**TITLE PAGE**

**PHENOTYPIC DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES PRODUCING ORGANISM AMONG GODFREY OKOYE UNIVERSITY STUDENTS**

**BY**

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**APPROVAL PAGE**

This work has been read and approved by Department of Microbiology, Faculty of Natural and Applied Science, Godfrey Okoye University, Enugu, in partial fulfillment of the requirement for the Award of Bachelor of Science, Degree in Microbiology, (B.Sc.).

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**DEDICATION**

This work is dedicated to almighty God and to my hardworking parents.

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My biggest appreciation goes to almighty God who made this research work a success. My appreciation also goes to my Head of Deapartment, Dr. (Mrs). Marian .N. Unachukwu for her encouragement.

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**TABLE OF CONTENTS**

Title page i

Approval page ii

Dedication iii

Acknowledgement iv

List of tables v

List of figures vi

Abstract xi

**CHAPTER ONE 1**

Introduction 1

Classification of ESBL 2

Diversity of the types of ESBL 2

Global epidemiology of ESBL 5

Phenotypic identification of ESBL 10

Description of ESBL detection tests 11

**CHAPTER TWO 15**

Literature review 15

Antibiotics resistance 15

Antibiotics resistance mechanism 16

Genetics of antibiotics resistance 17

Treatment 19

Prevention and control 20

Aim and objective 20

**CHAPTER THREE 21**

Materials 21

Sample collection 21

Culture media preparation 21

Identification of organism 23

Antibiotics susceptibility test 25

Double disc synergy test 26

**CHAPTER FOUR 27**

Results 27

**CHAPTER FIVE 33**

Discussion 33

Conclusion 34

**References 35**

**LIST OF FIGURES Pg**

**Figure 1:** phenotypic confirmation of ESBL production using DDST 12

**Figure 2:** DDST test 32

**LIST OF TABLES Pg**

**Table 1:** morphology of isolates 27

**Table 2:** frequency of probable organisms 28

**Table 3:** IMViC test result and coagulase test result 29

**Table 4:** antibiotics sensitivity test result 30

**Table 5:** double disc synergy test result 31

**ABSTRACT**

Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillin, cephalosporin, and the aztreonams. The aim of this present study is to phenotypically identify and establish the presence of ESBL-producing organism among students in the university community. Within the University community of Godfrey Okoye University, Enugu, early morning urine samples of midstream-catch were collected into sterile bottles from sixty (60) students between ages 18 and 25years from the 2ndMay to 31st May. Thirty (30) male students and thirty (30) female students were sampled. Eighteen (18) isolates were identified after the following biochemical test were carried out: Gram staining, IMViC test (Indole test, methyl red test, Vogesproskauer test and citrate utilization test), and coagulase test. Twelve (12) isolates were from female students and six (6) isolates were from male students. The organisms identified were: *Streptococcus* spp, *Corynebacterium*spp, *Staphylococcus* spp, and *Escherichia coli*. All theisolates were Gram positive except for one which was Gram negative. The double disc synergy test (DDST) was also carried out to phenotypically confirm the presence of ESBL producing organisms. All isolates were sensitive to the test drugs in the antimicrobial susceptibility test but there was no obvious DDST zones of inhibition. The result of the study suggests the absence of ESBL producing organisms among the students involved in this study.

**CHAPTER ONE**

**1.1 Introduction**

Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillin, cephalosporin, and aztreonams (Bush and Jacoby, 2010).

Extended-spectrum Beta(β)-lactamases (ESBLs) are a group which are mostly plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. Infections due to ESBL producers range from uncomplicated urinary tract infections (UTI) to life-threatening sepsis. These enzymes share the ability to hydrolyze third-generation cephalosporin and aztreonam and yet, are inhibited by clavulanic acid. In addition, ESBL-producing organisms exhibit co-resistance to many other classes of antibiotics, resulting in limitation of therapeutic option. Because of inoculum effect and substrate specificity, their detection is also a major challenge (Deepthi*et al* 2010).

Numerous studies have barbed towards high incidence rate of UTI associated with [*Escherichia coli*](https://en.wikipedia.org/wiki/Escherichia_coli) *(*[*E. coli*](https://en.wikipedia.org/wiki/Escherichia_coli)*)* and antibiotic resistance. The emergence of Multi Drug Resistant (MDR) variant of [*E. coli*](https://en.wikipedia.org/wiki/Escherichia_coli)has been accounted. MDR is defined as resistance to at least two antibiotics of different classes including aminoglycosides, chloramphenicol, tetracycline and/or erythromycin. MDR in many bacteria is due to the action of multi-drug efflux pumps and by the accumulation on Resistance (R) plasmids or transposons of genes with each coding for resistance to a specific agent. Nowadays, in UTIs, ESBL -expressing Gram-Negative Bacilli (ESBL-GNB) generally cause community-acquired infections. The resistance of Gram-negative bacteria is typically owed to plasmid mediated enzymes of ESBL. ESBL producing bacteria are typically associated with multi-drug resistance (MDR) and antibacterial choice is often complicated by multi-drug resistance (Prakash and Yadav, 2017).

**1.2 CLASSIFICATION OF ESBL**

There are two major classification systems for β-lactamases:

* + 1. **Molecular classification** is based on the amino acid sequence and divides β-lactamases Ambler classes into A (serine penicillinases), C (cephalosporinases), and D (oxa-cillinases) enzymes which utilize serine for β-lactam hydrolysis and class B metalloenzymes which require divalent zinc ions for substrate hydrolysis (Bush and Jacoby, 2010).
		2. **Functional classification** scheme was initially proposed by Bush in 1989 and then expanded in 1995. It takes into account substrate and inhibitor profiles in an attempt to group the enzymes in ways that can be correlated with their phenotype in clinical isolates (Bush and Jacoby, 2010).
	1. **DIVERSITYOF THE TYPES OF ESBL**
		1. **TEM beta-lactamases**

The first plasmid-mediated beta-lactamase in gram-negative bacteria was discovered in Greece in the 1960s. It was named TEM after the patient from whom it was isolated (Temoniera). Although TEM-type beta-lactamases are most often found in [*Escherichia coli*](https://en.wikipedia.org/wiki/Escherichia_coli) and [*Klebsiellapneumoniae*](https://en.wikipedia.org/wiki/K._pneumoniae), they are also found in other species of Gram-negative bacteria with increasing frequency (Clark *et al.,* 1990).

