ESBL producing-organisms.

4.8 OCCURRENCE OF ESBL-CODING GENES IN *K. PNEUMONIAE* AND *E. COLI* ISOLATES BY POLYMERASE CHAIN REACTION

Of the 202 ESBL producers, 100 were selected for genetic characterisation based on their antimicrobial susceptibility (MICs) to cefotaxime and ceftazidime as indicated in

table 4.5. Out of the 100 ESBL producers selected, 90 possessed CTX-M genes with amplicon sizes of 590bp as in plate 4.1, 70 of which possessed only CTX-M and 20 possessing both CTX-M and TEM ESBL genes. Twenty five (25) of the isolates possessed TEM genes with amplicon sizes of 966bp as shown in plate 4.2, 5 of which possessed TEM only. None of the ESBL producers possessed SHV genes as indicated in table 4.8. Of the 100 ESBL phenotypes, 78 and 2 were positive for CTX-M-1group and CTX-M-9 group ESBL genes respectively (Table 4.9). CTX-M-1group ESBL genes with amplicon size of 891bp and CTX-M-9group with band amplicon size of 857bp are indicated in plate 4.3 and plate 4.3 respectively.

Table 4.8: Occurrence of ESBL-coding genes in Isolates by Polymerase Chain Reaction (n=100)

ESBL genes	Isolates Number (%)
CTX-M	90(90)
CTX-M only	70(70)
TEM	25(25)
TEM only	5(5)
Both CTX-M and TEM	20(20)
Neither CTX-M nor TEM	5(5)
SHV	0(0)

Table 4.9: Occurrence of CTX-M-group ESBL coding genes in Isolates by Polymerase Chain Reaction

CTX-M ESBL genes	Isolates Number (%)
CTX-M-1group	78(78)
CTX-M-9group	2(2)
Neither CTX-M-1group nor CTX-M-9group	10 (10)

Results in Table 4.8 and 4.9 suggest that CTX-M and CTX-M-1group ESBL genes are more prevalent than TEM and SHV ESBL genes.



Plate 4.1 Representative agarose gel electrophoregram of PCR products (band size 590bp) of ESBL gene CTX-M

Lane M=100bp marker; Lanes 1-6=PCR positive CTX-M;

Lane 7 and 9 = PCR negative CTX-M;

Lanes 8, 10-15 = PCR positive CTX-M

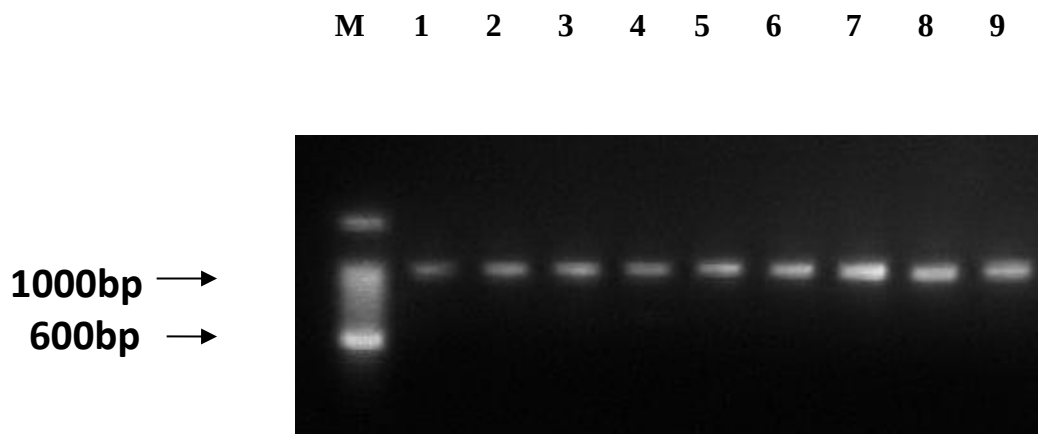


Plate 4.2 Representative agarose gel electrophoregram of PCR products (band size 966bp) of ESBL gene TEM

Lane M=100bp marker; Lanes 1-9 =PCR positive TEM



Plate 4.3 Representative agarose gel electrophoregram of PCR products (band size 891bp) of ESBL gene CTX-M-G1

Lane M=100bp marker; Lanes 1-6=PCR positive CTX-M-G1;

Lanes 8 and 9=PCR negative CTX-MG1;

Lanes 9-15 = PCR positive CTX-M-G1

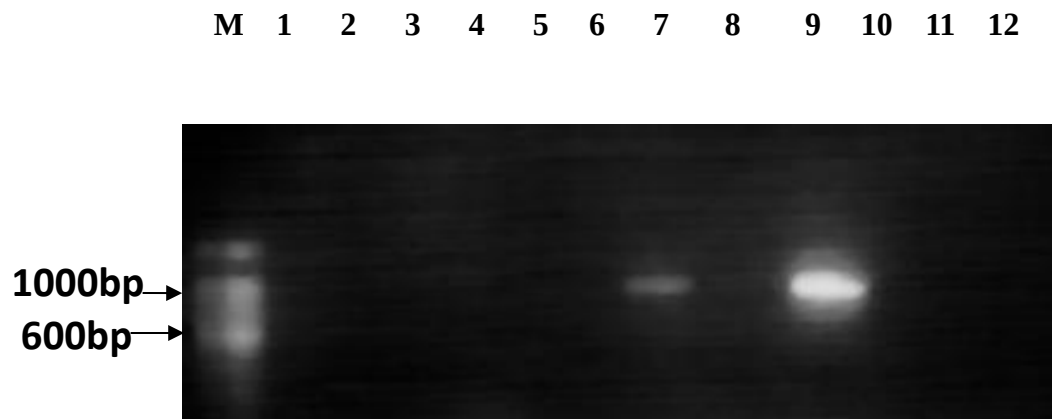


Plate 4.4 Representative agarose gel electrophoregram of PCR products (band size 857bp) of ESBL gene CTX-M-G9

Lane M=100bp marker; Lanes 7 and 9 =PCR positive CTX-M-G9

Lane 1-6,8,10-12=PCR negative CTX-M-G9

4.9 ANTIMICROBIAL RESISTANCE AMONG ONLY CTX-M ESBL-PRODUCING ISOLATES

Of the 70 CTX-M ESBL-producing organisms only, no isolate was resistant to imipenem and amikacin. All of the ampicillin, piperacillin, cefazolin and cefotaxime had MICs that were in the resistant breakpoint ranges. Of the 70 CTX-M ESBL-producing organisms only, 42.9% and 8.6% of the MICs of ceftazidime and cefepime respectively were in the resistant breakpoint ranges. Approximately 29% of the MIC of amoxicillin/clavulanic acid was in the resistant breakpoint and 61.4% of MIC of piperacillin/tazobactam was resistant. The antimicrobial resistant profiles of the other beta-lactams and non-beta-lactams are indicated in table 4.10.

Table 4.10 Antimicrobial Resistance among only CTX-M ESBL Producers based on their MICs (n=70)

Antimicrobial Agent	No.(%) of Resistant Isolates	MIC ₅₀ (µg/ml)	MIC ₉₀
Ampicillin	70 (100.0)	≥32	≥32
Amoxicillin/Clavulanic acid	20 (28.6)	≥32	≥32
Piperacillin	70 (100.0)	≥128	≥128
Piperacillin/Tazobactam	43 (61.4)	≥128	≥128
Cefazolin	70 (100.0)	≥64	≥64
Cefoxitin	9 (12.9)	32	≥64
Cefotaxime	70 (100.0)	≥64	≥64
Ceftazidime	30 (42.9)	≥64	≥64
Cefepime	6 (8.6)	≥64	≥64
Imipenem	0 (0.0)	0	0
Amikacin	0 (0.0)	0	0
Gentamicin	62 (88.6)	≥16	≥16
Ciprofloxacin	50 (71.4)	≥4	≥4
Norfloxacin	50 (71.4)	≥16	≥16
Tetracycline	50 (71.4)	≥16	≥16
Nitrofurantoin	47 (67.1)	≥512	≥512
Trimethoprim/Sulphamethoxazole	69 (98.6)	≥320	≥320

4.10 ANTIMICROBIAL RESISTANCE AMONG ONLY TEM ESBL-PRODUCING ISOLATES

All the 5 organisms producing TEM ESBL only were resistant to ampicillin, piperacillin, piperacillin/tazobactam, cefazolin, cefotaxime, ceftazidime, gentamicin, ciprofloxacin, norfloxacin, nitrofurantoin, tetracycline and trimethoprim/sulphamethoxazole. The TEM ESBL producers only indicated that 3 and 5 of amoxicillin/clavulanic acid and piperacillin/tazobactam respectively had MIC in resistant breakpoint with their MIC₉₀ being $\geq 32\mu\text{g/ml}$ and $\geq 128\mu\text{g/ml}$ respectively. None of TEM ESBL producers only were resistant to imipenem and amikacin as shown in table 4.11.

Table 4.11: Antimicrobial Resistance among only TEM ESBL Producers based on their MICs (n=5)

Antimicrobial Agent	No. of Resistant Isolates	MIC ₅₀ (µg/ml)	MIC ₉₀
Ampicillin	5	≥32	≥32
Amoxicillin/Clavulanic acid	3	≥32	≥32
Piperacillin	5	≥128	≥128
Piperacillin/Tazobactam	5	≥128	≥128
Cefazolin	5	≥64	≥64
Cefoxitin	3	32	≥64
Cefotaxime	5	≥64	≥64
Ceftazidime	5	≥64	≥64
Cefepime	1	≥64	≥64
Imipenem	0	0	0
Amikacin	0	0	0
Gentamicin	5	≥16	≥16
Ciprofloxacin	5	≥4	≥4
Norfloxacin	5	≥16	≥16
Tetracycline	2	≥16	≥16
Nitrofurantoin	5	≥512	≥512
Trimethoprim/Sulphamethoxazole	5	≥320	≥320

4.11 ANTIMICROBIAL RESISTANCE AMONG ISOLATES PRODUCING BOTH CTX-M AND TEM ESBLs

All of the 20 CTX-M and TEM ESBL-producing organisms were resistant to ampicillin, piperacillin, cefazolin and cefotaxime. None of the isolates was resistant to imipenem and amikacin. The CTX-M and TEM ESBL producers showed varied resistant rates to the other beta-lactams showed as shown in table 4.12.

Table 4.12 Antimicrobial Resistance among Isolates Producing both CTX-M and TEM ESBLs based on their MICs (n=20)

Antimicrobial Agent	No. of Resistant Isolates	MIC ₅₀ (µg/ml)	MIC ₉₀
Ampicillin	20	≥32	≥32
Amoxicillin/Clavulanic acid	7	≥32	≥32
Piperacillin	20	≥128	≥128
Piperacillin/Tazobactam	11	≥128	≥128
Cefazolin	20	≥64	≥64
Cefoxitin	4	32	≥64
Cefotaxime	20	≥64	≥64
Ceftazidime	12	≥64	≥64
Cefepime	3	≥64	≥64
Imipenem	0	0	0
Amikacin	0	0	0
Gentamicin	19	≥16	≥16
Ciprofloxacin	14	≥4	≥4
Norfloxacin	14	≥16	≥16
Tetracycline	13	≥16	≥16
Nitrofurantoin	10	≥512	≥512
Trimethoprim/Sulphamethoxazole	19	≥320	≥320

Results in Table 4.10, Table 4.11 and Table 4.12 demonstrate the characteristic antimicrobial resistance of CTX-M and TEM ESBL producers to beta-lactams, non-beta-lactams and beta-lactam/beta-lactamase inhibitor combination.

4.12 AMPC BETA-LACTAMASE-PRODUCING PHENOTYPES

Of the 400 bacterial isolates, 5 (1.3%) were AmpC beta-lactamase-producers of which 3 are ESBL producers and 2 are non-ESBL producers. Of the 202 ESBL-producing phenotypes, 3 (1.5%) were AmpC beta-lactamase-producers.

Table 4.13 AmpC Beta-lactamase-producing Phenotypes

Number (%)		
<i>K. pneumoniae</i> (n=175)	<i>E. coli</i> (n=225)	All Isolates (n=400)
2(1.1)	3(1.3)	5(1.3)

Table 4.14: AmpC Beta-lactamase-producing Phenotypes in ESBL producers

Number (%)		
<i>K. pneumoniae</i> (n=129)	<i>E. coli</i> (n=73)	All Isolates (n=202)
2(1.6)	1(1.4)	3(1.5)

Results in Table 4.13 and Table 4.14 show low occurrence of AmpC beta-lactamase producers among ESBL and non-ESBL phenotypes.

4.13 ANTIMICROBIAL RESISTANCE PROFILE AMONG AMPC BETA-LACTAMASE-PRODUCING PHENOTYPES

All AmpC beta-lactamase producing organisms were resistant to ampicillin, piperacillin, cefazolin, ceftiofur and tetracycline. AmpC beta-lactamase-producing phenotypes showed degree of resistances to piperacillin/tazobactam, amoxicillin/clavulanic acid and other non-beta-lactams as indicated in table 4.15.

Table 4.15: Antimicrobial Resistance among AmpC- Beta- lactamase-producing Phenotypes (n=5)

Antimicrobial Agent	No. of Resistant Isolates	MIC ₅₀ (µg/ml)	MIC ₉₀
Ampicillin	5	≥32	≥32
Amoxicillin/Clavulanic acid	2	8	≥32
Piperacillin	5	≥128	≥128
Piperacillin/Tazobactam	1	≥128	≥128
Cefazolin	5	≥64	≥64
Cefoxitin	5	32	≥64
Cefotaxime	3	≥64	≥64
Ceftazidime	2	32	32
Cefepime	3	32	32
Imipenem	0	0	0
Amikacin	1	≥64	≥64
Gentamicin	3	≥16	≥16
Ciprofloxacin	3	≥4	≥4
Norfloxacin	3	≥16	≥16
Tetracycline	3	≥16	≥16
Nitrofurantoin	0	0	0
Trimethoprim/Sulphamethoxazole	4	≥320	≥320

Results in Table 4.15 indicate that imipenem, amikacin and nitrofurantoin are the drugs of choice for the management of AmpC beta-lactamase producers.

CHAPTER FIVE

DISCUSSION

5.1 ESBL-PRODUCING PHENOTYPES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PROFILE

Infectious diseases account for the major cause of morbidity and mortality in Sub-Saharan Africa (Beitha, 2008). The success of antimicrobials against pathogens is one of the remarkable achievements of medical science and large quantities of different kinds of antimicrobials are now available. This remarkable achievement has been foiled by poor practices that promote drug resistance (Beitha, 2008). Increased consumption of antibiotics appears to correlate with drug resistance (Goossens & Lipsitch, 2006). The most common mode of resistant mechanism is enzymatic inactivation of the antibiotic (Todar, 2008).

There is a global upsurge in the occurrence of ESBL producers with its attending therapeutic challenges (Paterson & Bonomo, 2005). This study sought to determine the occurrence of ESBL-producers in clinical bacterial isolates and their antibiotic resistance profile in Accra. Of the bacterial isolates analysed, 50.5% were ESBL producers. The high ESBL phenotypes also imply that they will be resistant to penicillins and cephalosporins *in vivo*. Therefore in the absence of ESBL screening and antimicrobial susceptibility testing (AST), some of these infections may be wrongly treated with cephalosporins. This is expected to increase infectious disease burden due to treatment failure. This is another reason for clinicians to use evidence-based therapy in managing bacterial infections. The high ESBL phenotypes justify the need for routine ESBL phenotype screening in health facilities. The findings of this study is also consistent with the study of Olysegun and colleagues in 2006 which observed 50%

ESBL production rate in clinical isolates studied from Northwestern Nigeria. Kesah and Odugbemi (2002) reported more than 40% ESBL production among *Enterobacteriaceae* isolates in Lagos, Nigeria. These findings corroborate those of Feglo (2013) who reported 57.8% ESBL producers at Komfo Anokye Teaching Hospital, Kumasi.

It may seem that the rate of ESBL-producing bacteria is assuming alarming rates in Ghana and West Africa. The high prevalence of ESBL-producers may be attributed to indiscriminate antibiotic exposure especially to extended-spectrum beta-lactam antibiotics used for the treatment of blood, urinary tract infections and other infectious diseases (Paterson & Bonomo, 2005). This exerts selective antibiotic pressure for the emergence of ESBL-producing organisms in the population. Since extended spectrum beta-lactamases are plasmid mediated, the genes encoding these enzymes are easily transferable between other bacteria population thereby increasing the occurrence of ESBL-producing organisms (Paterson & Bonomo, 2005).

However, other published studies in Central, Northern and Southern Africa suggested lower occurrence rates of ESBL-producing bacteria in contrast to this current study. Twelve percent (12%) of *Enterobacteriaceae* strains isolated from Yaounde Central Hospital in Cameroon were shown to be positive for ESBLs (Gangoué-Piéboji *et al.*, 2005). In Tanzania, Blomberg and colleagues (2005) reported that 15% of enterobacteria isolates studied were ESBL-producing *E. coli* and *Klebsiella* species. Bouchillon and colleagues (2004) have documented ESBL prevalence of 38.5% in Egypt. It has been reported that 36.1% of *K. pneumoniae* isolates collected in a single South African hospital in 1998 and 1999 were ESBL producers (Bell *et al.*, 2002). These lower ESBL resistance rates may be due to improved antimicrobial usage and

prescription policy in North African countries and South Africa. This may also be a reflection of the literacy and economic condition of the populace in these countries.

In this current study ESBL producers were more common in *K. pneumoniae* than *E. coli*. This is corroborated by the study of Adu-Sarkodie (2010) where ESBL-producers were more common in *Klebsiella* than *E. coli* in Komfo Anokye Teaching Hospital, Kumasi.

There were high level of co-resistance of ESBL producers to gentamicin, ciprofloxacin, norfloxacin, tetracycline, nitrofurantoin and trimethoprim/sulphamethoxazole in this current study which was consistent with the work of Adu-Sarkodie (2010) but amikacin and imipenem retained high susceptibility against these strains. Aibinu and colleagues (2003) found co-resistance in ciprofloxacin, streptomycin and trimethoprim/sulphamethoxazole. However, both Adu-Sarkodie (2010) and Aibinu and colleagues (2003) interestingly had high resistances (20% and 63% respectively) to amikacin. This may be due to high exposure of patients to treatment with amikacin over the years. This might have led to the alarming development of resistance of the bacterial isolates to amikacin as reported in Kumasi and Nigeria. Furthermore, the bacterial isolates may possess resistance genes for the aminoglycoside on their ESBL plasmids which led to the abnormal high resistances of the bacterial isolates to amikacin as reported by Adu-Sarkodie (2010) and Aibinu and colleagues (2003).

However, 73.8% ESBL producers were active against cephamycins *in vitro*. Nevertheless, it has been reported that ESBL-producing strains that are apparently sensitive to cephamycins (73.8%) *in vitro* can become clinically resistant to this antibiotic due to the loss of an outer membrane porin protein (Pangon *et al*, 1989). The cefoxitin (cephamycin) resistance may be caused by AmpC beta-lactamases or other resistance mechanisms such as decreased permeability or alteration to the penicillin-

binding proteins (PBP) in both *K. pneumoniae* and *E. coli* (Peter-Getzlaff *et al.*, 2011) since cephamycins are not hydrolysed by ESBLs (Paterson & Bonomo, 2005).

As recommended by CLSI (2006), all penicillins and cephalosporins are considered to be resistant to ESBL producers irrespective of their MIC breakpoints as observed in this current study. Nevertheless, analysis of the MIC breakpoints of the cephalosporins used in this study suggested some *in vitro* susceptibility against the ESBL producers. This means that in the absence of ESBL screening tests, conversational antimicrobial susceptibility testing cannot reliably prevent the use of third and fourth generation cephalosporins in treating ESBL producing infections with its attendant therapeutic failure; justifying the need for routine ESBL screening in health facilities.

There are suggestions that the susceptible MIC breakpoints for third and fourth generation cephalosporins should be reduced so that ESBL producers will become resistant to cephalosporins during conversational antimicrobial susceptibility testing (Paterson & Bonomo, 2005). Reviewing of MIC breakpoints is not a substitute for routine ESBL screening in the clinical laboratories (Kahlmeter, 2008). The susceptible ESBL isolates already have low cephalosporins MICs (Table 4.5). These suggestions cannot discriminate between ESBL producers and non-ESBL producers and this will obviously limit the therapeutic options for managing non-ESBL producers which otherwise are susceptible to most of these cephalosporins with the existing MIC breakpoints.