* + 1. **SHV beta-lactamases**

Sulfhydryl variable, (SHV) shares 68 percent of its amino acids with TEM and has a similar overall structure. The SHV beta-lactamase is most commonly found in [*Klebsiellapneumoniae*](https://en.wikipedia.org/wiki/K._pneumoniae) and is responsible for up to 20% of the plasmid-mediated ampicillin resistance in this species. ESBLs in this family also have amino acid changes around the active site. More than 60 SHV varieties are known (Chow *et al*., 2010).

* + 1. **CTX-M beta-lactamases**

Cefotaximase Munich (CTX-M), these enzymes were named for their greater activity against [cefotaxime](https://en.wikipedia.org/wiki/Cefotaxime) than other oxyimino-beta-lactam substrates (e.g., [ceftazidime](https://en.wikipedia.org/wiki/Ceftazidime), [ceftriaxone](https://en.wikipedia.org/wiki/Ceftriaxone), or [cefepime](https://en.wikipedia.org/wiki/Cefepime)). Rather than arising by mutation, they represent examples of plasmid acquisition of beta-lactamase genes normally found on the chromosome of [*Kluyvera*](https://en.wikipedia.org/wiki/Kluyvera) species, a group of rarely pathogenic commensal organisms. These enzymes are not very closely related to TEM or SHV beta-lactamases in that they show only approximately 40% identity with these two commonly isolated beta-lactamases. More than 80 CTX-M enzymes are currently known. Despite their name, a few are more active on [ceftazidime](https://en.wikipedia.org/wiki/Ceftazidime) than [cefotaxime](https://en.wikipedia.org/wiki/Cefotaxime). They have mainly been found in strains of [*Salmonella enterica*](https://en.wikipedia.org/wiki/Salmonella_enterica)s*erovartyphimurium* and [*E. coli*](https://en.wikipedia.org/wiki/Escherichia_coli), but have also been described in other species of [*Enterobacteriaceae*](https://en.wikipedia.org/wiki/Enterobacteriaceae)(Chow *et al.,* 2010).

* + 1. **OXA beta-lactamases**

The OXA-type β-lactamases are so named because of their oxacillin-hydrolyzing abilities. OXA beta-lactamases were long recognized as a less common but also plasmid-mediated beta-lactamase variety that could hydrolyze [oxacillin](https://en.wikipedia.org/wiki/Oxacillin) and related anti-staphylococcal penicillin. These beta-lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d. The OXA-type beta-lactamases confer resistance to [ampicillin](https://en.wikipedia.org/wiki/Ampicillin) and [cephalothin](https://en.wikipedia.org/wiki/Cephalothin) and are characterized by their high hydrolytic activity against [oxacillin](https://en.wikipedia.org/wiki/Oxacillin) and [cloxacillin](https://en.wikipedia.org/wiki/Cloxacillin) and the fact that they are poorly inhibited by [clavulanic acid](https://en.wikipedia.org/wiki/Clavulanic_acid). Amino acid substitutions in OXA enzymes can also give the ESBL phenotype. While most ESBLs have been found in [*E. coli*](https://en.wikipedia.org/wiki/Escherichia_coli), [*Klebsiellapneumoniae*](https://en.wikipedia.org/wiki/K._pneumoniae), and other [*Enterobacteriaceae*](https://en.wikipedia.org/wiki/Enterobacteriaceae), the OXA-type ESBLs have been found mainly in [*Pneumoniaeaeruginosa*](https://en.wikipedia.org/wiki/P._aeruginosa). The OXA beta-lactamase family was originally created as a phenotypic rather than a genotypic group for a few beta-lactamases that had a specific hydrolysis profile. Therefore, there is as little as 20% sequence homology among some of the members of this family. However, recent additions to this family show some degree of homology to one or more of the existing members of the OXA beta-lactamase family. Some confer resistance predominantly to ceftazidime (Chow *et al*., 2010).

* + 1. **PER type**

The PER-type ESBLs share only around 25–27% homology with known TEM- and SHV-type ESBLs. PER-1 β-lactamase efficiently hydrolyzes penicillin and cephalosporin and is susceptible to clavulanic acid inhibition. PER was first detected in Pseudomonas aeruginosa, and later in *Salmonella* entericaserovarTyphimurium and Acinetobacter isolates as well. In Turkey, as many as 46% of nosocomial isolates of Acinetobacter spp. and 11% of P. aeruginosa were found to produce PER, which shares 86% homology to PER-1, has been detected in S. entericaserovarTyphimurium, E. coli, K. pneumoniae, Proteus mirabilis, and Vibrio cholerae (Danish *et al* 2015).

* + 1. **GES type**

GES was initially described in a K. pneumoniae isolate from a neonatal patient just transferred to France from French Guiana. GES has hydrolytic activity against penicillin and extended-spectrum cephalosporin, but not against cephamycin or carbapenem, and is inhibited by β-lactamase inhibitors. These enzymatic properties resemble those of other class A ESBLs; thus, GES was recognized as a member of ESBLs (Danish *et al* 2015).

* + 1. **VEB-1, BES-1, and other ESBL type**

Other unusual enzymes having ESBL have also been described (e.g. BES, CME, VE-B, PER, SFO, and GES). These novel enzymes are found infrequently (Danish *et al* 2015).