In a randomized trial of cefepime and imipenem, clinical response for infections with ESBL-producing organisms was 100% in patients treated with imipenem but only 69% in patients treated with cefepime (Yuan *et al.*, 1998). Subsequently, Yu and colleagues (2002) observed that cefepime resistance may be more frequent in strains which produce ESBLs. This current study agrees with the recommendation of Oteo and

colleagues (2010) to use carbapenems (imipenem and meropenem) and amikacin for the treatment of severe ESBL-producing infections. On the other hand, the suggestion made by Oteo and colleagues (2010) that beta-lactam/beta-lactamase inhibitor combinations may be used in treating ESBL producing infections contradicts this current study since there were high resistances in amoxicillin/clavulanic acid and piperacillin/tazobactam with a high probability of therapeutic failure when these beta-lactam/beta-lactamase inhibitor combinations are used for treating ESBL-producing infections.

Paterson and colleagues (2005) recommended that fluoroquinolones should be used in treating complicated urinary tract infections due to ESBL-producing organisms. However, it would seem this current study is at variance with this recommendation since ciprofloxacin and norfloxacin showed high resistances that will limit the use of these antibiotics for treating ESBL-producing infections.

5.2 NON-ESBL-PRODUCING ORGANISMS AND THEIR ANTIMICROBIAL RESISTANCE PROFILE

Antibiotics are among the most commonly prescribed drugs in hospitals and studies on their resistance patterns ensure quality healthcare. Antibiotics are widely and indiscriminately used in Ghana resulting to antimicrobial resistance. The observed resistance of non-ESBL producers to the beta-lactams, non-beta-lactams and beta-lactam/beta-lactamase inhibitor combination antimicrobials may be due to other resistance mechanisms resulting from indiscriminate use of antibiotics in Accra. Newman and colleagues (2006) who studied bacterial isolates from various clinical specimens in Ghana, recorded high resistance rates for ampicillin, tetracycline and co-trimoxazole, though their work did not specify the ESBL phenotypes of the bacterial isolates.

In Zimbabwe (Mbunga *et al.*, 2010), the high resistance to ampicillin, co-trimoxazole and trimethoprim/sulphamethoxazole reported correlate to this current study. Also, the findings of a study in Ethiopia (Kibret & Abera, 2011) with high resistance rate to tetracycline are consistent with this current study. On the other hand, lower rate of resistance was observed for ceftriazone (a third generation cephalosporin) and amikacin in Ghana by Newman and colleagues in 2006. This is consistent with this current study with non-ESBL-producers resistant rates of 2% to cefotaxime, ceftazidime and cefepime (which are third generation cephalosporins) and 1% for amikacin. However, there was increased resistant rate of 39.4% to ciprofloxacin and norfloxacin.

The steady rise in resistance of bacterial isolates to the fluoroquinolones such as ciprofloxacin and norfloxacin is alarming as cautioned by Newman and Seidu (2002). The rise in resistance to fluoroquinolones may be due to the ease with which mutations in the DNA gyrase are transferred to other fluoroquinolones (Nankanishi *et al.*, 1999). This may explain why both ciprofloxacin and norfloxacin have similar high resistance rates of 39.4% as observed in this current study.

The observed increase in resistance in non-ESBL producers to the beta-lactam/beta-lactamase inhibitor combination antimicrobials such as amoxicillin/clavulanic acid and piperacillin/tazobactam is worrying since these beta-lactam/beta-lactamase inhibitor combination antibiotics have become the empirical drug of choice for some clinicians for treating infectious diseases in Ghana.

Moreover, the over-the-counter sales and empirical prescription of ciprofloxacin and amoxicillin/clavulanic acid to treat various infections may be blamed for the alarming rates of resistance for these antimicrobial agents. As antibiotic resistance increases and the development of new antimicrobials declines, it would seem prudent to

take the caution of Kimang'a (2012) to manage infectious diseases with evidence based treatment seriously.

Nitrofurantoin continues to be effective against *K. pneumoniae* and *E. coli* infections especially in non-life threatening urinary tract infections. Considering the resistant rate of 1% and 0% for amikacin and imipenem respectively, it is appropriate to reserve these two antimicrobials for third-line treatment options.

5.3 RELIABILITY OF VITEK 2 SYSTEM AS ESBL DETECTION SYSTEM

VITEK 2 compact system (bioMérieux, France) is a semi-automated bacterial identification and susceptibility testing system enabling rapid determination of resistance mechanisms. This current study aimed at determining the reliability of using VITEK 2 system in detecting ESBL phenotypes in comparison with combined disc synergy method.

This would be the first time that Vitek 2 system would be used in Ghana on a large scientific scale and may be recommended to the Ministry of Health for routine use by laboratories in Ghana.

The outcome of this current work indicates that Vitek 2 system is concordant with the combined disc synergy method (Table 4.1, Figure 4.1). In a comparative study of combined disc method and Vitek 2 method of detecting ESBL, the sensitivity and specificity observed by Sorlozano and colleagues (2005) were comparable to findings in this current study.

The sensitivity and specificity values observed in this current study are however better than those reported by Leverstein-van Hall *et al.*, (2002), Sanders *et al.*, (2000) and Livermore *et al.*, (2002). Nevertheless, these investigators confirmed the reliability of Vitek 2 system to detect ESBL-producers as established in this study. This

conclusion was corroborated by Stefaniuk and colleagues (2005) in Poland who established that the comparison of the standard CDM method and the Vitek 2 system were similar in detecting ESBL in *Enterobacteriaceae*.

Teresa and colleagues (2006) also confirmed Vitek 2 system to be a rapid and reliable tool for routine identification of ESBL-producing isolates in Italy.

5.4 OCCURRENCE OF CTX-M, TEM AND SHV ESBL-GENES IN *K. PNEUMONIAE* AND *E. COLI* ISOLATES

CTX-M-type, TEM-type and SHV-type ESBLs are the main types of ESBL produced by bacteria. Some researchers have suggested that CTX-M-type ESBLs are now the most frequent ESBL type worldwide as compared to SHV and TEM-type ESBLs (Paterson & Bonomo, 2005). An objective of this current study was to characterize CTX-M-type, TEM-type and SHV-type ESBL-producing *K. pneumoniae* and *E. coli* isolates using polymerase chain reaction.

Of the 202 ESBL producers, 100 were selected for genetic characterisation based on the MICs of cefotaxime as indicated in table 4.5. It was observed in this current work that 194 (96%) of the ESBL producers had cefotaxime MICs in the resistant breakpoints ($\geq 64\mu\text{g/ml}$) (Appendix V). According to Paterson and Bonomo (2005), CTX-M ESBLs producers have cefotaxime MICs in the resistant breakpoints ($\geq 64\mu\text{g/ml}$). Consequently, all ESBL phenotypes with cefotaxime MIC less than $64\mu\text{g/ml}$ (8 isolates) were included in the molecular analysis together with those with cefotaxime MICs in the resistant breakpoint.

In this current study 90% isolates produced CTX-M-type ESBL confirming that CTX-M is the dominant ESBL-type in Accra as it is in most parts of the world (Falagas & Karageorgopoulos, 2009). This also reflects the high levels (96%) of the 202 ESBL-

producing phenotypes with cefotaxime MIC of >64µg/ml recorded in this current study as shown in appendix V; suggesting CTX-M-type ESBL producers according to Paterson & Bonomo (2005). The percentage of isolates producing CTX-M-type ESBL in this present study is consistent with the study in Kumasi by Feglo (2013) who reported 94.4% CTX-M-type ESBL. However, while Feglo (2013) reported that 64.5% of the ESBL isolates possess two genes and 29.9% possess three genes; only 20% of ESBL isolates in this present study had two genes and none possessed three ESBL genes. In a study in Southwest Nigeria, 30 selected multidrug-resistant *K. pneumoniae* strains isolated from patients with urinary tract infections showed 57% CTX-M enzymes (Olysegun *et al.*, 2006) which were slightly lower than the CTX-M ESBLs observed in *K. pneumoniae* strains in this study. Reports from Indonesia agreed with the high prevalence (94.5%) of CTX-M-type ESBL (Severin *et al.*, 2010). This was also corroborated by Tham and others (2010) who observed that 90% ESBL-positive genes were of CTX-M ESBL-type. This current study was consistent with the finding by Heffernan and colleagues (2007) in New Zealand who observed that 96% *E. coli* and *K. pneumoniae* isolates produced CTX-M ESBL genes which is also corroborated by a study done in Canada (Pitout *et al.*, 2007^b). So in this current study, Ghana joins North America, South America, Western Europe, Asia and other African nations with high prevalent CTX-M-ESBL producers. The remaining 5 unidentified ESBL producers may belong to other ESBL types (OXA, PER, VEB 1 etc) either than CTX-M, TEM and SHV.

This current study has shown that 78% of the CTX-M-type ESBLs were CTX-M-1 group with 2% belonging to CTX-M-9 group corroborating the study of Tham and colleagues in 2010 who reported that CTX-M-1 group was prevalent followed by the CTX-M-9 group as do the conclusions of Heffernan and colleagues who also showed

that CTX-M-1 group (CTX-M-15) and CTX-M-9 group (CTX-M-14) seem to be the most widespread (Heffernan *et al.*, 2007). The high prevalence of CTX-M-1group ESBL observed in this current study is consistent with studies in other parts of Africa. In Tanzania, Blomberg and colleagues (2005) discovered the presence of CTX-M-1group (CTX-M-15) ESBL-producing organism for the first time in Africa. In Tunisia, 43 of 47 isolates showed CTX-M-1 group (CTX-M-15) and 2 CTX-M-9 group (CTX-M-14) (Elhani *et al.*, 2010).

The PCR amplification of CTX-M-specific products without sequencing usually provides sufficient evidence that a CTX-M gene is responsible for the ESBL phenotype (Bradford, 2001). Nucleotide sequencing only helps to determine specific CTX-M-type ESBL subgroups in the strain (Heffernan *et al.*, 2007). CTX-M-15 (CTX-M-1group) may be the dominant CTX-M-type ESBL in this work when DNA sequencing is performed as reported in Tanzania (Blomberg *et al.*, 2005), Tunisia (Elhani *et al.*, 2010), Mexico (Garza-Gonza'lez *et al.*, 2011) and New Zealand (Heffernan *et al.*, 2007).

The remaining 10 unidentified CTX-M producers in this work may belong to other CTX-M sub-groups (CTX-M-2 group, CTX-M-8 group and CTX-M-25 group) either than CTX-M-1 group or CTX-M-9 group. However, this current study is at variance with the work of Pitout and colleagues in 2007 who found 48% and 37% CTX-M-1group and CTX-M-9 group respectively.

The dominance of CTX-M-type ESBLs (90%) and CTX-M-1 group ESBL (78%) phenotypes may be due to a selective pressure induced by indiscriminate exposure to cefotaxime since cefotaxime has been established to be the antimicrobial agent that induces selective pressure for the emergence of CTX-M genes (Du Bois *et al.*, 2005, Bonnet, 2004). Furthermore, the dominance of CTX-M-type ESBL producers can be attributed to the fact that CTX-M is easily transferred to other genetic sites since

encoding genes usually harbour insertion sequence ISEcp1 (Bonnet, 2004) and CTX-M-encoding plasmids are often transmissible by conjugation with high transfer frequencies of 10^{-7} to 10^{-2} per donor cell (Bonnet, 2004). Moreso, *Klyuvera* species are the reservoir of CTX-M genes (Bonnet, 2004). Their presence in the environment serves to heighten public health concerns since CTX-M genes are easily transferred to other bacteria plasmids in the Enterobacteriaceae family (Bonnet, 2004).

This consensus of CTX-M ESBL genes dominating the ESBL types was contradicted by the study of Feglo (2013) in Kumasi, Ghana which reported TEM-type ESBL as the dominating ESBL gene.

In this current study 25% of the bacterial isolates produced TEM-type ESBL; lower outcome was reported in Canada (Pitout *et al.*, 2007^b) and New Zealand (Heffernan *et al.*, 2007). Furthermore, Severin and colleagues (2010) in a study of 73 ESBL-positive *E. coli* and 72 *K. pneumoniae* strains in Indonesia, did not detect TEM-type ESBLs in any of the isolates. Interestingly Feglo in 2013, reported a high prevalent of 96.2% of TEM-type ESBL phenotypes in a study in Kumasi, Ghana in contrast to the findings of this current study and other reports. The dominance of TEM-type ESBL in Kumasi may be due to a selective pressure induced by indiscriminate exposure to ceftazidime, since ceftazidime has been established to be the antimicrobial agent that induces selective pressure for the emergence of TEM genes (Du Bois *et al.*, 1995; Bush *et al.*, 1995).

PCR amplification alone will not discriminate among different variants of TEM and SHV-type ESBLs. Sequencing is essential to discriminate between the non-ESBL parent enzymes (e.g., TEM 1, TEM 2, or SHV 1) and different variants of TEM or SHV ESBLs (e.g., TEM 3, TEM-10, or SHV 12 etc.) (Bradford, 2001). In this current study, DNA sequencing was not done due to technical and operational challenges.

Nevertheless, since all the isolates used for the PCR reaction were phenotypically pre-determined ESBL producers, all the TEM genes identified can be concluded to be TEM-type ESBLs.

Regarding SHV-type ESBL, none was identified in 100 strains of ESBL phenotypes as observed in Algiers by Ramdani-Bouguessa and colleagues in 2006. Low rates were reported in New Zealand (Heffernan *et al.*, 2007) and North Lebanon (Sana *et al.*, 2011) which contradicts findings in this current study. However, higher rates of 32.5% SHV-type ESBL were reported by Feglo (2013) in Kumasi contradicting this current work also in Ghana, 38.7% in Cameroon Gangoué-Piéboji *et al.*, 2005) while Jones and others also reported that SHV genes were found in 41% and 28% of the ESBL-positive *K. pneumoniae* and *E. coli* isolates respectively (Jones *et al.*, 2009) in contrast to this current study. SHV-type ESBL being the first ESBL isolated (Knothe *et al.*, 1983), initially dominated in most ESBL molecular studies, but due to the high transfer frequencies of CTX-M encoding genes (Bonnet, 2004), the dominance of SHV has been overshadowed by CTX-M-type ESBL phenotypes (Lee *et al.*, 2003).

The presence of TEM-type ESBL in Kumasi may be due to a selective pressure induced by indiscriminate exposure to ceftazidime, since ceftazidime has been established to be the antimicrobial agent that induces selective pressure for the emergence of SHV genes (Du Bois *et al.*, 1995; Bush *et al.*, 1995). However, one cannot entirely rule out possible DNA amplification difficulties relating to DNA template concentration, primers, reaction conditions and other factors.

5.5 CHARACTERISTIC ANTIMICROBIAL RESISTANCE AMONG PRODUCERS OF CTX-M AND TEM ESBL-CODING GENES

The genetic diversity in the various ESBL-producing organisms may reflect characteristic differences in relation to antibiotic resistance expression. This study sought to determine the characteristic resistance profile of beta-lactams, beta-lactam/beta-lactamase inhibitor combinations and non-beta-lactams among CTX-M-type and TEM-type ESBL producers based on their minimum inhibitory concentrations (MICs).

The suggestion that organisms producing CTX-M-type ESBL organisms typically have cefotaxime MICs in the resistant range (≥ 64 $\mu\text{g/ml}$) (Paterson & Bonomo, 2005) has been observed in this present study. This means that CTX-M producers hydrolyse cefotaxime with more efficiency. This gives credence to the fact that indiscriminate use of cefotaxime is the cause of the selective pressure leading to the emergence of CTX-M ESBL genes (Du Bois *et al.*, 1995, Bonnet, 2004). In this study the CTX-M-type ESBL hydrolysed ceftazidime with 42.9% of its MIC in the resistant range of ≥ 64 $\mu\text{g/ml}$ (Table 4.10) suggesting that CTX-M ESBLs producers have some ceftazidime MIC in the apparently susceptible breakpoints as observed by Sturenburg and colleagues (2004). This was also corroborated by Poirel and colleagues (2001) as well as Ramdani-Bougoussa and colleagues (2006). Therefore in the absence of ESBL screening, CTX-M producers may be wrongly treated with ceftazidime resulting in treatment failure.

In this current study, CTX-M-type ESBL showed co-resistances to gentamicin, tetracycline and trimethoprim/sulphamethoxazole (Table 4.10) similar to the findings of Ramdani-Bougoussa and others (2006) who observed co-resistance of CTX-M-type ESBL in gentamicin and trimethoprim/sulphamethoxazole in Algeria. Ben Ami and

colleagues (2006) also corroborated this study where CTX-M-producing *E. coli* were co-resistant to trimethoprim/sulphamethoxazole and gentamicin. According to Bonnet (2004), CTX-M-type ESBL co-resistance to non-beta-lactams may be attributed to genetic structures such as *sul1*-type integrons.

As in studies by Pitout and Laupland (2008) as well as Ben Ami (2006), this current study established that strains producing CTX-M enzymes were substantially resistant to ciprofloxacin. These reports contradict the findings of Ramdani-Bouguessa and colleagues (2006) who recorded low resistance rate to ciprofloxacin in Algeria.

The findings in this current study indicated that organisms producing TEM-type ESBL are efficient in hydrolysing cefotaxime and ceftazidime with their MIC in the resistant range of >64 µg/ml (Table 4.11). More so, TEM-type ESBLs had the tendency of hydrolysing ceftazidime more than CTX-M-types ESBL producers (Du Bois *et al.*, 1995; Bush *et al.*, 1995). This gives credence to that indiscriminate exposure to ceftazidime is the cause of the selective pressure leading to the emergence of TEM-ESBL genes (Du Bois *et al.*, 1995; Bush *et al.*, 1995).

TEM-type ESBL also showed co-resistances to gentamicin, ciprofloxacin, norfloxacin, nitrofurantoin, tetracycline and trimethoprim/sulphamethoxazole. TEM-type ESBLs seem to be efficient in hydrolysing piperacillin/tazobactam than amoxicillin/clavulanic acid which contradicted the work of Bush and colleagues (1993).

In this current study CTX-M and TEM-ESBL producing isolates were more resistant to piperacillin/tazobactam than amoxicillin/clavulanic acid. This contradicts the study of Bush and others (1993) in New York that tazobactam exhibits greater inhibitory activity to CTX-M-type ESBL than clavulanic acid.

Strains producing both CTX-M-type and TEM-type ESBLs showed co-resistances to beta-lactams, non-beta-lactams and beta-lactam/beta-lactamase inhibitor combination agreeing with the review of Pitout and Laupland (2008).

Both CTX-M and TEM ESBL producers hydrolyse cefepime with less efficiency with cefepime MIC in the susceptible breakpoints (Table 4.10; Table 4.11) at variance with Tzouvelekis and colleagues who reported in Greece that CTX-M-type beta-lactamases hydrolyse cefepime with high efficiency (Tzouvelekis *et al.*, 2000). This implies that in the absence of ESBL screening, CTX-M and TEM ESBL producers may be wrongly treated with cefepime leading to treatment failure.

This current study has indicated that none of the CTX-M-type and TEM-type ESBL showed resistance to imipenem and amikacin which confirm them as the drug of choice for treating CTX-M-type and TEM-type ESBL-producing infections. This is consistent with the findings of Sana and colleagues (2011) in Lebanon which concluded that imipenem and amikacin were ideal for the treatment of severe ESBL-producing organisms.

5.6 OCCURRENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF AMPC-BETA-LACTAMASE PRODUCING PHENOTYPES

AmpC beta-lactamases phenotypes hydrolyse penicillins, cephalosporins and cephamycins and resist inhibition by clavulanate, sulbactam and tazobactam. Many Gram-negative bacilli produce a chromosomally mediated AmpC which, when hyper-produced, may cause resistance to penicillins, aztreonam, cephamycins and cephalosporins (Thomson, 2010). It has been suggested by Thomson (2001) that AmpC beta-lactamases may interfere with the detection of ESBL producers. Consequently, the

occurrence of AmpC beta-lactamases phenotypes among ESBL producers and non-ESBL producers were determined in this work.

In the current study, only 5(1.3%) of the bacterial isolates analysed produced AmpC beta-lactamases. The occurrence of plasmid-mediated AmpC-producing strains is typically less common in most parts of the world (Jacoby, 2009). Of these 5 AmpC beta-lactamase producers, 3 were from *E. coli* and the remaining 2 were from *K. pneumoniae*. The occurrence of AmpC beta-lactamase producers was slightly higher in the finding of Heffernan and colleagues (2007) in New Zealand which reported that 18.2% of 33 *E. coli* isolates were AmpC beta-lactamase producers. However, in that report, none of the *K. pneumoniae* isolates carried AmpC genes in contrast to this current study. The low level of AmpC beta-lactamase phenotypes may be due to the low penetrance of the plasmid-mediated or chromosomally mediated AmpC-beta-lactamase genes in the environment. The low occurrence of AmpC beta-lactamase producers in this current study did not interfere with the detection of ESBL producers at variance with the suggestion of Thomson (2001).

Strains with AmpC genes are inherently resistant to multiple antibiotics, making the selection of an effective antibiotic difficult. All five (5) isolates were resistant to cefoxitin (cephamycins) which is a discriminative parameter for the detection of AmpC-producing strains (Peter-Getzlaff *et al.*, 2011).

In this current study AmpC-producing organisms showed co-resistance to gentamicin, ciprofloxacin, norfloxacin, tetracycline, trimethoprim/ sulphamethoxazole, amoxicillin/clavulanic acid and piperacillin/tazobactam. According to Jacoby (2009), most cephalosporins, penicillins and beta-lactam/beta-lactamase inhibitor combinations are ineffective in treating AmpC-producing infections.

In the current study, AmpC beta-lactamase producers showed little or no resistance to amikacin (Table 4.15), nitrofurantoin and imipenem as was reported by Jacoby (2009) who recommended that carbapenem and nitrofurantoin may be used for managing AmpC-producing infections.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The findings of this study showed an alarming rate of 50.5% ESBL-producing *E. coli* and *K. pneumoniae* in Accra with high co-resistance to beta-lactams, beta-lactam/beta-lactamase inhibitors and non-beta-lactams.

The high prevalence of ESBL producers in Accra requires routine, rapid and accurate diagnostic systems to ensure quality healthcare. This current study also showed that Vitek 2 system is concordant with combined disc synergy method in detecting ESBL phenotypes.

This current study showed a low level of AmpC beta-lactamase phenotypes among ESBL producers and non-ESBL phenotypes in *E. coli* and *K. pneumoniae* in Accra and did not seem to interfere with the detection of ESBL producers.

This work has underscored the significant differences between the antimicrobial co-resistance of ESBL producers and non-ESBL producers to beta-lactams (piperacillin, cefazolin, cefotaxime, ceftazidime, cefepime) non-beta-lactam (ciprofloxacin, norfloxacin, gentamicin, tetracycline, nitrofurantoin, trimethoprim/ sulphamethoxazole) and beta-lactam/beta-lactamase inhibitor combinations (amoxicillin/clavulanic acid and piperacillin/ tazobactam). The increasing resistance of non-ESBL producers to ciprofloxacin, gentamicin, amoxicillin/clavulanic acid and piperacillin/tazobactam is also worrying. Cephalosporins and nitrofurantoin were generally effective in inhibiting infections caused by non-ESBL producing *E. coli* and *K. pneumoniae*.

The findings of this current study suggested that organisms producing CTX-M-type and CTX-M-1 group ESBL are more prevalent in Accra than TEM-type and SHV-type ESBL.

It has been suggested in this present study that CTX-M and TEM-type ESBL producers expressed characteristic and high levels of antimicrobial resistance to beta-lactams, non-beta-lactams and beta-lactam/beta-lactamase inhibitor combinations.

CTX-M-type ESBLs are more efficient in hydrolysing cefotaxime with typical cefotaxime MIC of $\geq 64\mu\text{g/ml}$ and CTX-M-type ESBLs apparently hydrolyse ceftazidime with less efficiency with some of their MICs in the susceptible breakpoints.

The findings in this current study indicated that bacteria producing TEM-type ESBL seem to be efficient in hydrolysing ceftazidime more than CTX-M-types ESBL producers. TEM-type ESBL producers also hydrolyse both ceftazidime and cefotaxime effectively.

In this present study, CTX-M-type and TEM-type ESBL producers are more efficient in hydrolysing piperacillin/tazobactam than amoxicillin/clavulanic acid. CTX-M-type and TEM-type ESBL producers hydrolyse cefepime with less efficiency as compared with the third generation cephalosporins. This current study showed high co-resistances in *K. pneumoniae* and *E. coli* producing CTX-M-type and TEM-type ESBLs to penicillins, cephalosporins, gentamicin, ciprofloxacin, norfloxacin, nitrofurantoin, tetracycline, trimethoprim / sulphamethoxazole, piperacillin/tazobactam and amoxicillin/clavulanic acid.

It has been observed in this current study that imipenem and amikacin were the drugs of choice for managing CTX-M type and TEM type ESBL-producing organisms.

The limitation in this current study is that there was no sequencing of ESBL genes to determine specific ESBL genes present in a strain.

6.2 RECOMMENDATIONS

- i. There is the need to routinely screen for ESBL-producing phenotypes among clinical specimens in diagnostic laboratories using combined disc synergy method or the Vitek 2 system if appropriate financing strategies are enrolled with the support of Ministry of Health.
- ii. Regular monitoring and evaluation of antibiotic resistance must be prioritised.
- iii. Conscientious infection control efforts should be implemented to reduce the public health threat of increasing CTX-M-type and TEM-type ESBL producing organisms in Accra.
- iv. Evidence based clinical practices, systematic implementation of antimicrobial stewardship programs and enforcement of appropriate antimicrobial usage and prescription policies should be encouraged to curtail the alarming rate of antimicrobial resistance.
- v. There is the need for further studies into the risk factors associated to infection of ESBL producers and clinical therapeutic response to infections by CTX-M-type and TEM-type ESBL producers in Accra.
- vi. Further studies into the sequencing of ESBL genes is recommended to determine specific ESBL gene present in a strain.

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APPENDICES

Appendix I

Materials and Reagents

MAST IDTM ES β L Detection Disc (Mast Group, UK) were used for ESBL screening and confirmation according to CLSI standards. Vitek 2 Compact System (bioMérieux, France) was used to identify the isolates, determine minimum inhibitory concentration of selected antibiotics and interpret the MICs according to CSLI breakpoints. Cefotaxime, ceftazidime and boronic acid antibiotic discs were used for AmpC beta-lactamase phenotype confirmation. Water bath was used to heat the colony suspension and centrifuge was used to spin the suspension to extract the bacteria DNA. BIOER GenePro thermocycler (Bioer Technology, China) was used to perform the polymerase chain reaction (PCR) under controlled reaction conditions with specific primers. PCR products were used to perform agarose gel electrophoresis with 1X TAE buffer, 2% agarose gel and 0.5 μ g/ml ethidium bromide at 120V for 45minutes. The bands on the gels were visualized by ultraviolet trans-illumination and photographed using a Kodak EDAS 290 gel documentation system.

Glycerol Broth Preparation

The main materials include brain-heart infusion broth, distilled water and glycerol. The 20% brain heart infusion broth was prepared by weighing 200g in 1 liter of distilled water. The glycerol broth was prepared using the ratio of 4:1 distilled water to glycerol. The mixture of brain-heart infusion broth and glycerol broth was stirred until a uniform solution was obtained. A micropipette was used to pipette 1ml of the solution into Eppendorf tubes. The broth was then sterilized at 121°C for 15 minutes in an autoclave.

APPENDIX II

Diagrammatical Representation of the Operations of Vitek 2 Compact System

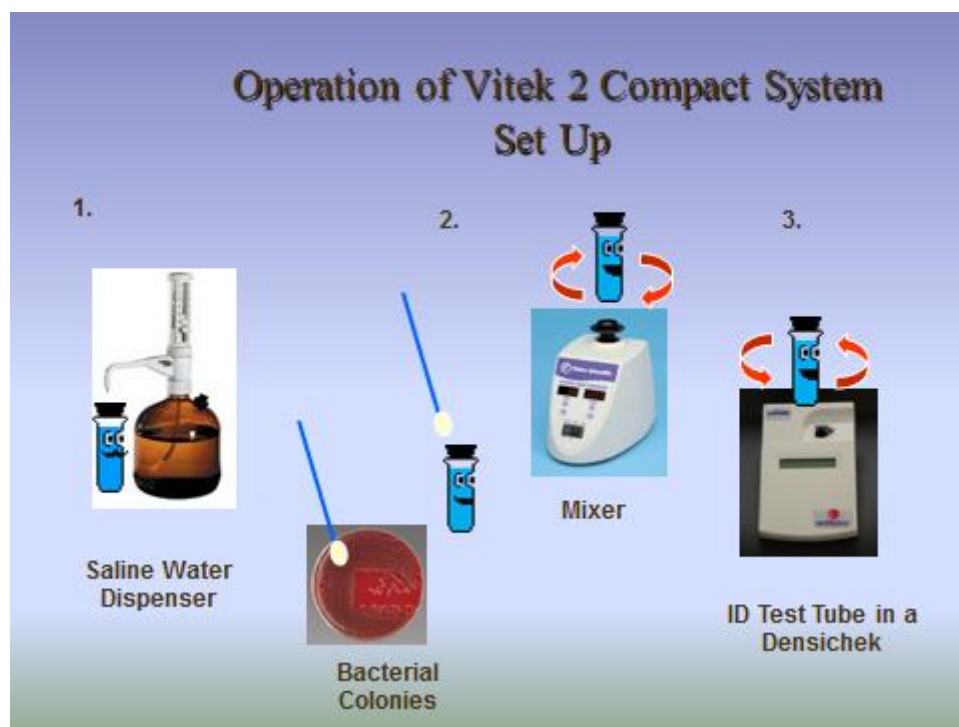


Plate A1: Operation of Vitek 2 System set up

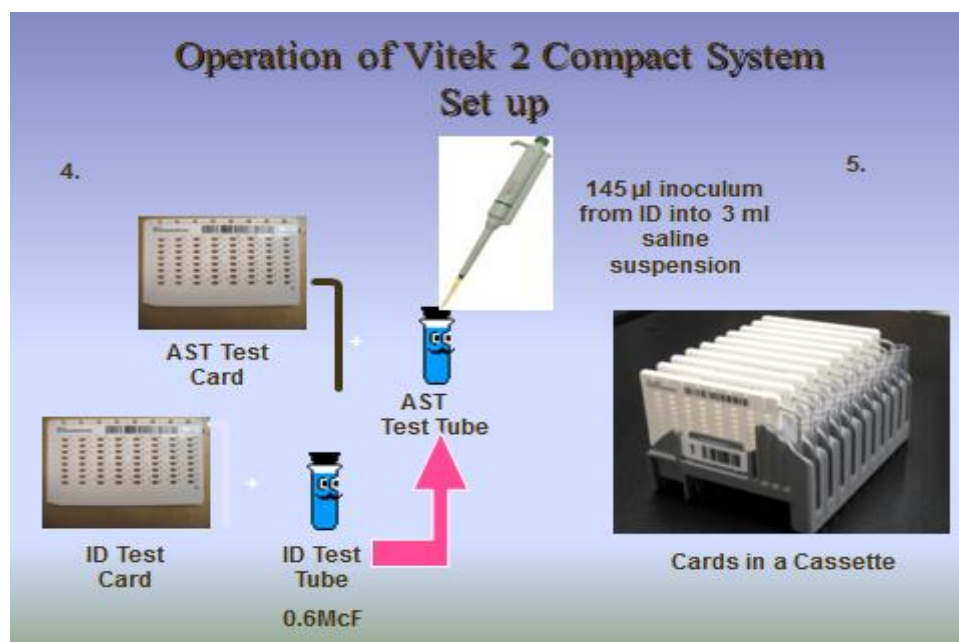


Plate A2: Operation of Vitek 2 compact system set up

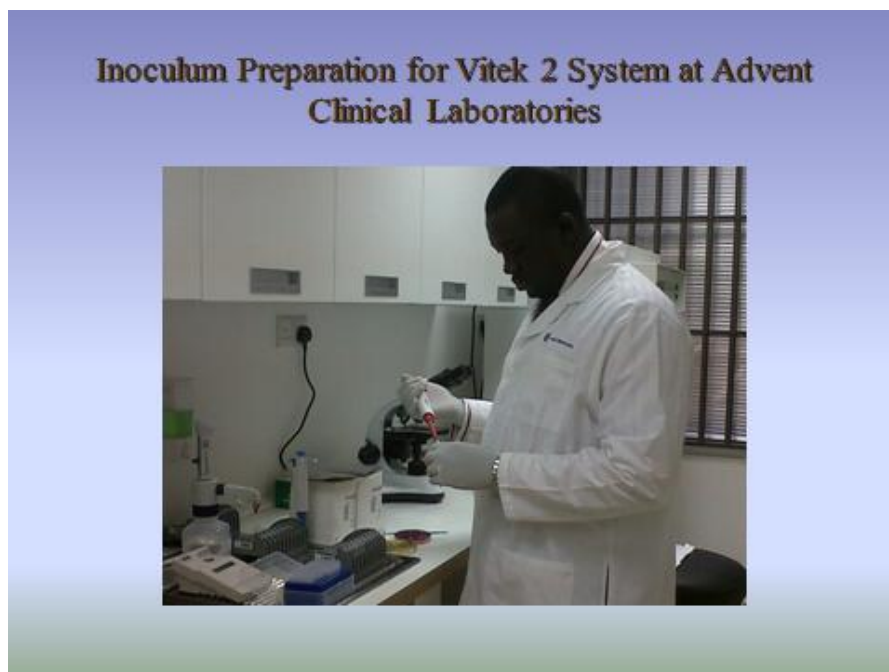


Plate A3: Inoculum preparation for Vitek 2 system at Advent Clinical Laboratory



Plate A4: Vitek 2 System (bioMérieux, France) at Advent Clinical Laboratory

Introducing the Cassette into the Filling Chamber



Plate A5: Introducing the cassette into the filling chamber of Vitek 2 system

APPENDIX III**Table A1:** Therapeutic Interpretation of Breakpoints of Antibiotics according to CLSI-2008

Antibiotics	MIC Breakpoint Range (µg/ml)		
	S	I	R
Ampicillin	≤2	16	≥ 32
Amoxicillin/Clavulanic acid	≤2	16	≥ 32
Piperacillin	≤4	32-64	≥128
Piperacillin/Tazobactam	≤4	32	≥128
Cefoxitin	≤4	16	≥ 64
Cefazolin	≤4	16	≥ 64
Cefotaxime	≤1	16	≥ 64
Ceftazidime	≤1	16	≥ 64
Cefepime	≤1	16	≥ 64
Imipenem	≤1	8	≥ 16
Amikacin	≤2	32	≥ 64
Gentamicin	≤1	8	≥ 16
Ciprofloxacin	≤0.25	2	≥ 4
Norfloxacin	≤0.5	8	≥ 16
Tetracycline	≤1	8	≥ 16
Nitrofurantoin	≤16	64	≥512
Trimethoprim/Sulphamethoxazole	≤20	80	≥320

S: susceptible

I: intermediate

R: resistant

APPENDIX IV

Zones of Inhibition

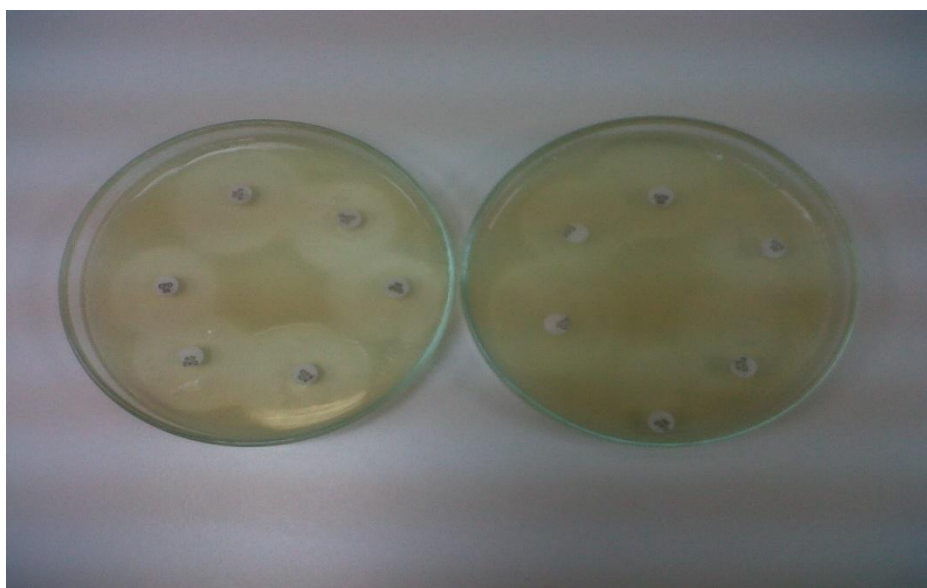


Plate A6: Zone of inhibition of MAST ID™ ESBL Detection Discs



Plate A7: Zone of inhibition of ESBL quality control strains of *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603

APPENDIX V

Table A2: Vitek 2 System Results of Species Identification, ESBL phenotypes and Antimicrobial Susceptibility Testing based on MICs

AMPICILLIN									
ID ^a	ORG ^b	ESBL ^c	AST ^d						
			MIC ^g						
2	EC ^e	+	>=32	R	55	KP	+	>=32	R
3	KP ^f	+	>=32	R	57	EC	+	>=32	R
4	KP	+	>=32	R	58	KP	+	>=32	R
8	KP	+	>=32	R	59	KP	+	>=32	R
9	KP	+	>=32	R	60	EC	+	>=32	R
11	EC	+	>=32	R	61	KP	+	>=32	R
12	KP	+	>=32	R	63	KP	+	>=32	R
14	KP	+	>=32	R	64	KP	+	>=32	R
15	KP	+	>=32	R	66	KP	+	>=32	R
17	KP	+	>=32	R	67	EC	+	>=32	R
19	KP	+	>=32	R	68	KP	+	>=32	R
23	EC	+	>=32	R	69	KP	+	>=32	R
25	KP	+	>=32	R	70	KP	+	>=32	R
27	KP	+	>=32	R	71	KP	+	>=32	R
28	EC	+	>=32	R	72	KP	+	>=32	R
29	KP	+	>=32	R	73	EC	+	>=32	R
30	EC	+	>=32	R	74	KP	+	>=32	R
31	KP	+	>=32	R	75	EC	+	>=32	R
32	KP	+	>=32	R	76	EC	+	>=32	R
33	KP	+	>=32	R	78	KP	+	>=32	R
34	KP	+	>=32	R	79	EC	+	>=32	R
36	KP	+	>=32	R	80	KP	+	>=32	R
38	KP	+	>=32	R	81	EC	+	>=32	R
40	KP	+	>=32	R	83	EC	+	>=32	R
41	KP	+	>=32	R	84	EC	+	>=32	R
43	KP	+	>=32	R	85	KP	+	>=32	R
44	KP	+	>=32	R	86	EC	+	>=32	R
47	KP	+	>=32	R	87	EC	+	>=32	R
48	KP	+	>=32	R	88	EC	+	>=32	R
49	KP	+	>=32	R	89	EC	+	>=32	R
50	KP	+	>=32	R	93	EC	+	>=32	R
51	KP	+	>=32	R	94	EC	+	>=32	R
53	KP	+	>=32	R	95	KP	+	>=32	R
54	KP	+	>=32	R	102	KP	+	>=32	R
					103	EC	+	>=32	R
					104	EC	+	>=32	R
					105	KP	+	>=32	R

107	EC	+	>=32	R	213	EC	+	>=32	R
108	EC	+	>=32	R	214	EC	+	>=32	R
109	EC	+	>=32	R	215	EC	+	>=32	R
112	EC	+	>=32	R	216	EC	+	>=32	R
116	EC	+	>=32	R	217	KP	+	>=32	R
117	EC	+	>=32	R	218	KP	+	>=32	R
118	EC	+	>=32	R	219	KP	+	>=32	R
119	EC	+	>=32	R	220	KP	+	>=32	R
120	EC	+	>=32	R	221	KP	+	>=32	R
121	KP	+	>=32	R	222	KP	+	>=32	R
122	EC	+	>=32	R	223	KP	+	>=32	R
123	EC	+	>=32	R	224	KP	+	>=32	R
124	EC	+	>=32	R	225	KP	+	>=32	R
126	EC	+	>=32	R	226	KP	+	>=32	R
129	KP	+	>=32	R	227	KP	+	>=32	R
130	KP	+	>=32	R	244	KP	+	>=32	R
133	KP	+	>=32	R	245	KP	+	>=32	R
135	KP	+	>=32	R	246	KP	+	>=32	R
145	KP	+	>=32	R	247	KP	+	>=32	R
147	KP	+	>=32	R	248	KP	+	>=32	R
149	EC	+	>=32	R	249	KP	+	>=32	R
151	KP	+	>=32	R	260	KP	+	>=32	R
154	KP	+	>=32	R	262	KP	+	>=32	R
156	EC	+	>=32	R	264	EC	+	>=32	R
158	EC	+	>=32	R	267	KP	+	>=32	R
162	EC	+	>=32	R	268	EC	+	>=32	R
164	KP	+	>=32	R	270	KP	+	>=32	R
165	KP	+	>=32	R	271	KP	+	>=32	R
166	KP	+	>=32	R	272	KP	+	>=32	R
167	EC	+	>=32	R	274	KP	+	>=32	R
169	EC	+	>=32	R	275	KP	+	>=32	R
175	EC	+	>=32	R	276	KP	+	>=32	R
177	EC	+	>=32	R	277	EC	+	>=32	R
187	EC	+	>=32	R	279	EC	+	>=32	R
195	EC	+	>=32	R	280	KP	+	>=32	R
196	EC	+	>=32	R	281	KP	+	>=32	R
198	KP	+	>=32	R	282	KP	+	>=32	R
202	KP	+	>=32	R	283	KP	+	>=32	R
203	KP	+	>=32	R	285	KP	+	>=32	R
206	KP	+	>=32	R	286	KP	+	>=32	R
207	KP	+	>=32	R	287	KP	+	>=32	R
211	KP	+	>=32	R	288	KP	+	>=32	R
212	KP	+	>=32	R	289	KP	+	>=32	R