* 1. **GLOBAL EPIDEMIOLOGY OF ESBL**

Antimicrobial resistance has been declared a global threat to public health, as a massive increase in this problem has been observed in different parts of the world (Kang and Song 2013). The reported frequency of MDRs is increasing, putting strain on the public health organizations that are attempting to control this issue in many countries. The alarming increase in the prevalence of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae has serious consequences for treatment outcomes (Pitout 2010). E. coli and Klebsiella species are important pathogens isolated from community-acquired and nosocomial-acquired infections, and have been studied extensively. The ESBL enzymes produced by these bacteria make them resistant to the first-choice antibiotic therapies that are commonly used. ESBL-positive strains are associated with a delay in the commencement of suitable antibiotic therapy, which consequently lengthens hospital stay and raises hospital costs. Failure of antibiotic therapy is responsible for higher mortality rates in patients infected with these bacteria. MDRs are posing a treatment challenge, and are emerging as a major cause of morbidity and mortality worldwide. Unfortunately, proper surveillance and documentation of such pathogens is very limited, especially in developing countries like Nigeria (Hayat *et al,* 2018).

The epidemiology of health-care associated infections has been characterized by the emergence of gram-negative multi drug resistant organisms, including ESBL-producing Enterobacteriaceae during the past decade. While nosocomial transmission was initially considered by their principal cause of spread, earlier report points to the importance of the food-chain as a continuous source of dissemination (Kluytmans*et al* 2013). In addition to a growing body of literature regarding the detection of ESBL- producing Enterobacteriaceae in retail meat and food worldwide, food has been reported as a vector for transmission of ESBL- producing *Klebsiellapneumoniae* in a hospital outbreak (Calbo*et al* 2011). This leads to the conclusion that control teams should consider extending their surveillance towards food as it is a vector of ESBL.

**1.4.1 AFRICA**

In Africa, the prevalence of ESBL in *Enterobacteriaceae* has been researched at local levels in various countries, but there is no summarizing research on how prevalent ESBL is on the continent, what type of genes are involved, and where research is missing (Victor, 2014).

In patients treated in African hospitals, the prevalence of ESBL-producing Enterobacteriaceae has been shown to vary between countries and the type of specimen studied. There is a trend of higher prevalence of ESBL in stool samples than in other specimens. There is also a trend of increasing prevalence over time. This is noticeable in the Tunisian setting, where a large amount of studies are available. In two hospitals studied (study periods: 1999–2005 and 2010), ESBLs have increased from 11.7 to 77.8% among K. pneumoniae. (Aouni*et al*, 2010). In other settings, the trend is not noticeable among the few studies available. In the studied countries in Africa, the prevalence is widely different: in Algeria, it was between 16.4 and 31.4% in mainly urine samples (Barguigua*et al,* 2012) and even 99% among Salmonella enterica in stool samples (Bentchouala*et al,* 2011) 19 and 42.9%, respectively, in urine and stool samples in Egypt (Domany*et al,* 2012); 32.6% among stool samples in Guinea-Bissau (Giske*et al,* 2012); 11.7–77.8% in mainly urine, blood, and stool samples from Tunisia (Kechrid*et al,* 2011); 62.8% in stool and blood samples from Ethiopia (Asrat*et al,* 2011); 38.3% in urine samples from Rwanda (Bayingana*et al,* 2011); 55.3 and 82.8% in stool samples from Cameroon (Assoumou*et al,* 2013); 10.3–27.5% in mainly urine and stool samples from Nigeria (Aibinu*et al,* 2012); and 8.8–13.1% in urine, nasopharyngeal, and wound samples from South Africa (Dube*et al,* 2009).

**1.4.1.1 Northern Africa**

**In Algerian hospitals**, ESBLs existed in 16.4–31.4% of the samples. Class A ESBLs were most common, but plasmid-encoded AmpC (pAmpC) was also present (Canica*et al,* 2011).

**In Egypt**, ESBLs were found in 11–42.9% of samples in both hospitals and communities; the genes involved were class A ESBLs (Eletreby*et al,* 2009).

**In Guinea-Bissau and Libya**, class A and D ESBLs and a carbapenemase were found in 32.6 and 16%, respectively, in rectal or stool samples (Giske*et al,* 2012).

**In Morocco,** class A and D ESBLs, pAmpC, and carbapenemases were found in hospital settings (Carattoli*et al,* 2012). In the community setting, class A and D ESBLs were found in between 1.3 and 7.5% of acquired urine samples (Amarouch*et al,* 2011).

**In Tunisia**, class A and D ESBLs, pAmpC, and carbapenemases were present, and the prevalence ranged from 11.7 to 77.8% in hospitals and was 0.7 and 7.3% in two communities (Kechrid*et al,* 2011).

### **1.4.1.2 Eastern Africa**

**In Ethiopia and Kenya**, 62.8 and 37.4%, respectively, of hospital and community samples were ESBLs (Asrat*et al,* 2011). Class A ESBLs and pAmpC were present in the Kenyan sample (Butaye*et al,* 2012). In samples taken from Kenya and Malawi, class A and D ESBLs were found (Boyle *et al,* 2011).

**In Rwanda,** ESBLs were found in 38.3% of hospital urine samples and in 5.9% of community urine samples (Bayingana*et al,* 2011).

**In Tanzania**, class A ESBLs were found in various samples from hospital settings (Chakraborty*et al,* 2011).

* + - 1. **Central Africa**

**In Cameroon**, class A and D ESBLs were found in 55.3 and 82.8% of hospital stool samples and in 17.2% of community stool samples (Assoumou*et al,* 2013).

**In the Central African Republic**, ESBLs were found in 11.3% of community urine samples (Bercion*et al,* 2009).

### **Southern Africa**

**In South Africa**, class A and D ESBLs and pAmpC were present, and the prevalence ranged from 8.8 to 13.1% in hospitals and was 0.3 and 4.7% in two communities (Chunderika*et al,* 2008).

### **1.4.1.5 Western Africa**

**In Ghana and Mali**, class A ESBLs were found in 49.4 and 63.4–96%, respectively, in hospital and community samples (Bougoudogo*et al,* 2009).

**In Niger**, 40% of hospital samples carried class A ESBLs or pAmpC (Andremont*et al,* 2011).

**In Senegal**, class A and D ESBLs were found in 10% of community stool samples. (Andremont*et al,* 2009)

**1.4.1.6NIGERIA (West Africa)**

In Nigeria, class A and D ESBLs and pAmpC were found in hospital settings, and the prevalence ranged from 10.3 to 27.5% (Aibinu*et al,* 2012). In a mixed sample from a hospital and a community, the prevalence was 11.7%. (Afunwa*et al,* 2011)

In Nigeria, an ESBL prevalence of 9.25% was recorded in a study conducted to screen for ESBLs production among isolates of *Enterobacteriaceae* (Aliyu*et al,* 2010).

In another study conducted in a tertiary health center in Nigeria to determine ESBL prevalence in *Escherichia coli* and *Klebsiella* Species; an ESBL prevalence of 2.5% for *Escherichia coli* and 5% for *Klebsiellapneumoniae* were recorded (Aboderin and Olowe, 2010).Kluytmans JA, Overdevest IT, Willemsen I, et al. Extended-spectrum β-lactamase-producing (Kl2013;56(4):478–487. producing Enterobacteriaceae The epidemiology of healthcare-associated infections has been characterized by the emergence of gram-negative multidrug-resistant organisms, including extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae, during the past decade. While nosocomial transmission was initially considered their principal cause of spread, recent reports point to the importance of the food chain as a continuous source of dissemination,[1](http://www.jstor.org/stable/10.1086/675831#rf1) explaining in part the expansion of such organisms to community settings.[2](http://www.jstor.org/stable/10.1086/675831#rf2) In addition to a growing body of literature regarding the detection of ESBL-producing Enterobacteriaceae in retail meat and food animals worldwide, food has been reported as a transmission vector for ESBL-producing *Klebsiella pneumoniae* in a hospital outbreak,[3](http://www.jstor.org/stable/10.1086/675831#rf3) leading to the conclusion that infection control teams should consider extending their surveillance to kitchen facilities and foodstuffs. We aimed to explore potential transmission pathways explaining the spread of ESBL-producing Enterobacteriaceae from the food chain to humans in both hospital and community settings, by examining cutting boards and gloves after use for food preparation.The epidemiology of healthcare-associated infections has been characterized by the emergence of gram-negative multidrug-resistant organisms, including extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae, during the past decade. 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**1.5 PHENOTYPIC IDENTIFICATION OF ESBL**

Extended-spectrum β-lactamase (ESBL) detection tests should accurately discriminate between bacteria producing these enzymes and those with other mechanisms of resistance to β-lactams, e.g., broad-spectrum β-lactamases, inhibitor-resistant β-lactamases and cephalosporinase overproduction. Several phenotypic detection tests, based on the synergy between a third-generation cephalosporin and clavulanate, have been designed: the double-disk synergy test (DDST), ESBL E-tests, and the combination disk method. These tests often need to be refined in order for them to detect an ESBL in some bacterial strains, such as those that also overproduce a cephalosporinase. The sensitivity of the DDST can be improved by reducing the distance between the disks of cephalosporins and clavulanate. The use of cefepime, a fourth-generation cephalosporin that is less rapidly inactivated by cephalosporinase than by ESBL, improves the detection of synergy with clavulanate when there is simultaneous stable hyperproduction of a cephalosporinase; alternatively, the cephalosporinase can be inactivated by performing phenotypic tests on a cloxacillin-containing agar. Some β-lactamases can hydrolyze both third-generation cephalosporins and carbapenems, such as the metallo-β-lactamases, which are not inhibited by clavulanate, but rather by Ethylenediaminetetraacetic acid (EDTA). The production of an ESBL masked by a metallo-β-lactamase can be detected by means of double inhibition by EDTA and clavulanate. Since extended-spectrum Ambler class D oxacillinases are weakly inhibited by clavulanate and not inhibited by EDTA, their detection is difficult in the routine laboratory (Danish *et al* 2015).

## **1.6 DESCRIPTION OF THE ESBL DETECTION TESTS**

## **1.6.1 DOUBLE-DISK SYNERGY TEST**

The first test specifically designed to detect ESBL production in *Enterobacteriaceae* was the double disk synergy test (DDST) (Jarlier *et al,* 1988). It was initially designed to differentiate between cefotaxime-resistant strains, that is, those overproducing cephalosporinase, and those producing ESBLs. The test is performed on agar with a 30-μg disk of cefotaxime (and/or ceftriaxone and/or ceftazidime and/or aztreonam) and a disk of amoxicillin–clavulanate (containing 10 μg of clavulanate) positioned at a distance of 30 mm (center to center). The test is considered as positive when a decreased susceptibility to cefotaxime is combined with a clear-cut enhancement of the inhibition zone of cefotaxime in front of the clavulanate-containing disk, often resulting in a characteristic shape-zone referred to as ‘champagne-cork’ or ‘keyhole’. The DDST was first used in epidemiological studies to assess the spread of ESBL-producing *Enterobacteriaceae* in French hospitals (Brossieur*et al,* 2008). It has been shown to work well with a wide range of *Enterobacteriaceae*species and ESBL types, and it is generally regarded as a reliable method for the detection of ESBLs, although it is sometimes necessary to adjust the disk spacing. It is important to note that reducing the distance between the clavulanate-containing disk and the third-generation cephalosporin disk (e.g., to 20 mm) significantly improves the test sensitivity (Brossieur*et al,* 2008).



**FIG 1: CAZ……………………AMC……………………CTX**

Phenotypic confirmation of ESBL production using DDST(Afunwa, 2018).

The image in FIG 1 shows an increased zones of inhibition indicating the presence of ESBL producing organism.

### **1.6.2 ESBL Etests**

ESBL Etests have been developed in order to quantify the synergy between extended-spectrum cephalosporins and clavulanate. The Etests called CT/CTL, TZ/TZL and PM/PML are two-sided strips containing gradients of cefotaxime (CT), or ceftazidime (TZ) or cefepime (PM), either alone (at one end of the strip), or combined with clavulanate 4 mg/L (on the other end). The ESBL test is considered as positive when the MIC value of the tested drug is reduced by more than three doubling dilution steps (MIC ratio ≥8) in the presence of clavulanate. The test is also considered as positive when there is either: (a) a rounded zone (phantom zone) just below the lowest concentration of CTL, TZL or PML gradients, or (b) a deformation of the CT, TZ or PM inhibition ellipse at the tapering end. The presence of a phantom zone or an ellipse deformation indicates ESBL production. Interpreting results of the ESBL Etest strips is delicate and requires training(Brossieur*et al,* 2008).