291	KP	+	>=32	R
292	KP	+	>=32	R
293	KP	+	>=32	R
294	KP	+	>=32	R
295	KP	+	>=32	R
296	KP	+	>=32	R
298	KP	+	>=32	R
304	KP	+	>=32	R
305	KP	+	>=32	R
306	KP	+	>=32	R
307	KP	+	>=32	R
308	KP	+	>=32	R
309	KP	+	>=32	R
310	KP	+	>=32	R
311	KP	+	>=32	R
312	EC	+	>=32	R
313	EC	+	>=32	R
314	KP	+	>=32	R
315	KP	+	>=32	R
316	KP	+	>=32	R
317	KP	+	>=32	R
318	EC	+	>=32	R
322	KP	+	>=32	R
323	KP	+	>=32	R
324	EC	+	>=32	R
325	EC	+	>=32	R
326	EC	+	>=32	R
327	EC	+	>=32	R
328	EC	+	>=32	R
329	EC	+	>=32	R
330	KP	+	>=32	R
331	KP	+	>=32	R
332	KP	+	>=32	R
333	KP	+	>=32	R
339	EC	+	>=32	R
346	KP	+	>=32	R
347	EC	+	>=32	R
350	EC	+	>=32	R
352	EC	+	>=32	R
357	EC	+	>=32	R
358	EC	+	>=32	R
360	EC	+	>=32	R
361	EC	+	>=32	R

363	EC	+	>=32	R
400	KP	+	>=32	R

AMOXICILLIN/CLAVULANIC ACID

ID ^a	ORG ^b	ESBL ^c	AST ^d	
MIC				
2	EC	+	16	I
3	KP	+	>=32	R
4	KP	+	>=32	R
8	KP	+	>=32	R
9	KP	+	>=32	R
11	EC	+	16	I
12	KP	+	16	I
14	KP	+	>=32	R
15	KP	+	>=32	R
17	KP	+	16	I
19	KP	+	16	I
23	EC	+	16	I
25	KP	+	16	I
27	KP	+	>=32	R
28	EC	+	>=32	R
29	KP	+	16	I
30	EC	+	16	I
31	KP	+	16	I
32	KP	+	16	I
33	KP	+	16	I
34	KP	+	16	I
36	KP	+	16	I
38	KP	+	16	I
40	KP	+	>=32	R
41	KP	+	16	I
43	KP	+	>=32	R
44	KP	+	16	I
47	KP	+	>=32	R
48	KP	+	>=32	R
49	KP	+	>=32	R
50	KP	+	>=32	R
51	KP	+	>=32	R
53	KP	+	4	S
54	KP	+	16	I
55	KP	+	16	I
57	EC	+	16	I

58	KP	+	16	I	120	EC	+	8	S
59	KP	+	>=32	R	121	KP	+	16	I
60	EC	+	8	S	122	EC	+	16	I
61	KP	+	>=32	R	123	EC	+	8	S
63	KP	+	>=32	R	124	EC	+	16	I
64	KP	+	>=32	R	126	EC	+	>=32	R
66	KP	+	>=32	R	129	KP	+	>=32	R
67	EC	+	>=32	R	130	KP	+	>=32	R
68	KP	+	>=32	R	133	KP	+	4	S
69	KP	+	>=32	R	135	KP	+	>=32	R
70	KP	+	>=32	R	145	KP	+	>=32	R
71	KP	+	16	I	147	KP	+	>=32	R
72	KP	+	16	I	149	EC	+	>=32	R
73	EC	+	16	I	151	KP	+	>=32	R
74	KP	+	>=32	R	154	KP	+	16	I
75	EC	+	16	I	156	EC	+	>=32	R
76	EC	+	16	I	158	EC	+	16	I
78	KP	+	16	I	162	EC	+	16	I
79	EC	+	16	I	164	KP	+	16	I
80	KP	+	>=32	R	165	KP	+	>=32	R
81	EC	+	8	S	166	KP	+	16	I
83	EC	+	16	I	167	EC	+	4	S
84	EC	+	16	I	169	EC	+	4	S
85	KP	+	>=32	R	175	EC	+	16	I
86	EC	+	16	I	177	EC	+	4	S
87	EC	+	16	I	187	EC	+	16	I
88	EC	+	16	I	195	EC	+	16	I
89	EC	+	16	I	196	EC	+	4	S
93	EC	+	16	I	198	KP	+	16	I
94	EC	+	16	I	202	KP	+	16	I
95	KP	+	16	I	203	KP	+	16	I
102	KP	+	8	S	206	KP	+	8	S
103	EC	+	16	I	207	KP	+	16	I
104	EC	+	16	I	211	KP	+	16	I
105	KP	+	8	S	212	KP	+	16	I
107	EC	+	16	I	213	EC	+	16	I
108	EC	+	16	I	214	EC	+	16	I
109	EC	+	16	I	215	EC	+	16	I
112	EC	+	16	I	216	EC	+	16	I
116	EC	+	8	S	217	KP	+	16	I
117	EC	+	8	S	218	KP	+	16	I
118	EC	+	8	S	219	KP	+	16	I
119	EC	+	8	S	220	KP	+	16	I

221	KP	+	16	I	305	KP	+	16	I
222	KP	+	16	I	306	KP	+	16	I
223	KP	+	≥ 32	R	307	KP	+	≥ 32	R
224	KP	+	≥ 32	R	308	KP	+	16	I
225	KP	+	≥ 32	R	309	KP	+	16	I
226	KP	+	≥ 32	R	310	KP	+	≥ 32	R
227	KP	+	≥ 32	R	311	KP	+	≥ 32	R
244	KP	+	16	I	312	EC	+	16	I
245	KP	+	16	I	313	EC	+	16	I
246	KP	+	16	I	314	KP	+	16	I
247	KP	+	16	I	315	KP	+	≥ 32	R
248	KP	+	16	I	316	KP	+	≥ 32	R
249	KP	+	16	I	317	KP	+	≥ 32	R
260	KP	+	16	I	318	EC	+	8	S
262	KP	+	≥ 32	R	322	KP	+	16	I
264	EC	+	16	I	323	KP	+	16	I
267	KP	+	16	I	324	EC	+	8	S
268	EC	+	16	I	325	EC	+	8	S
270	KP	+	≥ 32	R	326	EC	+	8	S
271	KP	+	≥ 32	R	327	EC	+	16	I
272	KP	+	≥ 32	R	328	EC	+	16	I
274	KP	+	16	I	329	EC	+	16	I
275	KP	+	16	I	330	KP	+	4	S
276	KP	+	16	I	331	KP	+	4	S
277	EC	+	16	I	332	KP	+	≥ 32	R
279	EC	+	16	I	333	KP	+	≥ 32	R
280	KP	+	≥ 32	R	339	EC	+	16	I
281	KP	+	≥ 32	R	346	KP	+	≥ 32	R
282	KP	+	≥ 32	R	347	EC	+	16	I
283	KP	+	≥ 32	R	350	EC	+	16	I
285	KP	+	16	I	352	EC	+	16	I
286	KP	+	16	I	357	EC	+	16	I
287	KP	+	16	I	358	EC	+	8	S
288	KP	+	≥ 32	R	360	EC	+	16	I
289	KP	+	≥ 32	R	361	EC	+	16	I
291	KP	+	16	I	363	EC	+	16	I
292	KP	+	16	I	400	KP	+	≥ 32	R
293	KP	+	16	I					
294	KP	+	16	I					
295	KP	+	16	I					
296	KP	+	≥ 32	R					
298	KP	+	16	I					
304	KP	+	16	I					

PIPERACILLIN

ID ^a	ORG ^b	ESBL ^c	AST ^d	
				M I C
2	EC	+	>=128	R
3	KP	+	>=128	R
4	KP	+	>=128	R
8	KP	+	>=128	R
9	KP	+	>=128	R
11	EC	+	>=128	R
12	KP	+	>=128	R
14	KP	+	>=128	R
15	KP	+	>=128	R
17	KP	+	>=128	R
19	KP	+	>=128	R
23	EC	+	>=128	R
25	KP	+	>=128	R
27	KP	+	>=128	R
28	EC	+	>=128	R
29	KP	+	>=128	R
30	EC	+	>=128	R
31	KP	+	>=128	R
32	KP	+	>=128	R
33	KP	+	>=128	R
34	KP	+	>=128	R
36	KP	+	>=128	R
38	KP	+	>=128	R
40	KP	+	>=128	R
41	KP	+	>=128	R
43	KP	+	>=128	R
44	KP	+	>=128	R
47	KP	+	>=128	R
48	KP	+	>=128	R
49	KP	+	>=128	R
50	KP	+	>=128	R
51	KP	+	>=128	R
53	KP	+	>=128	R
54	KP	+	>=128	R
55	KP	+	>=128	R
57	EC	+	>=128	R
58	KP	+	>=128	R
59	KP	+	>=128	R

60	EC	+	>=128	R
61	KP	+	>=128	R
63	KP	+	>=128	R
64	KP	+	>=128	R
66	KP	+	>=128	R
67	EC	+	>=128	R
68	KP	+	>=128	R
69	KP	+	>=128	R
70	KP	+	>=128	R
71	KP	+	>=128	R
72	KP	+	>=128	R
73	EC	+	>=128	R
74	KP	+	>=128	R
75	EC	+	>=128	R
76	EC	+	>=128	R
78	KP	+	>=128	R
79	EC	+	>=128	R
80	KP	+	>=128	R
81	EC	+	>=128	R
83	EC	+	>=128	R
84	EC	+	>=128	R
85	KP	+	>=128	R
86	EC	+	>=128	R
87	EC	+	>=128	R
88	EC	+	>=128	R
89	EC	+	>=128	R
93	EC	+	>=128	R
94	EC	+	>=128	R
95	KP	+	>=128	R
102	KP	+	>=128	R
103	EC	+	>=128	R
104	EC	+	>=128	R
105	KP	+	>=128	R
107	EC	+	>=128	R
108	EC	+	>=128	R
109	EC	+	>=128	R
112	EC	+	>=128	R
116	EC	+	>=128	R
117	EC	+	>=128	R
118	EC	+	>=128	R
119	EC	+	>=128	R
120	EC	+	>=128	R
121	KP	+	>=128	R

122	EC	+	>=128	R	223	KP	+	>=128	R
123	EC	+	>=128	R	224	KP	+	>=128	R
124	EC	+	>=128	R	225	KP	+	>=128	R
126	EC	+	>=128	R	226	KP	+	>=128	R
129	KP	+	>=128	R	227	KP	+	>=128	R
130	KP	+	>=128	R	244	KP	+	>=128	R
133	KP	+	>=128	R	245	KP	+	>=128	R
135	KP	+	>=128	R	246	KP	+	>=128	R
145	KP	+	>=128	R	247	KP	+	>=128	R
147	KP	+	>=128	R	248	KP	+	>=128	R
149	EC	+	>=128	R	249	KP	+	>=128	R
151	KP	+	>=128	R	260	KP	+	>=128	R
154	KP	+	>=128	R	262	KP	+	>=128	R
156	EC	+	>=128	R	264	EC	+	>=128	R
158	EC	+	>=128	R	267	KP	+	>=128	R
162	EC	+	>=128	R	268	EC	+	>=128	R
164	KP	+	>=128	R	270	KP	+	>=128	R
165	KP	+	>=128	R	271	KP	+	>=128	R
166	KP	+	>=128	R	272	KP	+	>=128	R
167	EC	+	>=128	R	274	KP	+	>=128	R
169	EC	+	>=128	R	275	KP	+	>=128	R
175	EC	+	>=128	R	276	KP	+	>=128	R
177	EC	+	>=128	R	277	EC	+	>=128	R
187	EC	+	>=128	R	279	EC	+	>=128	R
195	EC	+	>=128	R	280	KP	+	>=128	R
196	EC	+	>=128	R	281	KP	+	>=128	R
198	KP	+	>=128	R	282	KP	+	>=128	R
202	KP	+	>=128	R	283	KP	+	>=128	R
203	KP	+	>=128	R	285	KP	+	>=128	R
206	KP	+	>=128	R	286	KP	+	>=128	R
207	KP	+	>=128	R	287	KP	+	>=128	R
211	KP	+	>=128	R	288	KP	+	>=128	R
212	KP	+	>=128	R	289	KP	+	>=128	R
213	EC	+	>=128	R	291	KP	+	>=128	R
214	EC	+	>=128	R	292	KP	+	>=128	R
215	EC	+	>=128	R	293	KP	+	>=128	R
216	EC	+	>=128	R	294	KP	+	>=128	R
217	KP	+	>=128	R	295	KP	+	>=128	R
218	KP	+	>=128	R	296	KP	+	>=128	R
219	KP	+	>=128	R	298	KP	+	>=128	R
220	KP	+	>=128	R	304	KP	+	>=128	R
221	KP	+	>=128	R	305	KP	+	>=128	R
222	KP	+	>=128	R	306	KP	+	>=128	R

307	KP	+	>=128	R
308	KP	+	>=128	R
309	KP	+	>=128	R
310	KP	+	>=128	R
311	KP	+	>=128	R
312	EC	+	>=128	R
313	EC	+	>=128	R
314	KP	+	>=128	R
315	KP	+	>=128	R
316	KP	+	>=128	R
317	KP	+	>=128	R
318	EC	+	>=128	R
322	KP	+	>=128	R
323	KP	+	>=128	R
324	EC	+	>=128	R
325	EC	+	>=128	R
326	EC	+	>=128	R
327	EC	+	>=128	R
328	EC	+	>=128	R
329	EC	+	>=128	R
330	KP	+	>=128	R
331	KP	+	>=128	R
332	KP	+	>=128	R
333	KP	+	>=128	R
339	EC	+	>=128	R
346	KP	+	>=128	R
347	EC	+	>=128	R
350	EC	+	>=128	R
352	EC	+	>=128	R
357	EC	+	>=128	R
358	EC	+	>=128	R
360	EC	+	>=128	R
361	EC	+	>=128	R
363	EC	+	>=128	R
400	KP	+	>=128	R

PIPERACILLIN/TAZOBACTAM

ID ^a	ORG ^b	ESBL ^c	AST ^d
			M I C
2	EC	+	8
3	KP	+	>=128

4	KP	+	>=128	R
8	KP	+	>=128	R
9	KP	+	>=128	R
11	EC	+	16	S
12	KP	+	>=128	R
14	KP	+	>=128	R
15	KP	+	>=128	R
17	KP	+	>=128	R
19	KP	+	>=128	R
23	EC	+	16	S
25	KP	+	>=128	R
27	KP	+	>=128	R
28	EC	+	16	S
29	KP	+	>=128	R
30	EC	+	16	S
31	KP	+	>=128	R
32	KP	+	64	I
33	KP	+	>=128	R
34	KP	+	>=128	R
36	KP	+	>=128	R
38	KP	+	>=128	R
40	KP	+	>=128	R
41	KP	+	>=128	R
43	KP	+	>=128	R
44	KP	+	>=128	R
47	KP	+	>=128	R
48	KP	+	>=128	R
49	KP	+	>=128	R
50	KP	+	>=128	R
51	KP	+	>=128	R
53	KP	+	16	S
54	KP	+	32	I
55	KP	+	>=128	R
57	EC	+	8	S
58	KP	+	16	S
59	KP	+	>=128	R
60	EC	+	<=4	S
61	KP	+	>=128	R
63	KP	+	>=128	R
64	KP	+	>=128	R
66	KP	+	>=128	R
67	EC	+	64	I
68	KP	+	>=128	R

69	KP	+	>=128	R	135	KP	+	>=128	R
70	KP	+	>=128	R	145	KP	+	>=128	R
71	KP	+	>=128	R	147	KP	+	>=128	R
72	KP	+	>=128	R	149	EC	+	16	S
73	EC	+	<=4	S	151	KP	+	>=128	R
74	KP	+	>=128	R	154	KP	+	64	I
75	EC	+	<=4	S	156	EC	+	16	S
76	EC	+	<=4	S	158	EC	+	64	I
78	KP	+	32	I	162	EC	+	8	S
79	EC	+	>=128	R	164	KP	+	32	I
80	KP	+	>=128	R	165	KP	+	>=128	R
81	EC	+	<=4	S	166	KP	+	32	I
83	EC	+	>=128	R	167	EC	+	<=4	S
84	EC	+	>=128	R	169	EC	+	<=4	S
85	KP	+	>=128	R	175	EC	+	16	S
86	EC	+	>=128	R	177	EC	+	<=4	S
87	EC	+	>=128	S	187	EC	+	16	S
88	EC	+	>=128	R	195	EC	+	16	S
89	EC	+	>=128	R	196	EC	+	<=4	S
93	EC	+	16	S	198	KP	+	>=128	R
94	EC	+	16	S	202	KP	+	>=128	R
95	KP	+	8	S	203	KP	+	>=128	R
102	KP	+	16	S	206	KP	+	>=128	R
103	EC	+	32	I	207	KP	+	>=128	R
104	EC	+	32	I	211	KP	+	>=128	R
105	KP	+	<=4	S	212	KP	+	8	S
107	EC	+	32	I	213	EC	+	16	S
108	EC	+	32	I	214	EC	+	16	S
109	EC	+	32	I	215	EC	+	16	S
112	EC	+	32	I	216	EC	+	16	S
116	EC	+	<=4	S	217	KP	+	32	I
117	EC	+	<=4	S	218	KP	+	32	I
118	EC	+	<=4	S	219	KP	+	32	I
119	EC	+	<=4	S	220	KP	+	32	I
120	EC	+	<=4	S	221	KP	+	32	I
121	KP	+	64	I	222	KP	+	32	I
122	EC	+	<=4	S	223	KP	+	>=128	R
123	EC	+	<=4	S	224	KP	+	>=128	R
124	EC	+	8	S	225	KP	+	>=128	R
126	EC	+	>=128	R	226	KP	+	>=128	R
129	KP	+	>=128	R	227	KP	+	>=128	R
130	KP	+	>=128	R	244	KP	+	64	I
133	KP	+	<=4	S	245	KP	+	64	I

246	KP	+	64	I	314	KP	+	32	I
247	KP	+	64	I	315	KP	+	>=128	R
248	KP	+	64	I	316	KP	+	>=128	R
249	KP	+	64	I	317	KP	+	>=128	R
260	KP	+	16	S	318	EC	+	<=4	S
262	KP	+	>=128	R	322	KP	+	>=128	R
264	EC	+	<=4	S	323	KP	+	>=128	R
267	KP	+	16	S	324	EC	+	<=4	S
268	EC	+	>=128	R	325	EC	+	<=4	S
270	KP	+	>=128	R	326	EC	+	<=4	S
271	KP	+	>=128	R	327	EC	+	16	S
272	KP	+	>=128	R	328	EC	+	16	S
274	KP	+	>=128	R	329	EC	+	16	S
275	KP	+	>=128	R	330	KP	+	16	S
276	KP	+	>=128	R	331	KP	+	16	S
277	EC	+	<=4	S	332	KP	+	>=128	R
279	EC	+	<=4	S	333	KP	+	>=128	R
280	KP	+	>=128	R	339	EC	+	>=128	R
281	KP	+	>=128	R	346	KP	+	>=128	R
282	KP	+	>=128	R	347	EC	+	64	I
283	KP	+	>=128	R	350	EC	+	32	I
285	KP	+	8	S	352	EC	+	>=128	R
286	KP	+	8	S	357	EC	+	64	I
287	KP	+	8	S	358	EC	+	<=4	S
288	KP	+	>=128	R	360	EC	+	>=128	R
289	KP	+	>=128	R	361	EC	+	16	S
291	KP	+	>=128	R	363	EC	+	32	I
292	KP	+	>=128	R	400	KP	+	>=128	R
293	KP	+	>=128	R	CEFAZOLIN				
294	KP	+	>=128	R					
295	KP	+	>=128	R					
296	KP	+	>=128	R					
298	KP	+	>=128	R					
304	KP	+	>=128	R					
305	KP	+	>=128	R					
306	KP	+	>=128	R					
307	KP	+	>=128	R					
308	KP	+	32	I					
309	KP	+	32	I					
310	KP	+	>=128	R					
311	KP	+	>=128	R					
312	EC	+	16	S					
313	EC	+	16	S					