### **1.6.3 COMBINATION DISK METHOD**

Several manufacturers have developed ESBL detection tests based on the combination disk method. The principle of this method is to measure the inhibition zone around a disk of cephalosporin and around a disk of the same cephalosporin plus clavulanate. Depending on the disk type, a difference of ≥5 mm between the two diameters (i.e., corresponding to a two-fold dilution), or a zone expansion of 50% are considered as indicating ESBL production. The test is easy to perform and its interpretation is straightforward. Sensitivity and specificity for this method were first reported to be 96% and 100%, respectively. Evaluation of the performance of the Oxoidcefpodoxime 10 ng ± 1 μgclavulanate combination disks to distinguish ESBL producers from AmpC overproducers and Klebsiellaoxytoca isolates overexpressing K1 enzyme was done. The presence of clavulanate enlarged the zone of inhibition by ≥5 mm for all 180 ESBL-producing organisms, and by <1 mm for AmpC overproducers and K. oxytoca isolates overexpressing K1 enzyme (Brossieur*et al,* 2008).

### **1.6.4 AUTOMATED METHOD**

The VITEK 2 ESBL test is based on the simultaneous assessment of the antibacterial activity of cefepime, cefotaxime and ceftazidime, measured either alone or in the presence of clavulanate. This test relies on card wells containing 1.0 mg/L of cefepime, or 0.5 mg/L of cefotaxime or ceftazidime, either alone or associated with 10 or 4 mg/L of clavulanate, respectively. After inoculation, cards are introduced into the VITEK 2 machine, and for each antibiotic tested, turbidity is measured at regular intervals. The proportional reduction of growth in wells containing a cephalosporin combined with clavulanate is then compared with that achieved by the cephalosporin alone and is interpreted as ESBL-positive or – negative through a computerized expert system (Advanced Expert System). (Brossieur*et al,* 2008). The automated Phoenix ESBL test (Becton Dickinson, Sparks, MD, USA) also relies on the growth response to selected expanded-spectrum cephalosporins. This test is composed of five wells, each containing a cephalosporin alone or in combination with clavulanic acid (cefpodoxime, ceftazidime, and ceftazidime with clavulanic acid, cefotaxime with clavulanic acid and ceftriaxone with clavulanic acid). In this system, the results are also interpreted through a computerized system. (Brossieur*et al,* 2008)

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.1 ANTIBIOTIC RESISTANCE**

Antibiotics are medicines used to prevent and treat bacterial infections. Antibiotic resistance occurs when bacteria change in response to the use of these medicines. Bacteria, not humans or animals, become antibiotic-resistant. These bacteria may infect humans and animals, and the infections they cause are harder to treat than those caused by non-resistant bacteria. Antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality. The world urgently needs to change the way it prescribes and uses antibiotics. Even if new medicines are developed, without behavior change, antibiotic resistance will remain a major threat. Behavior changes must also include actions to reduce the spread of infections through vaccination, hand washing, practicing safer sex, and good food hygiene. Resistance to β-lactams has probably arisen throughout bacterial history but has become a useful and therefore selected trait since the β-lactam antibiotics came into clinical use. Alternately, it may be acquired through spontaneous mutation or DNA transfer. Functionally, β-lactam resistance may be a result of the production of β-lactamases, impermeability, efflux and target modification. These modalities may occur singly or in different combinations. ([Deepthi](https://www.ncbi.nlm.nih.gov/pubmed/?term=Nair%20D%5BAuthor%5D&cauthor=true&cauthor_uid=20927289)*et al* 2010)

The plasmids bearing genes-encoding ESBLs also frequently carry genes that encode resistance to other antimicrobial agents, such as aminoglycosides and quinolones. (Barbieret al. 2015). Therefore, the selection of antibacterial against ESBL organisms in clinical practice is often complicated.

* 1. **ANTIBIOTIC RESISTANCE MECHANISM**
		1. **By Hydrolysis**

Many antibiotics have chemical bonds such as amides and esters which are hydrolytically susceptible. Several enzymes are known to ruin antibiotic activity by targeting and cleaving these bonds. These enzymes can often be excreted. Extended-spectrum β-lactamases (ESBLs) mediate resistance to all penicillins, third generation cephalosporins (e.g. ceftazidime, cefotaxime, and ceftriaxone) and aztreonam, but not to cephamycins (cefoxitin and cefotetan) and carbapenems (Danish *et al* 2015)

* + 1. **By Redox process**

The pathogenic bacteria infrequently exploited oxidation or reduction of antibiotics. However, there are a few examples of this strategy. One is the oxidation of tetracycline antibiotics by the TetX enzyme. Streptomyces virginiae, a producer of the type a streptogramin antibiotic virginiamycin M1, protects itself from its own antibiotic by reducing a critical ketone group to an alcohol at position 16 (Danish *et al* 2015).

* + 1. **Antibiotic inactivation by group transfer**

The most diverse family of resistant enzymes is the group of transferases. These enzymes inactivate antibiotics (aminoglycosides, chloramphenicol, streptogramin, macrolides or rifampicin) by chemical substitution (adenylyl, phosphoryl or acetyl groups are added to the periphery of the antibiotic molecule). The modified antibiotics are impaired in their binding to a target. Chemical strategies include O-acetylation and N-acetylation, O-phosphorylation, O-nucleotidylation, O-ribosylation, O-glycosylation, and thiol transfer. These covalent modification strategies all require a co-substrate such as ATP, acetyl-CoA, NAD+, UDP-glucose, or glutathione for their activity and consequently these processes are restricted to the cytoplasm. (Danish *et al* 2015)

* + 1. **Antibiotic resistance via target modification**

The second major resistance mechanism is the modification of the antibiotic target site so that the antibiotic is impotent to bind properly. However, it is possible for mutational changes to occur in the target that reduce susceptibility to inhibition while retaining cellular function (Danish *et al* 2015)

## **GENETICS OF ANTIBIOTIC RESISTANCE:** There are two types

## **Antibiotic resistance via mutations:**

## There is a substantial number of biochemical mechanisms of antibiotic resistance that are based on mutational events, like the mutations of the sequences of genes encoding the target of certain antibiotics (e.g. resistance to rifampicin and fluoroquinolones is caused by mutations in the genes encoding the targets of these molecules, RpoB and DNA-topoisomerases, respectively) The variation in the expression of antibiotic uptake or of the efflux systems may also be modified by mutation (e.g. the reduced expression or absence of the OprDporin of Pseudomonas aeruginosa reduces the permeability of the cell wall to carbapenems) (Danish *et al* 2015)

## **Antibiotic resistance via horizontal gene transfer**

## A principal mechanism for the spread of antibiotic resistance is by horizontal transfer of genetic material. Antibiotic resistance genes may be transferred by different mechanisms of conjugation, transformation or transduction. Over the last 15 years, β-lactamase enzymes that have an extended spectrum of activity (ESBL) against the majority of β-lactams, including cephalosporins but not carbapenemases, have evolved. One of these, CTX-M-15, initially found in E. coli but now found in other members of Enterobacteriaceae and frequently associated with a specific lineage, uropathogenic clone ST131 ([Bush and Fisher, 2011; Woodford](https://www.sciencedirect.com/science/article/pii/S1319562X14000941%22%20%5Cl%20%22b0085) *[et al](https://www.sciencedirect.com/science/article/pii/S1319562X14000941%22%20%5Cl%20%22b0085)*[., 2011](https://www.sciencedirect.com/science/article/pii/S1319562X14000941%22%20%5Cl%20%22b0085)), has spread worldwide. It is often located on highly mobile IncFII plasmids and associated with mobile genetic element IS26. The risk of infection is particularly high in individuals in association with prolonged hospitalization, catheterization, nursing home residency, previous antibiotic treatment, underlying renal or liver pathology, and travel to high-risk areas ([Livermore](https://www.sciencedirect.com/science/article/pii/S1319562X14000941%22%20%5Cl%20%22b0310) *[et al](https://www.sciencedirect.com/science/article/pii/S1319562X14000941%22%20%5Cl%20%22b0310)*[., 2011](https://www.sciencedirect.com/science/article/pii/S1319562X14000941%22%20%5Cl%20%22b0310)).

Clinical isolates of *Escherichia coli* were collected from Microbiology Laboratory unit of a tertiary hospital (Ebonyi State University teaching hospital, Abakaliki, EBSUTH) and a secondary hospital (Federal Medical Center, Abakaliki, FMC) from four different clinical specimens (urine, stool, blood and sputum) between February to November 2006. Sixty-three clinical isolates of *Escherichia coli* were isolated from EBSUTH while forty-six were from FMC Abakaliki. These organisms were characterized and identified to species level using standard identification technique. Sensitivity studies were carried out on the test organisms using disc diffusion method and later the organisms were characterized phenotypically for ESBL production using the Double Disc Synergy Test (DDST). A preliminary molecular characterization of the ESBL producing isolates were further carried out based on the evaluation of their plasmid profile via agarose gel electrophoresis. The over-all result of the study revealed that the prevalence of ESBL producing organisms was high 18 (16.5%) in our environment. The rate of occurrence varied within the two hospitals with 11 (23.9%) from FMC (urine 2 (18.2%), blood 5 (35.7%), wound 3 (30%) semen 1 (33.3%) and none was isolated from sputum while 7(11.1%) were from EBSUTH (urine 2(9.5%), blood 3 (21.4%), wound 2 (18.2%) respectively and none was isolated from sputum and semen. The plasmid profile studies revealed the presence of low molecular weight plasmid DNA within the ranges of 21.3-29.4 kb. The ESBL-producing isolates were predominantly isolated from blood samples followed by wound, urine and sputum. The prevalence rate of ESBL producers in FMC (a secondary hospital) was higher than in EBSUTH (a tertiary hospital). Previous studies show that ESBL producers are more prevalent in a tertiary hospital than in secondary hospitals (Adikwu*et al*., 2003). This is because it is thought that 2nd and 3rd generation cephalosporins are used more often in tertiary hospitals for life threatening infections than in secondary hospitals (Adikwu*et al.,* 2008).

**2.4 TREATMENT**

The carbapenems (imipenem, meropenem, ertapenem, doripenem) are still the first choice of treatment for serious infections with ESBL-producing E. coli and K. pneumoniae. It has been reported that more than 98% of the ESBL-producing E. coli, K. pneumoniae and *Proteus mirabilis* (P. mirabilis) are still susceptible to these drugs. But with the emergence of the carbapenem-resistant Enterobacteriaceae, the “magic bullet” is actually difficult to find. There are some older drugs which can be used to treat the ESBL-producing E. coli or K. pneumoniae infections. Fosfomycin was reported of having admirable in vitro activity against the ESBL-producing E. coli or K. pneumoniae. In Hong Kong, most of the ESBL-producing E. coli isolates were reported to be sensitive to fosfomycin ([Ho et al., 2010](https://www.sciencedirect.com/science/article/pii/S1319562X14000941%22%20%5Cl%20%22b0195)).

Colistin is another choice which we can consider for the treatment of these organisms. Although once considered as quite a toxic antibiotic, it is a last resort that we can consider at the present moment as there is no new anti-gram negative antibiotics available for the treatment of these multidrug resistant organisms. Other than ESBL-producing organisms, actually colistin is used in the treatment of multidrug resistant *Proteus*aeruginosa, carbapenem resistant *Acinetobacterbaumannii (*A. baumannii)*.* Close monitoring for the development of side effects can improve the safety margin when prescribing the drug. Tigecycline is also one of the drugs in the pipeline which can be considered for treatment (Danish *et al* 2015).