17	KP	+	>=64	R	76	EC	+	>=64	R
19	KP	+	>=64	R	78	KP	+	>=64	R
23	EC	+	>=64	R	79	EC	+	>=64	R
25	KP	+	>=64	R	80	KP	+	>=64	R
27	KP	+	>=64	R	81	EC	+	>=64	R
28	EC	+	>=64	R	83	EC	+	>=64	R
29	KP	+	>=64	R	84	EC	+	>=64	R
30	EC	+	>=64	R	85	KP	+	8	R
31	KP	+	>=64	R	86	EC	+	>=64	R
32	KP	+	>=64	R	87	EC	+	>=64	R
33	KP	+	>=64	R	88	EC	+	>=64	R
34	KP	+	>=64	R	89	EC	+	>=64	R
36	KP	+	>=64	R	93	EC	+	>=64	R
38	KP	+	>=64	R	94	EC	+	>=64	R
40	KP	+	>=64	R	95	KP	+	>=64	R
41	KP	+	>=64	R	102	KP	+	>=64	R
43	KP	+	>=64	R	103	EC	+	>=64	R
44	KP	+	>=64	R	104	EC	+	>=64	R
47	KP	+	>=64	R	105	KP	+	>=64	R
48	KP	+	>=64	R	107	EC	+	>=64	R
49	KP	+	>=64	R	108	EC	+	>=64	R
50	KP	+	>=64	R	109	EC	+	>=64	R
51	KP	+	>=64	R	112	EC	+	>=64	R
53	KP	+	>=64	R	116	EC	+	>=64	R
54	KP	+	>=64	R	117	EC	+	>=64	R
55	KP	+	>=64	R	118	EC	+	>=64	R
57	EC	+	>=64	R	119	EC	+	>=64	R
58	KP	+	>=64	R	120	EC	+	>=64	R
59	KP	+	>=64	R	121	KP	+	>=64	R
60	EC	+	>=64	R	122	EC	+	>=64	R
61	KP	+	>=64	R	123	EC	+	>=64	R
63	KP	+	>=64	R	124	EC	+	>=64	R
64	KP	+	>=64	R	126	EC	+	>=64	R
66	KP	+	>=64	R	129	KP	+	>=64	R
67	EC	+	>=64	R	130	KP	+	>=64	R
68	KP	+	>=64	R	133	KP	+	>=64	R
69	KP	+	>=64	R	135	KP	+	>=64	R
70	KP	+	>=64	R	145	KP	+	>=64	R
71	KP	+	>=64	R	147	KP	+	>=64	R
72	KP	+	>=64	R	149	EC	+	>=64	R
73	EC	+	>=64	R	151	KP	+	>=64	R
74	KP	+	>=64	R	154	KP	+	>=64	R
75	EC	+	>=64	R	156	EC	+	>=64	R

158	EC	+	>=64	R	267	KP	+	>=64	R
162	EC	+	>=64	R	268	EC	+	>=64	R
164	KP	+	>=64	R	270	KP	+	>=64	R
165	KP	+	>=64	R	271	KP	+	>=64	R
166	KP	+	>=64	R	272	KP	+	>=64	R
167	EC	+	>=64	R	274	KP	+	>=64	R
169	EC	+	>=64	R	275	KP	+	>=64	R
175	EC	+	>=64	R	276	KP	+	>=64	R
177	EC	+	>=64	R	277	EC	+	>=64	R
187	EC	+	>=64	R	279	EC	+	>=64	R
195	EC	+	>=64	R	280	KP	+	8	R
196	EC	+	>=64	R	281	KP	+	8	R
198	KP	+	>=64	R	282	KP	+	8	R
202	KP	+	>=64	R	283	KP	+	8	R
203	KP	+	>=64	R	285	KP	+	>=64	R
206	KP	+	>=64	R	286	KP	+	>=64	R
207	KP	+	>=64	R	287	KP	+	>=64	R
211	KP	+	>=64	R	288	KP	+	>=64	R
212	KP	+	>=64	R	289	KP	+	>=64	R
213	EC	+	>=64	R	291	KP	+	>=64	R
214	EC	+	>=64	R	292	KP	+	>=64	R
215	EC	+	>=64	R	293	KP	+	>=64	R
216	EC	+	>=64	R	294	KP	+	>=64	R
217	KP	+	>=64	R	295	KP	+	>=64	R
218	KP	+	>=64	R	296	KP	+	>=64	R
219	KP	+	>=64	R	298	KP	+	>=64	R
220	KP	+	>=64	R	304	KP	+	>=64	R
221	KP	+	>=64	R	305	KP	+	>=64	R
222	KP	+	>=64	R	306	KP	+	>=64	R
223	KP	+	>=64	R	307	KP	+	>=64	R
224	KP	+	>=64	R	308	KP	+	>=64	R
225	KP	+	>=64	R	309	KP	+	>=64	R
226	KP	+	>=64	R	310	KP	+	>=64	R
227	KP	+	>=64	R	311	KP	+	>=64	R
244	KP	+	>=64	R	312	EC	+	>=64	R
245	KP	+	>=64	R	313	EC	+	>=64	R
246	KP	+	>=64	R	314	KP	+	>=64	R
247	KP	+	>=64	R	315	KP	+	>=64	R
248	KP	+	>=64	R	316	KP	+	>=64	R
249	KP	+	>=64	R	317	KP	+	>=64	R
260	KP	+	>=64	R	318	EC	+	>=64	R
262	KP	+	>=64	R	322	KP	+	>=64	R
264	EC	+	>=64	R	323	KP	+	>=64	R

324	EC	+	>=64	R
325	EC	+	>=64	R
326	EC	+	>=64	R
327	EC	+	>=64	R
328	EC	+	>=64	R
329	EC	+	>=64	R
330	KP	+	>=64	R
331	KP	+	>=64	R
332	KP	+	>=64	R
333	KP	+	>=64	R
339	EC	+	>=64	R
346	KP	+	>=64	R
347	EC	+	>=64	R
350	EC	+	>=64	R
352	EC	+	>=64	R
357	EC	+	>=64	R
358	EC	+	>=64	R
360	EC	+	>=64	R
361	EC	+	>=64	R
363	EC	+	>=64	R
400	KP	+	>=64	R

CEFOXITIN

ID ^a	ORG ^b	ESBL ^c	AST ^d	
			M I C	
2	EC	+	<=4	S
3	KP	+	>=64	R
4	KP	+	>=64	R
8	KP	+	>=64	R
9	KP	+	>=64	R
11	EC	+	<=4	S
12	KP	+	<=4	S
14	KP	+	>=64	R
15	KP	+	>=64	R
17	KP	+	<=4	S
19	KP	+	8	S
23	EC	+	<=4	S
25	KP	+	8	S
27	KP	+	<=4	S
28	EC	+	<=4	S
29	KP	+	16	I
30	EC	+	8	S

31	KP	+	8	S
32	KP	+	<=4	S
33	KP	+	8	S
34	KP	+	8	S
36	KP	+	<=4	S
38	KP	+	8	S
40	KP	+	<=4	S
41	KP	+	<=4	S
43	KP	+	>=64	R
44	KP	+	16	I
47	KP	+	<=4	S
48	KP	+	<=4	S
49	KP	+	<=4	S
50	KP	+	<=4	S
51	KP	+	<=4	S
53	KP	+	<=4	S
54	KP	+	<=4	S
55	KP	+	<=4	S
57	EC	+	<=4	S
58	KP	+	<=4	S
59	KP	+	<=4	S
60	EC	+	16	I
61	KP	+	<=4	S
63	KP	+	<=4	S
64	KP	+	<=4	S
66	KP	+	<=4	S
67	EC	+	32	R
68	KP	+	<=4	S
69	KP	+	8	S
70	KP	+	<=4	S
71	KP	+	8	S
72	KP	+	8	S
73	EC	+	<=4	S
74	KP	+	<=4	S
75	EC	+	<=4	S
76	EC	+	<=4	S
78	KP	+	<=4	S
79	EC	+	32	R
80	KP	+	8	S
81	EC	+	<=4	S
83	EC	+	<=4	S
84	EC	+	<=4	S
85	KP	+	<=4	S

86	EC	+	<=4	S	177	EC	+	<=4	S
87	EC	+	<=4	S	187	EC	+	<=4	S
88	EC	+	<=4	S	195	EC	+	8	S
89	EC	+	<=4	S	196	EC	+	8	S
93	EC	+	<=4	S	198	KP	+	16	I
94	EC	+	<=4	S	202	KP	+	8	S
95	KP	+	32	R	203	KP	+	8	S
102	KP	+	32	R	206	KP	+	<=4	S
103	EC	+	<=4	S	207	KP	+	8	S
104	EC	+	<=4	S	211	KP	+	<=4	S
105	KP	+	<=4	S	212	KP	+	8	S
107	EC	+	<=4	S	213	EC	+	32	R
108	EC	+	<=4	S	214	EC	+	32	R
109	EC	+	<=4	S	215	EC	+	32	R
112	EC	+	<=4	S	216	EC	+	32	R
116	EC	+	<=4	S	217	KP	+	<=4	S
117	EC	+	<=4	S	218	KP	+	<=4	S
118	EC	+	<=4	S	219	KP	+	<=4	S
119	EC	+	<=4	S	220	KP	+	<=4	S
120	EC	+	<=4	S	221	KP	+	<=4	S
121	KP	+	<=4	S	222	KP	+	<=4	S
122	EC	+	16	I	223	KP	+	8	S
123	EC	+	<=4	S	224	KP	+	8	S
124	EC	+	<=4	S	225	KP	+	8	S
126	EC	+	>=64	R	226	KP	+	8	S
129	KP	+	>=64	R	227	KP	+	8	S
130	KP	+	>=64	R	244	KP	+	8	S
133	KP	+	<=4	S	245	KP	+	8	S
135	KP	+	>=64	R	246	KP	+	8	S
145	KP	+	32	R	247	KP	+	8	S
147	KP	+	>=64	R	248	KP	+	8	S
149	EC	+	<=4	S	249	KP	+	8	S
151	KP	+	<=4	S	260	KP	+	<=4	S
154	KP	+	<=4	S	262	KP	+	<=4	S
156	EC	+	<=4	S	264	EC	+	<=4	S
158	EC	+	16	I	267	KP	+	<=4	S
162	EC	+	<=4	S	268	EC	+	8	S
164	KP	+	<=4	S	270	KP	+	32	R
165	KP	+	>=64	R	271	KP	+	32	R
166	KP	+	<=4	S	272	KP	+	>=64	R
167	EC	+	8	S	274	KP	+	<=4	S
169	EC	+	8	S	275	KP	+	<=4	S
175	EC	+	<=4	S	276	KP	+	<=4	S

277	EC	+	16	I	332	KP	+	<=4	S
279	EC	+	16	I	333	KP	+	<=4	S
280	KP	+	<=4	S	339	EC	+	<=4	S
281	KP	+	<=4	S	346	KP	+	<=4	S
282	KP	+	<=4	S	347	EC	+	<=4	S
283	KP	+	<=4	S	350	EC	+	16	I
285	KP	+	32	R	352	EC	+	<=4	S
286	KP	+	32	R	357	EC	+	<=4	S
287	KP	+	32	R	358	EC	+	<=4	S
288	KP	+	32	R	360	EC	+	<=4	S
289	KP	+	32	R	361	EC	+	<=4	S
291	KP	+	16	I	363	EC	+	<=4	S
292	KP	+	16	I	400	KP	+	<=4	S
293	KP	+	16	I	CEFOTAXIME				
294	KP	+	16	I	ID^a	ORG^b	ESBL^c	AST^d	
295	KP	+	16	I					
296	KP	+	<=4	S				MIC	
298	KP	+	16	I	2	EC	+	>=64	R
304	KP	+	8	S	3	KP	+	>=64	R
305	KP	+	<=4	S	4	KP	+	>=64	R
306	KP	+	<=4	S	8	KP	+	>=64	R
307	KP	+	8	S	9	KP	+	>=64	R
308	KP	+	<=4	S	11	EC	+	>=64	R
309	KP	+	<=4	S	12	KP	+	>=64	R
310	KP	+	32	R	14	KP	+	>=64	R
311	KP	+	32	R	15	KP	+	>=64	R
312	EC	+	32	R	17	KP	+	>=64	R
313	EC	+	32	R	19	KP	+	>=64	R
314	KP	+	<=4	S	23	EC	+	>=64	R
315	KP	+	8	S	25	KP	+	>=64	R
316	KP	+	32	R	27	KP	+	>=64	R
317	KP	+	32	R	28	EC	+	>=64	R
318	EC	+	<=4	S	29	KP	+	>=64	R
322	KP	+	16	I	30	EC	+	>=64	R
323	KP	+	16	I	31	KP	+	>=64	R
324	EC	+	<=4	S	32	KP	+	>=64	R
325	EC	+	<=4	S	33	KP	+	>=64	R
326	EC	+	<=4	S	34	KP	+	>=64	R
327	EC	+	<=4	S	36	KP	+	>=64	R
328	EC	+	<=4	S	38	KP	+	>=64	R
329	EC	+	<=4	S	40	KP	+	>=64	R
330	KP	+	<=4	S	41	KP	+	>=64	R
331	KP	+	<=4	S					

43	KP	+	>=64	R	103	EC	+	>=64	R
44	KP	+	>=64	R	104	EC	+	>=64	R
47	KP	+	>=64	R	105	KP	+	>=64	R
48	KP	+	>=64	R	107	EC	+	>=64	R
49	KP	+	>=64	R	108	EC	+	>=64	R
50	KP	+	>=64	R	109	EC	+	>=64	R
51	KP	+	>=64	R	112	EC	+	>=64	R
53	KP	+	>=64	R	116	EC	+	>=64	R
54	KP	+	>=64	R	117	EC	+	>=64	R
55	KP	+	>=64	R	118	EC	+	>=64	R
57	EC	+	>=64	R	119	EC	+	>=64	R
58	KP	+	>=64	R	120	EC	+	>=64	R
59	KP	+	>=64	R	121	KP	+	>=64	R
60	EC	+	>=64	R	122	EC	+	>=64	R
61	KP	+	>=64	R	123	EC	+	>=64	R
63	KP	+	>=64	R	124	EC	+	>=64	R
64	KP	+	>=64	R	126	EC	+	>=64	R
66	KP	+	>=64	R	129	KP	+	>=64	R
67	EC	+	>=64	R	130	KP	+	>=64	R
68	KP	+	>=64	R	133	KP	+	>=64	R
69	KP	+	>=64	R	135	KP	+	>=64	R
70	KP	+	>=64	R	145	KP	+	>=64	R
71	KP	+	>=64	R	147	KP	+	>=64	R
72	KP	+	>=64	R	149	EC	+	>=64	R
73	EC	+	>=64	R	151	KP	+	>=64	R
74	KP	+	>=64	R	154	KP	+	>=64	R
75	EC	+	>=64	R	156	EC	+	>=64	R
76	EC	+	>=64	R	158	EC	+	>=64	R
78	KP	+	>=64	R	162	EC	+	>=64	R
79	EC	+	>=64	R	164	KP	+	>=64	R
80	KP	+	>=64	R	165	KP	+	>=64	R
81	EC	+	>=64	R	166	KP	+	>=64	R
83	EC	+	>=64	R	167	EC	+	32	R
84	EC	+	>=64	R	169	EC	+	32	R
85	KP	+	<=1	R	175	EC	+	>=64	R
86	EC	+	>=64	R	177	EC	+	>=64	R
87	EC	+	>=64	R	187	EC	+	>=64	R
88	EC	+	>=64	R	195	EC	+	>=64	R
89	EC	+	>=64	R	196	EC	+	32	R
93	EC	+	>=64	R	198	KP	+	>=64	R
94	EC	+	>=64	R	202	KP	+	>=64	R
95	KP	+	>=64	R	203	KP	+	>=64	R
102	KP	+	>=64	R	206	KP	+	>=64	R

207	KP	+	>=64	R	287	KP	+	>=64	R
211	KP	+	>=64	R	288	KP	+	>=64	R
212	KP	+	>=64	R	289	KP	+	>=64	R
213	EC	+	>=64	R	291	KP	+	>=64	R
214	EC	+	>=64	R	292	KP	+	>=64	R
215	EC	+	>=64	R	293	KP	+	>=64	R
216	EC	+	>=64	R	294	KP	+	>=64	R
217	KP	+	>=64	R	295	KP	+	>=64	R
218	KP	+	>=64	R	296	KP	+	>=64	R
219	KP	+	>=64	R	298	KP	+	>=64	R
220	KP	+	>=64	R	304	KP	+	>=64	R
221	KP	+	>=64	R	305	KP	+	>=64	R
222	KP	+	>=64	R	306	KP	+	>=64	R
223	KP	+	>=64	R	307	KP	+	>=64	R
224	KP	+	>=64	R	308	KP	+	>=64	R
225	KP	+	>=64	R	309	KP	+	>=64	R
226	KP	+	>=64	R	310	KP	+	>=64	R
227	KP	+	>=64	R	311	KP	+	>=64	R
244	KP	+	>=64	R	312	EC	+	>=64	R
245	KP	+	>=64	R	313	EC	+	>=64	R
246	KP	+	>=64	R	314	KP	+	>=64	R
247	KP	+	>=64	R	315	KP	+	>=64	R
248	KP	+	>=64	R	316	KP	+	>=64	R
249	KP	+	>=64	R	317	KP	+	>=64	R
260	KP	+	>=64	R	318	EC	+	>=64	R
262	KP	+	>=64	R	322	KP	+	>=64	R
264	EC	+	>=64	R	323	KP	+	>=64	R
267	KP	+	>=64	R	324	EC	+	>=64	R
268	EC	+	>=64	R	325	EC	+	>=64	R
270	KP	+	>=64	R	326	EC	+	>=64	R
271	KP	+	>=64	R	327	EC	+	>=64	R
272	KP	+	>=64	R	328	EC	+	>=64	R
274	KP	+	>=64	R	329	EC	+	>=64	R
275	KP	+	>=64	R	330	KP	+	>=64	R
276	KP	+	>=64	R	331	KP	+	>=64	R
277	EC	+	>=64	R	332	KP	+	>=64	R
279	EC	+	>=64	R	333	KP	+	>=64	R
280	KP	+	<=1	R	339	EC	+	>=64	R
281	KP	+	<=1	R	346	KP	+	>=64	R
282	KP	+	<=1	R	347	EC	+	>=64	R
283	KP	+	<=1	R	350	EC	+	>=64	R
285	KP	+	>=64	R	352	EC	+	>=64	R
286	KP	+	>=64	R	357	EC	+	>=64	R

358	EC	+	>=64	R
360	EC	+	>=64	R
361	EC	+	>=64	R
363	EC	+	>=64	R
400	KP	+	>=64	R

CEFTAZIDIME

ID ^a	ORG ^b	ESBL ^c	AST ^d	
				M I C
2	EC	+	16	R
3	KP	+	>=64	R
4	KP	+	>=64	R
8	KP	+	>=64	R
9	KP	+	>=64	R
11	EC	+	16	R
12	KP	+	>=64	R
14	KP	+	>=64	R
15	KP	+	>=64	R
17	KP	+	4	R
19	KP	+	>=64	R
23	EC	+	4	R
25	KP	+	>=64	R
27	KP	+	16	R
28	EC	+	16	R
29	KP	+	>=64	R
30	EC	+	16	R
31	KP	+	>=64	R
32	KP	+	>=64	R
33	KP	+	>=64	R
34	KP	+	>=64	R
36	KP	+	>=64	R
38	KP	+	>=64	R
40	KP	+	16	R
41	KP	+	>=64	R
43	KP	+	16	R
44	KP	+	>=64	R
47	KP	+	16	R
48	KP	+	16	R
49	KP	+	16	R
50	KP	+	16	R
51	KP	+	16	R
53	KP	+	16	R

54	KP	+	16	R
55	KP	+	16	R
57	EC	+	16	R
58	KP	+	4	R
59	KP	+	16	R
60	EC	+	16	R
61	KP	+	>=64	R
63	KP	+	>=64	R
64	KP	+	>=64	R
66	KP	+	>=64	R
67	EC	+	16	R
68	KP	+	>=64	R
69	KP	+	16	R
70	KP	+	>=64	R
71	KP	+	>=64	R
72	KP	+	>=64	R
73	EC	+	16	R
74	KP	+	16	R
75	EC	+	16	R
76	EC	+	16	R
78	KP	+	>=64	R
79	EC	+	16	R
80	KP	+	>=64	R
81	EC	+	16	R
83	EC	+	16	R
84	EC	+	16	R
85	KP	+	<=1	R
86	EC	+	16	R
87	EC	+	>=64	R
88	EC	+	16	R
89	EC	+	16	R
93	EC	+	16	R
94	EC	+	16	R
95	KP	+	16	R
102	KP	+	>=64	R
103	EC	+	16	R
104	EC	+	16	R
105	KP	+	16	R
107	EC	+	16	R
108	EC	+	16	R
109	EC	+	16	R
112	EC	+	16	R
116	EC	+	4	R