**2.5 PREVENTION AND CONTROL**

Proper infection-control practices and barriers are essential to prevent spreading and outbreaks of ESBL-producing bacteria. The reservoir for these bacteria seems to be the gastrointestinal tract of patients. Alternative reservoirs could be the oropharynx, colonized wounds and urine. The contaminated hands and stethoscopes of healthcare providers are important factors in spreading infection between patients. Essential infection-control practices should include avoiding unnecessary use of invasive devices such as indwelling urinary catheters or IV lines, hand washing by hospital personnel, increased barrier precautions, and isolation of patients colonized or infected with ESBL producers. (Deepthi*et al* 2010)

**2.6 OBJECTIVES**

The aim of this present study is to phenotypically identify and establish the presence of ESBL-producing organism among students in the university community.

**CHAPTER THREE**

**3.1 MATERIALS**

Nutrient agar, Mueller hinton agar, MacConkey agar, distilled water, crystal violet, Gram’s iodine, 95% alcohol, safranin , tryptone broth, xylene, Kovac’s reagent, glucose phosphate broth, methyl red indicator, peptone water, alpha naphthol, potassium hydroxide, antibiotics disc, test tubes, bunsen burner, sterile bottles, bijour bottles, wireloop, petri dishes, sulphuric acid, barium chloride, pipette, conical flask, etc.

**3.2 METHODS**

**3.2.1 SAMPLE COLLECTION**

Within the university community of Godfrey okoye university, Enugu, early morning urine samples of midstream-catch were collected into sterile bottles from sixty students between ages 18 – 25years within the month of May. Thirty male and thirty female.

**3.2.2 CULTURE MEDIA PREPARATION:**

**3.2.2.1 Preparation of Nutrient agar**

A 3.8g of Nutrient agar was dissolved in 100ml of distilled water by heating over a Bunsen flame. It was dissolved at 121oC for 15minutes and allowed to cool to a temperature of 400C after which it was poured aseptically into petri dishes and left to solidify.

**3.2.2.2 Preparation of MacConkey agar**

A 5.2g of MacConkey agar was dissolved in 100ml of distilled water by heating over a Bunsen flame. It was dissolved at 1210C for 15minutes and allowed to cool to a temperature of 400C after which it was poured aseptically into petri dishes and left to solidify.

**3.2.2.3 Preparation of Mueller Hinton agar**

A 5.2g of Mueller Hinton agar was dissolved in 100ml of distilled water by heating over a Bunsen flame. It was dissolved at 1210C for 15minutes and allowed to cool to a temperature of 400C after which it was poured aseptically into petri dishes and left to solidify.

**3.2.2.4 Preparation of McFarland turbidity equivalent standard**

A 1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water. Also, 1%w/v barium chloride was prepared by dissolving 0.5g of dehydrated barium chloride in 50ml distilled water. Then 0.5 McFarland standard equivalent was prepared by adding 0.6ml of barium chloride solution to 99.4ml of Sulphuric acid solution.

**3.2.2.5 Preparation of culture broth**

A 3ml volume of sterile nutrient broth was prepared and test organisms were inoculated into the nutrient broth and allowed to cool. It was poured into plates with combs and left to set.

**3.3 IDENTIFICATION OF ORGANISM**

**3.3.1 Biochemical: Gram staining**

A clean, grease free slide was taken. The smear of suspension was prepared on the clean slide with a loopful of sample. It was air dried and heat fixed. Crystal Violet was then poured on the slide and kept for 40 seconds for primary staining and rinsed with water. It was then flooded with gram’s iodine for 1 minute and washed with water. It was thereafter washed with 95% alcohol for about 15 seconds and rinsed with water. Safranin was added for about 1 minute for counterstaining and washed with water. Eventually, the slide was air dried, and observed under microscope.

**3.3.2 IMViC**: Each of the letters in “IMViC” stands for one of these tests. “I” is for indole; “M” is for methyl red; “V” is for Voges Proskauer, and “C” is for citrate, lowercase “i” is added for the ease of pronunciation. IMViC is an acronym that stands for four different tests. (Tankeshwar, 2013).

**3.3.2.1 Indole Test**

Tryptone broth was prepared, distributed in test tube and sterilized by autoclaving. The sterile tryptone broth tubes were inoculated with loopful of suspension and incubated at 37° C for 24 hours. After incubation, 3-4 drops of xylene was added in tubes and shook vigorously. Then the tubes were kept still so that two layers get separated. After separation of two layers, 1 ml of Kovac’s reagent or Ehrlich’s reagent were added and tubes were observed for formation of pink colour ring.

**3.3.2.2 Methyl Red Test**

Glucose phosphate broth was prepared and distributed in test tubes. This test tubes were further sterilized by autoclaving. The sterile test tubes were then inoculated with test culture and incubated at 37°C for 24 hours. After incubation, five drops of methyl-red indicator was added to the medium and the tubes were observed for development of red colour.

**3.3.2.3 Voges-Proskauer (V-P) Test**

Glucose phosphate broth was prepared and distributed in test tubes. These test tubes were further sterilized by autoclaving. The sterile test tubes were inoculated with test culture and incubated at 37°C for 24 hours. After incubation, 0.6 ml of alpha naphthol and 0.2 ml of KOH solution per ml of culture broth media were added. Further after addition of these two reagents, the culture tubes were shook properly and kept in slanting position to increase aeration. These tubes were kept in slanting position for about one hour and then results were noted down.

**3.3.2.4 Citrate Utilization Test**

**Composition of Simmons’s citrate agar**

* Sodium citrate                                     –0.2 gm
* Magnesium sulphate                          –0.02 gm
* Sodium chloride                                  –0.5 gm
* Ammonium dihydrogen phosphate – 0.1 gm
* Dipotassium phosphate                      – 0.1 gm
* Bromothymol blue                               – 0.008 gm
* Agar-agar                                               – 3 gm
* Distill water                                            – 100 ml
* pH                                                             – 7

The Simmons’s agar was prepared according to the composition given above, sterilized and after sterilization the slants of this media were prepared. Further this slant are streaked heavily on the slant and incubated for 24 hours at about 37°C. After incubation the slant were observed for change in colour and the results were recorded.