117	EC	+	4	R	218	KP	+	16	R
118	EC	+	4	R	219	KP	+	16	R
119	EC	+	4	R	220	KP	+	16	R
120	EC	+	4	R	221	KP	+	16	R
121	KP	+	>=64	R	222	KP	+	16	R
122	EC	+	16	R	223	KP	+	16	R
123	EC	+	4	R	224	KP	+	16	R
124	EC	+	16	R	225	KP	+	16	R
126	EC	+	>=64	R	226	KP	+	16	R
129	KP	+	>=64	R	227	KP	+	16	R
130	KP	+	>=64	R	244	KP	+	16	R
133	KP	+	>=64	R	245	KP	+	16	R
135	KP	+	>=64	R	246	KP	+	16	R
145	KP	+	16	R	247	KP	+	16	R
147	KP	+	>=64	R	248	KP	+	16	R
149	EC	+	16	R	249	KP	+	16	R
151	KP	+	16	R	260	KP	+	16	R
154	KP	+	>=64	R	262	KP	+	>=64	R
156	EC	+	16	R	264	EC	+	16	R
158	EC	+	16	R	267	KP	+	16	R
162	EC	+	16	R	268	EC	+	>=64	R
164	KP	+	16	R	270	KP	+	16	R
165	KP	+	>=64	R	271	KP	+	16	R
166	KP	+	16	R	272	KP	+	>=64	R
167	EC	+	4	R	274	KP	+	16	R
169	EC	+	4	R	275	KP	+	16	R
175	EC	+	16	R	276	KP	+	16	R
177	EC	+	4	R	277	EC	+	16	R
187	EC	+	>=64	R	279	EC	+	16	R
195	EC	+	16	R	280	KP	+	<=1	R
196	EC	+	4	R	281	KP	+	<=1	R
198	KP	+	>=64	R	282	KP	+	<=1	R
202	KP	+	>=64	R	283	KP	+	<=1	R
203	KP	+	>=64	R	285	KP	+	16	R
206	KP	+	>=64	R	286	KP	+	16	R
207	KP	+	>=64	R	287	KP	+	16	R
211	KP	+	>=64	R	288	KP	+	16	R
212	KP	+	32	R	289	KP	+	16	R
213	EC	+	16	R	291	KP	+	>=64	R
214	EC	+	16	R	292	KP	+	>=64	R
215	EC	+	16	R	293	KP	+	>=64	R
216	EC	+	16	R	294	KP	+	>=64	R
217	KP	+	16	R	295	KP	+	>=64	R

296	KP	+	16	R					
298	KP	+	>=64	R					
304	KP	+	>=64	R					
305	KP	+	4	R					
306	KP	+	4	R					
307	KP	+	16	R					
308	KP	+	>=64	R					
309	KP	+	>=64	R					
310	KP	+	16	R					
311	KP	+	16	R					
312	EC	+	16	R					
313	EC	+	16	R					
314	KP	+	16	R					
315	KP	+	16	R					
316	KP	+	16	R					
317	KP	+	16	R					
318	EC	+	16	R					
322	KP	+	>=64	R					
323	KP	+	>=64	R					
324	EC	+	4	R					
325	EC	+	4	R					
326	EC	+	4	R					
327	EC	+	4	R					
328	EC	+	4	R					
329	EC	+	4	R					
330	KP	+	16	R					
331	KP	+	16	R					
332	KP	+	16	R					
333	KP	+	16	R					
339	EC	+	16	R					
346	KP	+	>=64	R					
347	EC	+	16	R					
350	EC	+	16	R					
352	EC	+	16	R					
357	EC	+	16	R					
358	EC	+	4	R					
360	EC	+	16	R					
361	EC	+	16	R					
363	EC	+	16	R					
400	KP	+	>=64	R					
CEFEPIME									
ID^a	ORG^b	ESBL^c	AST^d						
2	EC	+	2	R					
3	KP	+	16	R					
4	KP	+	16	R					
8	KP	+	16	R					
9	KP	+	16	R					
11	EC	+	2	R					
12	KP	+	>=64	R					
14	KP	+	16	R					
15	KP	+	16	R					
17	KP	+	2	R					
19	KP	+	2	R					
23	EC	+	2	R					
25	KP	+	2	R					
27	KP	+	2	R					
28	EC	+	2	R					
29	KP	+	16	R					
30	EC	+	>=64	R					
31	KP	+	2	R					
32	KP	+	16	R					
33	KP	+	2	R					
34	KP	+	2	R					
36	KP	+	>=64	R					
38	KP	+	2	R					
40	KP	+	2	R					
41	KP	+	8	R					
43	KP	+	4	R					
44	KP	+	16	R					
47	KP	+	2	R					
48	KP	+	2	R					
49	KP	+	2	R					
50	KP	+	8	R					
51	KP	+	2	R					
53	KP	+	2	R					
54	KP	+	2	R					
55	KP	+	2	R					
57	EC	+	2	R					
58	KP	+	<=1	R					
59	KP	+	2	R					
60	EC	+	8	R					
61	KP	+	2	R					
63	KP	+	2	R					

64	KP	+	2	R	126	EC	+	>=64	R
66	KP	+	2	R	129	KP	+	16	R
67	EC	+	8	R	130	KP	+	16	R
68	KP	+	2	R	133	KP	+	32	R
69	KP	+	2	R	135	KP	+	16	R
70	KP	+	2	R	145	KP	+	2	R
71	KP	+	>=64	R	147	KP	+	>=64	R
72	KP	+	>=64	R	149	EC	+	2	R
73	EC	+	2	R	151	KP	+	2	R
74	KP	+	2	R	154	KP	+	16	R
75	EC	+	2	R	156	EC	+	2	R
76	EC	+	2	R	158	EC	+	8	R
78	KP	+	8	R	162	EC	+	2	R
79	EC	+	8	R	164	KP	+	2	R
80	KP	+	16	R	165	KP	+	16	R
81	EC	+	4	R	166	KP	+	2	R
83	EC	+	8	R	167	EC	+	2	R
84	EC	+	8	R	169	EC	+	2	R
85	KP	+	<=1	R	175	EC	+	>=64	R
86	EC	+	8	R	177	EC	+	4	R
87	EC	+	16	R	187	EC	+	16	R
88	EC	+	8	R	195	EC	+	32	R
89	EC	+	8	R	196	EC	+	2	R
93	EC	+	>=64	R	198	KP	+	8	R
94	EC	+	4	R	202	KP	+	16	R
95	KP	+	2	R	203	KP	+	2	R
102	KP	+	32	R	206	KP	+	>=64	R
103	EC	+	2	R	207	KP	+	>=64	R
104	EC	+	2	R	211	KP	+	>=64	R
105	KP	+	2	R	212	KP	+	4	R
107	EC	+	2	R	213	EC	+	8	R
108	EC	+	2	R	214	EC	+	8	R
109	EC	+	2	R	215	EC	+	8	R
112	EC	+	2	R	216	EC	+	8	R
116	EC	+	4	R	217	KP	+	2	R
117	EC	+	4	R	218	KP	+	2	R
118	EC	+	4	R	219	KP	+	2	R
119	EC	+	4	R	220	KP	+	2	R
120	EC	+	4	R	221	KP	+	2	R
121	KP	+	16	R	222	KP	+	2	R
122	EC	+	2	R	223	KP	+	4	R
123	EC	+	4	R	224	KP	+	4	R
124	EC	+	2	R	225	KP	+	4	R

226	KP	+	4	R	310	KP	+	32	R
227	KP	+	4	R	311	KP	+	32	R
244	KP	+	2	R	312	EC	+	8	R
245	KP	+	2	R	313	EC	+	8	R
246	KP	+	2	R	314	KP	+	2	R
247	KP	+	2	R	315	KP	+	4	R
248	KP	+	2	R	316	KP	+	32	R
249	KP	+	2	R	317	KP	+	32	R
260	KP	+	2	R	318	EC	+	2	R
262	KP	+	2	R	322	KP	+	8	R
264	EC	+	2	R	323	KP	+	8	R
267	KP	+	2	R	324	EC	+	4	R
268	EC	+	>=64	R	325	EC	+	4	R
270	KP	+	8	R	326	EC	+	4	R
271	KP	+	8	R	327	EC	+	2	R
272	KP	+	16	R	328	EC	+	2	R
274	KP	+	2	R	329	EC	+	2	R
275	KP	+	2	R	330	KP	+	2	R
276	KP	+	2	R	331	KP	+	2	R
277	EC	+	2	R	332	KP	+	2	R
279	EC	+	2	R	333	KP	+	2	R
280	KP	+	<=1	R	339	EC	+	4	R
281	KP	+	<=1	R	346	KP	+	4	R
282	KP	+	<=1	R	347	EC	+	>=64	R
283	KP	+	<=1	R	350	EC	+	4	R
285	KP	+	2	R	352	EC	+	8	R
286	KP	+	2	R	357	EC	+	>=64	R
287	KP	+	2	R	358	EC	+	4	R
288	KP	+	2	R	360	EC	+	>=64	R
289	KP	+	2	R	361	EC	+	2	R
291	KP	+	16	R	363	EC	+	2	R
292	KP	+	16	R	400	KP	+	4	R
293	KP	+	16	R	IMIPENEM				
294	KP	+	16	R	ID^a	ORG^b	ESBL^c	AST^d	
295	KP	+	16	R					
296	KP	+	2	R				MIC	
298	KP	+	16	R	2	EC	+	<=1	S
304	KP	+	>=64	R	3	KP	+	<=1	S
305	KP	+	2	R	4	KP	+	<=1	S
306	KP	+	2	R	8	KP	+	<=1	S
307	KP	+	2	R	9	KP	+	<=1	S
308	KP	+	8	R	11	EC	+	<=1	S
309	KP	+	8	R	12	KP	+	<=1	S

14	KP	+	<=1	S	74	KP	+	<=1	S
15	KP	+	<=1	S	75	EC	+	<=1	S
17	KP	+	<=1	S	76	EC	+	<=1	S
19	KP	+	<=1	S	78	KP	+	<=1	S
23	EC	+	<=1	S	79	EC	+	<=1	S
25	KP	+	<=1	S	80	KP	+	<=1	S
27	KP	+	<=1	S	81	EC	+	<=1	S
28	EC	+	<=1	S	83	EC	+	<=1	S
29	KP	+	<=1	S	84	EC	+	<=1	S
30	EC	+	<=1	S	85	KP	+	<=1	S
31	KP	+	<=1	S	86	EC	+	<=1	S
32	KP	+	<=1	S	87	EC	+	<=1	S
33	KP	+	<=1	S	88	EC	+	<=1	S
34	KP	+	<=1	S	89	EC	+	<=1	S
36	KP	+	<=1	S	93	EC	+	<=1	S
38	KP	+	<=1	S	94	EC	+	<=1	S
40	KP	+	<=1	S	95	KP	+	<=1	S
41	KP	+	<=1	S	102	KP	+	<=1	S
43	KP	+	<=1	S	103	EC	+	<=1	S
44	KP	+	<=1	S	104	EC	+	<=1	S
47	KP	+	<=1	S	105	KP	+	<=1	S
48	KP	+	<=1	S	107	EC	+	<=1	S
49	KP	+	<=1	S	108	EC	+	<=1	S
50	KP	+	<=1	S	109	EC	+	<=1	S
51	KP	+	<=1	S	112	EC	+	<=1	S
53	KP	+	<=1	S	116	EC	+	<=1	S
54	KP	+	<=1	S	117	EC	+	<=1	S
55	KP	+	<=1	S	118	EC	+	<=1	S
57	EC	+	<=1	S	119	EC	+	<=1	S
58	KP	+	<=1	S	120	EC	+	<=1	S
59	KP	+	<=1	S	121	KP	+	<=1	S
60	EC	+	<=1	S	122	EC	+	<=1	S
61	KP	+	<=1	S	123	EC	+	<=1	S
63	KP	+	<=1	S	124	EC	+	<=1	S
64	KP	+	<=1	S	126	EC	+	<=1	R
66	KP	+	<=1	S	129	KP	+	<=1	S
67	EC	+	<=1	S	130	KP	+	<=1	S
68	KP	+	<=1	S	133	KP	+	<=1	S
69	KP	+	<=1	S	135	KP	+	<=1	S
70	KP	+	<=1	S	145	KP	+	<=1	S
71	KP	+	<=1	S	147	KP	+	<=1	S
72	KP	+	<=1	S	149	EC	+	<=1	S
73	EC	+	<=1	S	151	KP	+	<=1	S

154	KP	+	<=1	S	262	KP	+	<=1	S
156	EC	+	<=1	S	264	EC	+	<=1	S
158	EC	+	<=1	S	267	KP	+	<=1	S
162	EC	+	<=1	S	268	EC	+	<=1	R
164	KP	+	<=1	S	270	KP	+	<=1	S
165	KP	+	<=1	S	271	KP	+	<=1	S
166	KP	+	<=1	S	272	KP	+	<=1	S
167	EC	+	<=1	S	274	KP	+	<=1	S
169	EC	+	<=1	S	275	KP	+	<=1	S
175	EC	+	<=1	S	276	KP	+	<=1	S
177	EC	+	<=1	S	277	EC	+	<=1	S
187	EC	+	<=1	S	279	EC	+	<=1	S
195	EC	+	<=1	S	280	KP	+	<=1	S
196	EC	+	<=1	S	281	KP	+	<=1	S
198	KP	+	<=1	S	282	KP	+	<=1	S
202	KP	+	<=1	S	283	KP	+	<=1	S
203	KP	+	<=1	S	285	KP	+	<=1	S
206	KP	+	<=1	S	286	KP	+	<=1	S
207	KP	+	<=1	S	287	KP	+	<=1	S
211	KP	+	<=1	S	288	KP	+	<=1	S
212	KP	+	<=1	S	289	KP	+	<=1	S
213	EC	+	<=1	S	291	KP	+	<=1	S
214	EC	+	<=1	S	292	KP	+	<=1	S
215	EC	+	<=1	S	293	KP	+	<=1	S
216	EC	+	<=1	S	294	KP	+	<=1	S
217	KP	+	<=1	S	295	KP	+	<=1	S
218	KP	+	<=1	S	296	KP	+	<=1	S
219	KP	+	<=1	S	298	KP	+	<=1	S
220	KP	+	<=1	S	304	KP	+	<=1	S
221	KP	+	<=1	S	305	KP	+	<=1	S
222	KP	+	<=1	S	306	KP	+	<=1	S
223	KP	+	<=1	S	307	KP	+	<=1	S
224	KP	+	<=1	S	308	KP	+	<=1	S
225	KP	+	<=1	S	309	KP	+	<=1	S
226	KP	+	<=1	S	310	KP	+	<=1	S
227	KP	+	<=1	S	311	KP	+	<=1	S
244	KP	+	<=1	S	312	EC	+	<=1	S
245	KP	+	<=1	S	313	EC	+	<=1	S
246	KP	+	<=1	S	314	KP	+	<=1	S
247	KP	+	<=1	S	315	KP	+	<=1	S
248	KP	+	<=1	S	316	KP	+	<=1	S
249	KP	+	<=1	S	317	KP	+	<=1	S
260	KP	+	<=1	S	318	EC	+	<=1	S

322	KP	+	<=1	S	30	EC	+	8	S
323	KP	+	<=1	S	31	KP	+	<=2	S
324	EC	+	<=1	S	32	KP	+	4	S
325	EC	+	<=1	S	33	KP	+	<=2	S
326	EC	+	<=1	S	34	KP	+	<=2	S
327	EC	+	<=1	S	36	KP	+	16	S
328	EC	+	<=1	S	38	KP	+	<=2	S
329	EC	+	<=1	S	40	KP	+	<=2	S
330	KP	+	<=1	S	41	KP	+	<=2	S
331	KP	+	<=1	S	43	KP	+	<=2	S
332	KP	+	<=1	S	44	KP	+	<=2	S
333	KP	+	<=1	S	47	KP	+	<=2	S
339	EC	+	<=1	S	48	KP	+	<=2	S
346	KP	+	<=1	S	49	KP	+	<=2	S
347	EC	+	<=1	S	50	KP	+	<=2	S
350	EC	+	<=1	S	51	KP	+	<=2	S
352	EC	+	<=1	S	53	KP	+	<=2	S
357	EC	+	<=1	S	54	KP	+	<=2	S
358	EC	+	<=1	S	55	KP	+	4	S
360	EC	+	<=1	S	57	EC	+	<=2	S
361	EC	+	<=1	S	58	KP	+	<=2	S
363	EC	+	<=1	S	59	KP	+	<=2	S
400	KP	+	<=1	S	60	EC	+	<=2	S
AMIKACIN					61	KP	+	16	S
ID^a	ORG^b	ESBL^c	AST^d		63	KP	+	16	S
			MIC		64	KP	+	16	S
2	EC	+	8	S	66	KP	+	16	S
3	KP	+	32	I	67	EC	+	32	I
4	KP	+	32	I	68	KP	+	16	S
8	KP	+	32	I	69	KP	+	8	S
9	KP	+	32	I	70	KP	+	16	S
11	EC	+	4	S	71	KP	+	<=2	S
12	KP	+	16	S	72	KP	+	<=2	S
14	KP	+	32	I	73	EC	+	<=2	S
15	KP	+	32	I	74	KP	+	<=2	S
17	KP	+	<=2	S	75	EC	+	<=2	S
19	KP	+	<=2	S	76	EC	+	<=2	S
23	EC	+	4	S	78	KP	+	<=2	S
25	KP	+	<=2	S	79	EC	+	16	S
27	KP	+	<=2	S	80	KP	+	>=64	R
28	EC	+	4	S	81	EC	+	<=2	S
29	KP	+	<=2	S	83	EC	+	8	S
					84	EC	+	8	S

85	KP	+	<=2	S	175	EC	+	16	S
86	EC	+	8	S	177	EC	+	<=2	S
87	EC	+	16	S	187	EC	+	16	S
88	EC	+	8	S	195	EC	+	4	S
89	EC	+	8	S	196	EC	+	<=2	S
93	EC	+	16	S	198	KP	+	16	S
94	EC	+	8	S	202	KP	+	<=2	S
95	KP	+	<=2	S	203	KP	+	<=2	S
102	KP	+	8	S	206	KP	+	<=2	S
103	EC	+	<=2	S	207	KP	+	<=2	S
104	EC	+	<=2	S	211	KP	+	<=2	S
105	KP	+	<=2	S	212	KP	+	8	S
107	EC	+	<=2	S	213	EC	+	16	S
108	EC	+	<=2	S	214	EC	+	16	S
109	EC	+	<=2	S	215	EC	+	16	S
112	EC	+	<=2	S	216	EC	+	16	S
116	EC	+	<=2	S	217	KP	+	<=2	S
117	EC	+	<=2	S	218	KP	+	<=2	S
118	EC	+	<=2	S	219	KP	+	<=2	S
119	EC	+	<=2	S	220	KP	+	<=2	S
120	EC	+	<=2	S	221	KP	+	<=2	S
121	KP	+	4	S	222	KP	+	<=2	S
122	EC	+	<=2	S	223	KP	+	8	S
123	EC	+	<=2	S	224	KP	+	8	S
124	EC	+	<=2	S	225	KP	+	8	S
126	EC	+	4	S	226	KP	+	8	S
129	KP	+	32	I	227	KP	+	8	S
130	KP	+	32	I	244	KP	+	<=2	S
133	KP	+	16	S	245	KP	+	<=2	S
135	KP	+	32	I	246	KP	+	<=2	S
145	KP	+	4	S	247	KP	+	<=2	S
147	KP	+	4	S	248	KP	+	<=2	S
149	EC	+	4	S	249	KP	+	<=2	S
151	KP	+	<=2	S	260	KP	+	<=2	S
154	KP	+	4	S	262	KP	+	16	S
156	EC	+	4	S	264	EC	+	<=2	S
158	EC	+	16	S	267	KP	+	<=2	S
162	EC	+	<=2	S	268	EC	+	4	S
164	KP	+	<=2	S	270	KP	+	<=2	S
165	KP	+	32	I	271	KP	+	<=2	S
166	KP	+	<=2	S	272	KP	+	32	I
167	EC	+	<=2	S	274	KP	+	4	S
169	EC	+	<=2	S	275	KP	+	4	S

276	KP	+	4	S	331	KP	+	<=2	S
277	EC	+	<=2	S	332	KP	+	<=2	S
279	EC	+	<=2	S	333	KP	+	<=2	S
280	KP	+	<=2	S	339	EC	+	16	S
281	KP	+	<=2	S	346	KP	+	<=2	S
282	KP	+	<=2	S	347	EC	+	8	S
283	KP	+	<=2	S	350	EC	+	8	S
285	KP	+	<=2	S	352	EC	+	8	S
286	KP	+	<=2	S	357	EC	+	16	S
287	KP	+	<=2	S	358	EC	+	<=2	S
288	KP	+	4	S	360	EC	+	<=2	S
289	KP	+	4	S	361	EC	+	16	S
291	KP	+	<=2	S	363	EC	+	<=2	S
292	KP	+	<=2	S	400	KP	+	<=2	S
293	KP	+	<=2	S	GENTAMICIN				
294	KP	+	<=2	S	ID^a	ORG^b	ESBL^c	AST^d	
295	KP	+	<=2	S				MIC	
296	KP	+	<=2	S	2	EC	+	>=16	R
298	KP	+	<=2	S	3	KP	+	>=16	R
304	KP	+	<=2	S	4	KP	+	>=16	R
305	KP	+	<=2	S	8	KP	+	>=16	R
306	KP	+	<=2	S	9	KP	+	>=16	R
307	KP	+	8	S	11	EC	+	<=1	S
308	KP	+	<=2	S	12	KP	+	>=16	R
309	KP	+	<=2	S	14	KP	+	>=16	R
310	KP	+	8	S	15	KP	+	>=16	R
311	KP	+	8	S	17	KP	+	>=16	R
312	EC	+	16	S	19	KP	+	>=16	R
313	EC	+	16	S	23	EC	+	<=1	S
314	KP	+	<=2	S	25	KP	+	>=16	R
315	KP	+	8	S	27	KP	+	>=16	R
316	KP	+	8	S	28	EC	+	>=16	R
317	KP	+	8	S	29	KP	+	>=16	R
318	EC	+	4	S	30	EC	+	>=16	R
322	KP	+	16	S	31	KP	+	>=16	R
323	KP	+	16	S	32	KP	+	>=16	R
324	EC	+	<=2	S	33	KP	+	>=16	R
325	EC	+	<=2	S	34	KP	+	>=16	R
326	EC	+	<=2	S	36	KP	+	>=16	R
327	EC	+	4	S	38	KP	+	>=16	R
328	EC	+	4	S	40	KP	+	>=16	R
329	EC	+	4	S	41	KP	+	>=16	R
330	KP	+	<=2	S					

43	KP	+	≥ 16	R	103	EC	+	≥ 16	R
44	KP	+	≥ 16	R	104	EC	+	≥ 16	R
47	KP	+	≥ 16	R	105	KP	+	≤ 1	S
48	KP	+	≥ 16	R	107	EC	+	≥ 16	R
49	KP	+	≥ 16	R	108	EC	+	≥ 16	R
50	KP	+	≥ 16	R	109	EC	+	≥ 16	R
51	KP	+	≥ 16	R	112	EC	+	≥ 16	R
53	KP	+	≤ 1	S	116	EC	+	≥ 16	R
54	KP	+	≤ 1	S	117	EC	+	≥ 16	R
55	KP	+	≥ 16	R	118	EC	+	≥ 16	R
57	EC	+	≥ 16	R	119	EC	+	≥ 16	R
58	KP	+	≥ 16	R	120	EC	+	≥ 16	R
59	KP	+	≥ 16	R	121	KP	+	≥ 16	R
60	EC	+	≤ 1	S	122	EC	+	≤ 1	S
61	KP	+	≥ 16	R	123	EC	+	≥ 16	R
63	KP	+	≥ 16	R	124	EC	+	≥ 16	R
64	KP	+	≥ 16	R	126	EC	+	≥ 16	R
66	KP	+	≥ 16	R	129	KP	+	≥ 16	R
67	EC	+	≥ 16	R	130	KP	+	≥ 16	R
68	KP	+	≥ 16	R	133	KP	+	≤ 1	S
69	KP	+	≥ 16	R	135	KP	+	≥ 16	R
70	KP	+	≥ 16	R	145	KP	+	≥ 16	R
71	KP	+	≥ 16	R	147	KP	+	≥ 16	R
72	KP	+	≥ 16	R	149	EC	+	≥ 16	R
73	EC	+	≥ 16	R	151	KP	+	≥ 16	R
74	KP	+	≥ 16	R	154	KP	+	≥ 16	R
75	EC	+	≥ 16	R	156	EC	+	≥ 16	R
76	EC	+	≥ 16	R	158	EC	+	≥ 16	R
78	KP	+	≥ 16	R	162	EC	+	≥ 16	R
79	EC	+	≥ 16	R	164	KP	+	≤ 1	S
80	KP	+	≥ 16	R	165	KP	+	≥ 16	R
81	EC	+	≤ 1	S	166	KP	+	≤ 1	S
83	EC	+	≥ 16	R	167	EC	+	≥ 16	R
84	EC	+	≥ 16	R	169	EC	+	≥ 16	R
85	KP	+	≥ 16	R	175	EC	+	≥ 16	R
86	EC	+	≥ 16	R	177	EC	+	≤ 1	S
87	EC	+	≥ 16	R	187	EC	+	≥ 16	R
88	EC	+	≥ 16	R	195	EC	+	≥ 16	R
89	EC	+	≥ 16	R	196	EC	+	≥ 16	R
93	EC	+	≥ 16	R	198	KP	+	≥ 16	R
94	EC	+	≥ 16	R	202	KP	+	≥ 16	R
95	KP	+	≥ 16	R	203	KP	+	≥ 16	R
102	KP	+	≥ 16	R	206	KP	+	≥ 16	R

207	KP	+	>=16	R	287	KP	+	>=16	R
211	KP	+	>=16	R	288	KP	+	>=16	R
212	KP	+	>=16	R	289	KP	+	>=16	R
213	EC	+	>=16	R	291	KP	+	>=16	R
214	EC	+	>=16	R	292	KP	+	>=16	R
215	EC	+	>=16	R	293	KP	+	>=16	R
216	EC	+	>=16	R	294	KP	+	>=16	R
217	KP	+	<=1	S	295	KP	+	>=16	R
218	KP	+	<=1	S	296	KP	+	>=16	R
219	KP	+	<=1	S	298	KP	+	>=16	R
220	KP	+	<=1	S	304	KP	+	>=16	R
221	KP	+	<=1	S	305	KP	+	>=16	R
222	KP	+	<=1	S	306	KP	+	>=16	R
223	KP	+	2	S	307	KP	+	>=16	R
224	KP	+	2	S	308	KP	+	>=16	R
225	KP	+	2	S	309	KP	+	>=16	R
226	KP	+	2	S	310	KP	+	>=16	R
227	KP	+	2	S	311	KP	+	>=16	R
244	KP	+	>=16	R	312	EC	+	>=16	R
245	KP	+	>=16	R	313	EC	+	>=16	R
246	KP	+	>=16	R	314	KP	+	<=1	S
247	KP	+	>=16	R	315	KP	+	2	S
248	KP	+	>=16	R	316	KP	+	>=16	R
249	KP	+	>=16	R	317	KP	+	>=16	R
260	KP	+	<=1	S	318	EC	+	<=1	S
262	KP	+	>=16	R	322	KP	+	>=16	R
264	EC	+	>=16	R	323	KP	+	>=16	R
267	KP	+	<=1	S	324	EC	+	>=16	R
268	EC	+	>=16	R	325	EC	+	>=16	R
270	KP	+	>=16	R	326	EC	+	>=16	R
271	KP	+	>=16	R	327	EC	+	<=1	S
272	KP	+	>=16	R	328	EC	+	<=1	S
274	KP	+	>=16	R	329	EC	+	<=1	S
275	KP	+	>=16	R	330	KP	+	<=1	S
276	KP	+	>=16	R	331	KP	+	<=1	S
277	EC	+	<=1	S	332	KP	+	>=16	R
279	EC	+	<=1	S	333	KP	+	>=16	R
280	KP	+	>=16	R	339	EC	+	>=16	R
281	KP	+	>=16	R	346	KP	+	>=16	R
282	KP	+	>=16	R	347	EC	+	>=16	R
283	KP	+	>=16	R	350	EC	+	>=16	R
285	KP	+	>=16	R	352	EC	+	>=16	R
286	KP	+	>=16	R	357	EC	+	>=16	R

358	EC	+	>=16	R	55	KP	+	>=4	R
360	EC	+	2	S	57	EC	+	>=4	R
361	EC	+	>=16	R	58	KP	+	>=4	R
363	EC	+	>=16	R	59	KP	+	0.5	S
400	KP	+	>=16	R	60	EC	+	>=4	R
CIPROFLOXACIN					61	KP	+	<=0.25	S
ID^a	ORG^b	ESBL^c	AST^d		63	KP	+	<=0.25	S
			MIC		64	KP	+	<=0.25	S
2	EC	+	>=4	R	66	KP	+	<=0.25	S
3	KP	+	>=4	R	67	EC	+	>=4	R
4	KP	+	>=4	R	68	KP	+	<=0.25	S
8	KP	+	>=4	R	69	KP	+	>=4	R
9	KP	+	>=4	R	70	KP	+	<=0.25	S
11	EC	+	>=4	R	71	KP	+	>=4	R
12	KP	+	>=4	R	72	KP	+	>=4	R
14	KP	+	>=4	R	73	EC	+	>=4	R
15	KP	+	>=4	R	74	KP	+	<=0.25	S
17	KP	+	>=4	R	75	EC	+	>=4	R
19	KP	+	>=4	R	76	EC	+	>=4	R
23	EC	+	>=4	R	78	KP	+	>=4	R
25	KP	+	>=4	R	79	EC	+	>=4	R
27	KP	+	<=0.25	S	80	KP	+	>=4	R
28	EC	+	>=4	R	81	EC	+	<=0.25	S
29	KP	+	>=4	R	83	EC	+	>=4	R
30	EC	+	>=4	R	84	EC	+	>=4	R
31	KP	+	>=4	R	85	KP	+	>=4	R
32	KP	+	>=4	R	86	EC	+	>=4	R
33	KP	+	>=4	R	87	EC	+	>=4	R
34	KP	+	>=4	R	88	EC	+	>=4	R
36	KP	+	>=4	R	89	EC	+	>=4	R
38	KP	+	>=4	R	93	EC	+	>=4	R
40	KP	+	0.5	S	94	EC	+	>=4	R
41	KP	+	>=4	R	95	KP	+	1	S
43	KP	+	>=4	R	102	KP	+	>=4	R
44	KP	+	>=4	R	103	EC	+	>=4	R
47	KP	+	0.5	S	104	EC	+	>=4	R
48	KP	+	0.5	S	105	KP	+	>=4	R
49	KP	+	0.5	S	107	EC	+	>=4	R
50	KP	+	2	I	108	EC	+	>=4	R
51	KP	+	0.5	S	109	EC	+	>=4	R
53	KP	+	>=4	R	112	EC	+	>=4	R
54	KP	+	>=4	R	116	EC	+	1	S
					117	EC	+	1	S

118	EC	+	1	S	219	KP	+	≥ 4	R
119	EC	+	1	S	220	KP	+	≥ 4	R
120	EC	+	1	S	221	KP	+	≥ 4	R
121	KP	+	≥ 4	R	222	KP	+	≥ 4	R
122	EC	+	≥ 4	R	223	KP	+	0.5	S
123	EC	+	1	S	224	KP	+	0.5	S
124	EC	+	≥ 4	R	225	KP	+	0.5	S
126	EC	+	≥ 4	R	226	KP	+	0.5	S
129	KP	+	≥ 4	R	227	KP	+	0.5	S
130	KP	+	≥ 4	R	244	KP	+	≥ 4	R
133	KP	+	≥ 4	R	245	KP	+	≥ 4	R
135	KP	+	≥ 4	R	246	KP	+	≥ 4	R
145	KP	+	≥ 4	R	247	KP	+	≥ 4	R
147	KP	+	≥ 4	R	248	KP	+	≥ 4	R
149	EC	+	≥ 4	R	249	KP	+	≥ 4	R
151	KP	+	0.5	S	260	KP	+	≥ 4	R
154	KP	+	≥ 4	R	262	KP	+	≤ 0.25	S
156	EC	+	≥ 4	R	264	EC	+	≥ 4	R
158	EC	+	≥ 4	R	267	KP	+	≥ 4	R
162	EC	+	≥ 4	R	268	EC	+	≥ 4	R
164	KP	+	≥ 4	R	270	KP	+	≥ 4	R
165	KP	+	≥ 4	R	271	KP	+	≥ 4	R
166	KP	+	≥ 4	R	272	KP	+	≥ 4	R
167	EC	+	≥ 4	R	274	KP	+	≥ 4	R
169	EC	+	≥ 4	R	275	KP	+	≥ 4	R
175	EC	+	≥ 4	R	276	KP	+	≥ 4	R
177	EC	+	2	I	277	EC	+	≥ 4	R
187	EC	+	≥ 4	R	279	EC	+	≥ 4	R
195	EC	+	≥ 4	R	280	KP	+	≥ 4	R
196	EC	+	≥ 4	R	281	KP	+	≥ 4	R
198	KP	+	≥ 4	R	282	KP	+	≥ 4	R
202	KP	+	≥ 4	R	283	KP	+	≥ 4	R
203	KP	+	≥ 4	R	285	KP	+	1	S
206	KP	+	≥ 4	R	286	KP	+	1	S
207	KP	+	≥ 4	R	287	KP	+	1	S
211	KP	+	≥ 4	R	288	KP	+	≥ 4	R
212	KP	+	≥ 4	R	289	KP	+	≥ 4	R
213	EC	+	≥ 4	R	291	KP	+	≥ 4	R
214	EC	+	≥ 4	R	292	KP	+	≥ 4	R
215	EC	+	≥ 4	R	293	KP	+	≥ 4	R
216	EC	+	≥ 4	R	294	KP	+	≥ 4	R
217	KP	+	≥ 4	R	295	KP	+	≥ 4	R
218	KP	+	≥ 4	R	296	KP	+	≤ 0.25	S

					NORFLOXACIN			
					ID ^a	ORG ^b	ESBL ^c	AST ^d
298	KP	+	>=4	R				MIC
304	KP	+	>=4	R				
305	KP	+	>=4	R				
306	KP	+	>=4	R	2	EC	+	>=16 R
307	KP	+	>=4	R	3	KP	+	>=16 R
308	KP	+	>=4	R	4	KP	+	>=16 R
309	KP	+	>=4	R	8	KP	+	>=16 R
310	KP	+	>=4	R	9	KP	+	>=16 R
311	KP	+	>=4	R	11	EC	+	>=16 R
312	EC	+	>=4	R	12	KP	+	>=16 R
313	EC	+	>=4	R	14	KP	+	>=16 R
314	KP	+	>=4	R	15	KP	+	>=16 R
315	KP	+	0.5	S	17	KP	+	>=16 R
316	KP	+	>=4	R	19	KP	+	>=16 R
317	KP	+	>=4	R	23	EC	+	>=16 R
318	EC	+	>=4	R	25	KP	+	>=16 R
322	KP	+	>=4	R	27	KP	+	<=0.5 S
323	KP	+	>=4	R	28	EC	+	>=16 R
324	EC	+	1	S	29	KP	+	>=16 R
325	EC	+	1	S	30	EC	+	>=16 R
326	EC	+	1	S	31	KP	+	>=16 R
327	EC	+	>=4	R	32	KP	+	>=16 R
328	EC	+	>=4	R	33	KP	+	>=16 R
329	EC	+	>=4	R	34	KP	+	>=16 R
330	KP	+	>=4	R	36	KP	+	>=16 R
331	KP	+	>=4	R	38	KP	+	>=16 R
332	KP	+	<=0.25	S	40	KP	+	2 S
333	KP	+	<=0.25	S	41	KP	+	>=16 R
339	EC	+	>=4	R	43	KP	+	>=16 R
346	KP	+	>=4	R	44	KP	+	>=16 R
347	EC	+	>=4	R	47	KP	+	2 S
350	EC	+	>=4	R	48	KP	+	2 S
352	EC	+	>=4	R	49	KP	+	2 S
357	EC	+	>=4	R	50	KP	+	1 S
358	EC	+	1	S	51	KP	+	2 S
360	EC	+	>=4	R	53	KP	+	>=16 R
361	EC	+	>=4	R	54	KP	+	>=16 R
363	EC	+	>=4	R	55	KP	+	>=16 R
400	KP	+	>=4	R	57	EC	+	>=16 R
					58	KP	+	>=16 R
					59	KP	+	2 S
					60	EC	+	>=16 R

61	KP	+	≤ 0.5	S	123	EC	+	2	S
63	KP	+	≤ 0.5	S	124	EC	+	≥ 16	R
64	KP	+	≤ 0.5	S	126	EC	+	≥ 16	R
66	KP	+	≤ 0.5	S	129	KP	+	≥ 16	R
67	EC	+	≥ 16	R	130	KP	+	≥ 16	R
68	KP	+	≤ 0.5	S	133	KP	+	≥ 16	R
69	KP	+	≥ 16	R	135	KP	+	≥ 16	R
70	KP	+	≤ 0.5	S	145	KP	+	≥ 16	R
71	KP	+	≥ 16	R	147	KP	+	≥ 16	R
72	KP	+	≥ 16	R	149	EC	+	≥ 16	R
73	EC	+	≥ 16	R	151	KP	+	2	S
74	KP	+	≤ 0.5	S	154	KP	+	≥ 16	R
75	EC	+	≥ 16	R	156	EC	+	≥ 16	R
76	EC	+	≥ 16	R	158	EC	+	≥ 16	R
78	KP	+	≥ 16	R	162	EC	+	≥ 16	R
79	EC	+	≥ 16	R	164	KP	+	≥ 16	R
80	KP	+	≥ 16	R	165	KP	+	≥ 16	R
81	EC	+	2	S	166	KP	+	≥ 16	R
83	EC	+	≥ 16	R	167	EC	+	≥ 16	R
84	EC	+	≥ 16	R	169	EC	+	≥ 16	R
85	KP	+	≥ 16	R	175	EC	+	≥ 16	R
86	EC	+	≥ 16	R	177	EC	+	2	S
87	EC	+	≥ 16	R	187	EC	+	≥ 16	R
88	EC	+	≥ 16	R	195	EC	+	≥ 16	R
89	EC	+	≥ 16	R	196	EC	+	≥ 16	R
93	EC	+	≥ 16	R	198	KP	+	≥ 16	R
94	EC	+	≥ 16	R	202	KP	+	≥ 16	R
95	KP	+	2	S	203	KP	+	≥ 16	R
102	KP	+	≥ 16	R	206	KP	+	≥ 16	R
103	EC	+	≥ 16	R	207	KP	+	≥ 16	R
104	EC	+	≥ 16	R	211	KP	+	≥ 16	R
105	KP	+	≥ 16	R	212	KP	+	≥ 16	R
107	EC	+	≥ 16	R	213	EC	+	≥ 16	R
108	EC	+	≥ 16	R	214	EC	+	≥ 16	R
109	EC	+	≥ 16	R	215	EC	+	≥ 16	R
112	EC	+	≥ 16	R	216	EC	+	≥ 16	R
116	EC	+	2	S	217	KP	+	≥ 16	R
117	EC	+	2	S	218	KP	+	≥ 16	R
118	EC	+	2	S	219	KP	+	≥ 16	R
119	EC	+	2	S	220	KP	+	≥ 16	R
120	EC	+	2	S	221	KP	+	≥ 16	R
121	KP	+	≥ 16	R	222	KP	+	≥ 16	R
122	EC	+	≥ 16	R	223	KP	+	1	S

224	KP	+	1	S	308	KP	+	>=16	R
225	KP	+	1	S	309	KP	+	>=16	R
226	KP	+	1	S	310	KP	+	>=16	R
227	KP	+	1	S	311	KP	+	>=16	R
244	KP	+	>=16	R	312	EC	+	>=16	R
245	KP	+	>=16	R	313	EC	+	>=16	R
246	KP	+	>=16	R	314	KP	+	>=16	R
247	KP	+	>=16	R	315	KP	+	1	S
248	KP	+	>=16	R	316	KP	+	>=16	R
249	KP	+	>=16	R	317	KP	+	>=16	R
260	KP	+	>=16	R	318	EC	+	>=16	R
262	KP	+	<=0.5	S	322	KP	+	>=16	R
264	EC	+	>=16	R	323	KP	+	>=16	R
267	KP	+	>=16	R	324	EC	+	2	S
268	EC	+	>=16	R	325	EC	+	2	S
270	KP	+	>=16	R	326	EC	+	2	S
271	KP	+	>=16	R	327	EC	+	>=16	R
272	KP	+	>=16	R	328	EC	+	>=16	R
274	KP	+	>=16	R	329	EC	+	>=16	R
275	KP	+	>=16	R	330	KP	+	>=16	R
276	KP	+	>=16	R	331	KP	+	>=16	R
277	EC	+	>=16	R	332	KP	+	<=0.5	S
279	EC	+	>=16	R	333	KP	+	<=0.5	S
280	KP	+	>=16	R	339	EC	+	>=16	R
281	KP	+	>=16	R	346	KP	+	>=16	R
282	KP	+	>=16	R	347	EC	+	>=16	R
283	KP	+	>=16	R	350	EC	+	>=16	R
285	KP	+	2	S	352	EC	+	>=16	R
286	KP	+	2	S	357	EC	+	>=16	R
287	KP	+	2	S	358	EC	+	2	S
288	KP	+	>=16	R	360	EC	+	>=16	R
289	KP	+	>=16	R	361	EC	+	>=16	R
291	KP	+	>=16	R	363	EC	+	>=16	R
292	KP	+	>=16	R	400	KP	+	>=16	R
293	KP	+	>=16	R	TETRACYCLINE				
294	KP	+	>=16	R	ID^a	ORG^b	ESBL^c	AST^d	
295	KP	+	>=16	R				MIC	
296	KP	+	<=0.5	S					
298	KP	+	>=16	R	2	EC	+	>=16	R
304	KP	+	>=16	R	3	KP	+	8	I
305	KP	+	>=16	R	4	KP	+	8	I
306	KP	+	>=16	R	8	KP	+	8	I
307	KP	+	>=16	R	9	KP	+	8	I

11	EC	+	≥ 16	R	72	KP	+	2	S
12	KP	+	≥ 16	R	73	EC	+	≥ 16	R
14	KP	+	8	I	74	KP	+	≥ 16	R
15	KP	+	8	I	75	EC	+	≥ 16	R
17	KP	+	≤ 1	S	76	EC	+	≥ 16	R
19	KP	+	≥ 16	R	78	KP	+	≥ 16	R
23	EC	+	≥ 16	R	79	EC	+	≥ 16	R
25	KP	+	≥ 16	R	80	KP	+	≥ 16	R
27	KP	+	≤ 1	S	81	EC	+	≥ 16	R
28	EC	+	≥ 16	R	83	EC	+	≥ 16	R
29	KP	+	≥ 16	R	84	EC	+	≥ 16	R
30	EC	+	≥ 16	R	85	KP	+	≤ 1	S
31	KP	+	≥ 16	R	86	EC	+	≥ 16	R
32	KP	+	≥ 16	R	87	EC	+	≥ 16	R
33	KP	+	≥ 16	R	88	EC	+	≥ 16	R
34	KP	+	≥ 16	R	89	EC	+	≥ 16	R
36	KP	+	≥ 16	R	93	EC	+	≥ 16	R
38	KP	+	≥ 16	R	94	EC	+	≥ 16	R
40	KP	+	≥ 16	R	95	KP	+	8	I
41	KP	+	≥ 16	R	102	KP	+	≥ 16	R
43	KP	+	8	I	103	EC	+	≥ 16	R
44	KP	+	≥ 16	R	104	EC	+	≥ 16	R
47	KP	+	≥ 16	R	105	KP	+	≤ 1	S
48	KP	+	≥ 16	R	107	EC	+	≥ 16	R
49	KP	+	≥ 16	R	108	EC	+	≥ 16	R
50	KP	+	≥ 16	R	109	EC	+	≥ 16	R
51	KP	+	≥ 16	R	112	EC	+	≥ 16	R
53	KP	+	≥ 16	R	116	EC	+	≥ 16	R
54	KP	+	≥ 16	R	117	EC	+	≥ 16	R
55	KP	+	≥ 16	R	118	EC	+	≥ 16	R
57	EC	+	≥ 16	R	119	EC	+	≥ 16	R
58	KP	+	≤ 1	S	120	EC	+	≥ 16	R
59	KP	+	≥ 16	R	121	KP	+	≥ 16	R
60	EC	+	≥ 16	R	122	EC	+	≥ 16	R
61	KP	+	≤ 1	S	123	EC	+	≥ 16	R
63	KP	+	≤ 1	S	124	EC	+	≥ 16	R
64	KP	+	≤ 1	S	126	EC	+	≥ 16	R
66	KP	+	≤ 1	S	129	KP	+	8	I
67	EC	+	≥ 16	R	130	KP	+	8	I
68	KP	+	≤ 1	S	133	KP	+	≥ 16	R
69	KP	+	≥ 16	R	135	KP	+	8	I
70	KP	+	≤ 1	S	145	KP	+	≥ 16	R
71	KP	+	2	S	147	KP	+	2	S

149	EC	+	≥ 16	R	249	KP	+	8	I
151	KP	+	≥ 16	R	260	KP	+	≥ 16	R
154	KP	+	≥ 16	R	262	KP	+	≤ 1	S
156	EC	+	≥ 16	R	264	EC	+	≥ 16	R
158	EC	+	≥ 16	R	267	KP	+	≥ 16	R
162	EC	+	≥ 16	R	268	EC	+	≥ 16	R
164	KP	+	≥ 16	R	270	KP	+	≥ 16	R
165	KP	+	8	I	271	KP	+	≥ 16	R
166	KP	+	≥ 16	R	272	KP	+	8	I
167	EC	+	4	S	274	KP	+	≥ 16	R
169	EC	+	4	S	275	KP	+	≥ 16	R
175	EC	+	≥ 16	R	276	KP	+	≥ 16	R
177	EC	+	≥ 16	R	277	EC	+	≥ 16	R
187	EC	+	≥ 16	R	279	EC	+	≥ 16	R
195	EC	+	≥ 16	R	280	KP	+	≤ 1	S
196	EC	+	4	S	281	KP	+	≤ 1	S
198	KP	+	≥ 16	R	282	KP	+	≤ 1	S
202	KP	+	2	S	283	KP	+	≤ 1	S
203	KP	+	≥ 16	R	285	KP	+	8	I
206	KP	+	8	I	286	KP	+	8	I
207	KP	+	2	S	287	KP	+	8	I
211	KP	+	≤ 1	S	288	KP	+	≥ 16	R
212	KP	+	≥ 16	R	289	KP	+	≥ 16	R
213	EC	+	4	S	291	KP	+	≥ 16	R
214	EC	+	4	S	292	KP	+	≥ 16	R
215	EC	+	4	S	293	KP	+	≥ 16	R
216	EC	+	4	S	294	KP	+	≥ 16	R
217	KP	+	≥ 16	R	295	KP	+	≥ 16	R
218	KP	+	≥ 16	R	296	KP	+	≤ 1	S
219	KP	+	≥ 16	R	298	KP	+	≥ 16	R
220	KP	+	≥ 16	R	304	KP	+	2	S
221	KP	+	≥ 16	R	305	KP	+	≤ 1	S
222	KP	+	≥ 16	R	306	KP	+	≤ 1	S
223	KP	+	≥ 16	R	307	KP	+	≥ 16	R
224	KP	+	≥ 16	R	308	KP	+	≥ 16	R
225	KP	+	≥ 16	R	309	KP	+	≥ 16	R
226	KP	+	≥ 16	R	310	KP	+	≥ 16	R
227	KP	+	≥ 16	R	311	KP	+	≥ 16	R
244	KP	+	8	I	312	EC	+	4	S
245	KP	+	8	I	313	EC	+	4	S
246	KP	+	8	I	314	KP	+	≥ 16	R
247	KP	+	8	I	315	KP	+	≥ 16	R
248	KP	+	8	I	316	KP	+	≥ 16	R

317	KP	+	>=16	R	27	KP	+	64	I
318	EC	+	>=16	R	28	EC	+	<=16	S
322	KP	+	>=16	R	29	KP	+	>=512	R
323	KP	+	>=16	R	30	EC	+	128	R
324	EC	+	>=16	R	31	KP	+	128	R
325	EC	+	>=16	R	32	KP	+	64	I
326	EC	+	>=16	R	33	KP	+	128	R
327	EC	+	>=16	R	34	KP	+	128	R
328	EC	+	>=16	R	36	KP	+	128	R
329	EC	+	>=16	R	38	KP	+	128	R
330	KP	+	>=16	R	40	KP	+	64	I
331	KP	+	>=16	R	41	KP	+	128	R
332	KP	+	>=16	R	43	KP	+	64	I
333	KP	+	>=16	R	44	KP	+	>=512	R
339	EC	+	>=16	R	47	KP	+	64	I
346	KP	+	<=1	S	48	KP	+	64	I
347	EC	+	>=16	R	49	KP	+	64	I
350	EC	+	>=16	R	50	KP	+	<=16	S
352	EC	+	>=16	R	51	KP	+	64	I
357	EC	+	>=16	R	53	KP	+	64	I
358	EC	+	>=16	R	54	KP	+	256	R
360	EC	+	>=16	R	55	KP	+	64	I
361	EC	+	>=16	R	57	EC	+	32	S
363	EC	+	>=16	R	58	KP	+	<=16	S
400	KP	+	<=1	S	59	KP	+	64	I
NITROFURANTOIN					60	EC	+	<=16	S
ID^a	ORG^b	ESBL^c	AST^d		61	KP	+	64	I
			MIC		63	KP	+	64	I
2	EC	+	<=16	S	64	KP	+	64	I
3	KP	+	>=512	R	66	KP	+	64	I
4	KP	+	>=512	R	67	EC	+	128	R
8	KP	+	>=512	R	68	KP	+	64	I
9	KP	+	>=512	R	69	KP	+	256	R
11	EC	+	<=16	S	70	KP	+	64	I
12	KP	+	>=512	R	71	KP	+	>=512	R
14	KP	+	>=512	R	72	KP	+	>=512	R
15	KP	+	>=512	R	73	EC	+	32	S
17	KP	+	<=16	S	74	KP	+	32	S
19	KP	+	128	R	75	EC	+	32	S
23	EC	+	64	I	76	EC	+	32	S
25	KP	+	128	R	78	KP	+	256	R
					79	EC	+	<=16	S
					80	KP	+	>=512	R

81	EC	+	<=16	S	166	KP	+	128	R
83	EC	+	128	R	167	EC	+	64	S
84	EC	+	128	R	169	EC	+	64	S
85	KP	+	>=512	R	175	EC	+	<=16	S
86	EC	+	128	R	177	EC	+	<=16	S
87	EC	+	<=16	S	187	EC	+	<=16	S
88	EC	+	128	R	195	EC	+	<=16	S
89	EC	+	128	R	196	EC	+	64	S
93	EC	+	<=16	S	198	KP	+	>=512	R
94	EC	+	64	I	202	KP	+	>=512	R
95	KP	+	256	R	203	KP	+	128	R
102	KP	+	64	I	206	KP	+	256	R
103	EC	+	32	S	207	KP	+	>=512	R
104	EC	+	32	S	211	KP	+	256	R
105	KP	+	>=512	R	212	KP	+	128	R
107	EC	+	32	S	213	EC	+	<=16	S
108	EC	+	32	S	214	EC	+	<=16	S
109	EC	+	32	S	215	EC	+	<=16	S
112	EC	+	32	S	216	EC	+	<=16	S
116	EC	+	<=16	S	217	KP	+	128	R
117	EC	+	<=16	S	218	KP	+	128	R
118	EC	+	<=16	S	219	KP	+	128	R
119	EC	+	<=16	S	220	KP	+	128	R
120	EC	+	<=16	S	221	KP	+	128	R
121	KP	+	64	I	222	KP	+	128	R
122	EC	+	<=16	S	223	KP	+	<=16	S
123	EC	+	<=16	S	224	KP	+	<=16	S
124	EC	+	32	S	225	KP	+	<=16	S
126	EC	+	128	R	226	KP	+	<=16	S
129	KP	+	>=512	R	227	KP	+	<=16	S
130	KP	+	>=512	R	244	KP	+	>=512	R
133	KP	+	32	S	245	KP	+	>=512	R
135	KP	+	>=512	R	246	KP	+	>=512	R
145	KP	+	64	I	247	KP	+	>=512	R
147	KP	+	>=512	R	248	KP	+	>=512	R
149	EC	+	<=16	S	249	KP	+	>=512	R
151	KP	+	64	I	260	KP	+	256	R
154	KP	+	64	I	262	KP	+	64	I
156	EC	+	<=16	S	264	EC	+	32	S
158	EC	+	<=16	S	267	KP	+	256	R
162	EC	+	32	S	268	EC	+	<=16	R
164	KP	+	128	R	270	KP	+	128	R
165	KP	+	>=512	R	271	KP	+	128	R

272	KP	+	>=512	R	TRIMETHOPRIM SULPHAMETHOXAZOLE	328	EC	+	64	I
274	KP	+	64	I		329	EC	+	64	I
275	KP	+	64	I		330	KP	+	64	I
276	KP	+	64	I		331	KP	+	64	I
277	EC	+	<=16	S		332	KP	+	32	S
279	EC	+	<=16	S		333	KP	+	32	S
280	KP	+	>=512	R		339	EC	+	32	S
281	KP	+	>=512	R		346	KP	+	128	R
282	KP	+	>=512	R		347	EC	+	<=16	S
283	KP	+	>=512	R		350	EC	+	<=16	S
285	KP	+	256	R		352	EC	+	128	R
286	KP	+	256	R		357	EC	+	32	S
287	KP	+	256	R		358	EC	+	<=16	S
288	KP	+	64	I		360	EC	+	<=16	S
289	KP	+	64	I		361	EC	+	64	I
291	KP	+	>=512	R		363	EC	+	32	S
292	KP	+	>=512	R		400	KP	+	128	R
293	KP	+	>=512	R						
294	KP	+	>=512	R						
295	KP	+	>=512	R						
296	KP	+	64	I						
298	KP	+	>=512	R						
304	KP	+	>=512	R						
305	KP	+	<=16	S						
306	KP	+	<=16	S						
307	KP	+	256	R						
308	KP	+	256	R						
309	KP	+	256	R						
310	KP	+	128	R						
311	KP	+	128	R						
312	EC	+	<=16	S						
313	EC	+	<=16	S						
314	KP	+	128	R						
315	KP	+	<=16	S						
316	KP	+	128	R						
317	KP	+	128	R						
318	EC	+	<=16	S						
322	KP	+	>=512	R						
323	KP	+	>=512	R						
324	EC	+	<=16	S						
325	EC	+	<=16	S						
326	EC	+	<=16	S						
327	EC	+	64	I						

36	KP	+	>=320	R	93	EC	+	>=320	R
38	KP	+	>=320	R	94	EC	+	>=320	R
40	KP	+	>=320	R	95	KP	+	>=320	R
41	KP	+	>=320	R	102	KP	+	>=320	R
43	KP	+	>=320	R	103	EC	+	>=320	R
44	KP	+	>=320	R	104	EC	+	>=320	R
47	KP	+	>=320	R	105	KP	+	>=320	R
48	KP	+	>=320	R	107	EC	+	>=320	R
49	KP	+	>=320	R	108	EC	+	>=320	R
50	KP	+	>=320	R	109	EC	+	>=320	R
51	KP	+	>=320	R	112	EC	+	>=320	R
53	KP	+	>=320	R	116	EC	+	>=320	R
54	KP	+	>=320	R	117	EC	+	>=320	R
55	KP	+	>=320	R	118	EC	+	>=320	R
57	EC	+	>=320	R	119	EC	+	>=320	R
58	KP	+	>=320	R	120	EC	+	>=320	R
59	KP	+	>=320	R	121	KP	+	>=320	R
60	EC	+	>=320	R	122	EC	+	>=320	R
61	KP	+	>=320	R	123	EC	+	>=320	R
63	KP	+	>=320	R	124	EC	+	>=320	R
64	KP	+	>=320	R	126	EC	+	>=320	R
66	KP	+	>=320	R	129	KP	+	>=320	R
67	EC	+	>=320	R	130	KP	+	>=320	R
68	KP	+	>=320	R	133	KP	+	>=320	R
69	KP	+	80	R	135	KP	+	>=320	R
70	KP	+	>=320	R	145	KP	+	>=320	R
71	KP	+	>=320	R	147	KP	+	>=320	R
72	KP	+	>=320	R	149	EC	+	>=320	R
73	EC	+	>=320	R	151	KP	+	>=320	R
74	KP	+	>=320	R	154	KP	+	>=320	R
75	EC	+	>=320	R	156	EC	+	>=320	R
76	EC	+	>=320	R	158	EC	+	>=320	R
78	KP	+	>=320	R	162	EC	+	>=320	R
79	EC	+	>=320	R	164	KP	+	>=320	R
80	KP	+	>=320	R	165	KP	+	>=320	R
81	EC	+	>=320	R	166	KP	+	>=320	R
83	EC	+	>=320	R	167	EC	+	>=320	R
84	EC	+	>=320	R	169	EC	+	>=320	R
85	KP	+	>=320	R	175	EC	+	>=320	R
86	EC	+	>=320	R	177	EC	+	>=320	R
87	EC	+	>=320	R	187	EC	+	>=320	R
88	EC	+	>=320	R	195	EC	+	>=320	R
89	EC	+	>=320	R	196	EC	+	>=320	R

198	KP	+	>=320	R	282	KP	+	>=320	R
202	KP	+	>=320	R	283	KP	+	>=320	R
203	KP	+	>=320	R	285	KP	+	>=320	R
206	KP	+	>=320	R	286	KP	+	>=320	R
207	KP	+	>=320	R	287	KP	+	>=320	R
211	KP	+	>=320	R	288	KP	+	>=320	R
212	KP	+	>=320	R	289	KP	+	>=320	R
213	EC	+	>=320	R	291	KP	+	>=320	R
214	EC	+	>=320	R	292	KP	+	>=320	R
215	EC	+	>=320	R	293	KP	+	>=320	R
216	EC	+	>=320	R	294	KP	+	>=320	R
217	KP	+	>=320	R	295	KP	+	>=320	R
218	KP	+	>=320	R	296	KP	+	>=320	R
219	KP	+	>=320	R	298	KP	+	>=320	R
220	KP	+	>=320	R	304	KP	+	>=320	R
221	KP	+	>=320	R	305	KP	+	<=20	S
222	KP	+	>=320	R	306	KP	+	<=20	S
223	KP	+	>=320	R	307	KP	+	80	R
224	KP	+	>=320	R	308	KP	+	>=320	R
225	KP	+	>=320	R	309	KP	+	>=320	R
226	KP	+	>=320	R	310	KP	+	>=320	R
227	KP	+	>=320	R	311	KP	+	>=320	R
244	KP	+	>=320	R	312	EC	+	>=320	R
245	KP	+	>=320	R	313	EC	+	>=320	R
246	KP	+	>=320	R	314	KP	+	>=320	R
247	KP	+	>=320	R	315	KP	+	>=320	R
248	KP	+	>=320	R	316	KP	+	>=320	R
249	KP	+	>=320	R	317	KP	+	>=320	R
260	KP	+	>=320	R	318	EC	+	>=320	R
262	KP	+	>=320	R	322	KP	+	>=320	R
264	EC	+	>=320	R	323	KP	+	>=320	R
267	KP	+	>=320	R	324	EC	+	>=320	R
268	EC	+	>=320	R	325	EC	+	>=320	R
270	KP	+	>=320	R	326	EC	+	>=320	R
271	KP	+	>=320	R	327	EC	+	>=320	R
272	KP	+	>=320	R	328	EC	+	>=320	R
274	KP	+	>=320	R	329	EC	+	>=320	R
275	KP	+	>=320	R	330	KP	+	>=320	R
276	KP	+	>=320	R	331	KP	+	>=320	R
277	EC	+	>=320	R	332	KP	+	>=320	R
279	EC	+	>=320	R	333	KP	+	>=320	R
280	KP	+	>=320	R	339	EC	+	>=320	R
281	KP	+	>=320	R	346	KP	+	<=20	S

347	EC	+	≥ 320	R
350	EC	+	≥ 320	R
352	EC	+	≥ 320	R
357	EC	+	≤ 20	S
358	EC	+	≥ 320	R
360	EC	+	≥ 320	R
361	EC	+	≥ 320	R
363	EC	+	≥ 320	R
400	KP	+	≤ 20	S

KEY

ID ^a	Isolate ID
ORG ^b	Organism
ESBL ^c	ESBL phenotype
AST ^d	Antimicrobial Susceptibility Test
EC ^e	<i>E. Coli</i>
KP ^f	<i>K. Pneumoniae</i>
MIC ^g	Minimum inhibitory concentration