**3.3.3COAGULASE TEST**

An emulsification of the staphylococcal colony was made in a drop of water on a clean and grease free glass slide with a minimum of spreading and showed a milky color. Similar suspensions of control positive and negative strains were made to confirm the proper reactivity of the plasma. A sterile wire loop was used to pick the undiluted plasma into the staphylococcal suspension, and was stirred on the slide. The wire loop was flamed and same process was repeated for the control suspensions. A clumping was observed and recorded.

 **3.4 ANTIBIOTIC SUSCEPTIBILITY TESTING:**

A 20ml volume of Mueller Hinton agar was prepared and dispensed aseptically into petri dishes. A 0.1ml suspension of each of the isolates equivalent to 0.5ml McFarland standard was aseptically seeded into the Mueller Hinton agar plate. This was allowed to dry. The antibiotic disc was aseptically placed on the surface of the Mueller Hinton agar and allowed for 30minutes to pre-diffuse. The set up was done in triplicate with a control containing no antibiotic disc. It was incubated for 24hours at 370C and thereafter, the inhibition zone diameters were observed.

**3.5 DOUBLE DISC SYNERGY TEST: DDST**

A 20ml volume of Mueller Hinton agar was prepared and dispensed aseptically into petri dishes. A 0.1ml suspension of each of the isolates equivalent to 0.5ml McFarland standard was aseptically seeded into the petri dishes together with Mueller Hinton agar. This was allowed to dry. A combination disc (Amoxicillin 20ug and Clavulanic acid 10ug combination) was placed at the center of the dish and antibiotics (Ceftazidime-CAZ 30ug and Cefotaxime - CTX 30ug) were placed 15mm apart center to center on both sides of the plates.

The set up was done in triplicate and it was left for 30minutes to pre-diffuse into the medium. It was incubated at 370C for 24 hours after which the various inhibition zone diameters were measured.

**CHAPTER FOUR**

**4.1 RESULTS**

Culture growth: Of the sixty (60) samples cultured, eighteen (18) isolates were obtained. Twelve (12) isolates from the female’s samples and six (6) isolates from the male.

**TABLE 1: RESULT OF GRAM REACTION**

|  |  |  |
| --- | --- | --- |
| s/n | GRAM REACTION | PROBABLE ORGANISM |
| G01 | + cocci in clusters | *Staphylococcus* sp |
| G02 | + cocci in clusters | *Staphylococcus* sp |
| G03 | + cocci in clusters | *Staphylococcus* sp |
| G04 | - bacilli (scattered) | *Escherichia coli* |
| G05 | + cocci in chains | *Streptococcus* sp |
| G06 | + cocci in chains | *Streptococcus* sp |
| G07 | + cocci in chains | *Streptococcus* sp |
| G08 | + cocci in clusters | *Staphylococcus* sp |
| G09 | + cocci in chains | *Streptococcus* sp |
| G10 | + cocci in chains | *Streptococcus* sp |
| G11 | + cocci in chains | *Streptococcus* sp |
| G12 | + bacilli in chains  | *Corynebacterium*sp |
| B13 | + bacilli in chains  | *Corynebacterium*sp |
| B14 | + diplococci in chains | *Streptococcus* sp |
| B15 | + cocci in chains | *Streptococcus* sp |
| B16 | + bacilli in chains  | *Corynebacterium*sp |
| B17 | + cocci in clusters | *Staphylococcus* sp |
| B18 | + cocci in chains | *Streptococcus* sp |

**TABLE 2: FREQUENCY OF PROBABLE ORGANISMS FROM ISOLATES**

|  |  |
| --- | --- |
| PROBABLE ORGANISMS | FREQUENCY OF ISOLATES |
| ***Streptococcus* spp** | **9** |
| ***Corynebacterium*spp** | **3** |
| ***Staphylococcus* spp** | **5** |
| ***Escherichia coli*** | **1** |
| **TOTAL NUMBER OF PROBABLE ORGANISM** | **18** |

**TABLE 3: IMViC TEST RESULT AND COOAGULASE TEST RESULT**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S/N** | **PROBABLE ORGANISMS** | **FREQUENCY OF PROBABLE ORGANISMS** | **INDOLE TEST** | **METHYL RED** | **VOGES PROSKAURE** | **CITRATE UTILISATION TEST** | **COAGULASE TEST** |
| **1** | *Streptococcus* spp | **9** | **-** | **+** | **-** | **+** | **-** |
| **2** | *Staphylococcus* spp | **5** | **+** | **+** | **-** | **+** | **+** |
| **3** | *Corynebacterium*spp | **3** | **-** | **+** | **-** | **+** | **-** |
| **4** | *Escherichia coli* | **1** | **+** | **+** | **-** | **-** | **-** |

**KEY:** + Positive

 - Negative

**TABLE 4: ANTIBIOTICS SENSITIVITY TEST RESULT**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S/N** | **PROBABLE ORGANISMS** | **PEF** | **GN** | **APX** | **Z** | **AM** | **R** | **CPX** | **S** | **SXT** | **E** |
| **G01** | *Staphylococcus* sp | **0** | **0** | **0** | **0** | **0** | **15** | **0** | **15** | **0** | **0** |
| **G02** | *Staphylococcus* sp | **0** | **15** | **0** | **0** | **0** | **0** | **28** | **0** | **16** | **0** |
| **G03** | *Staphylococcus* sp | **27** | **19** | **0** | **0** | **0** | **21** | **28** | **0** | **16** | **0** |
| **G04** | *Escherichia coli* | **12** | **15** | **26** | **12** | **27** | **26** | **26** | **27** | **12** | **18** |
| **G05** | *Streptococcus* sp | **21**  | **16** | **0** | **0** | **0** | **19** | **25** | **19** | **18** | **17** |
| **G06** | *Streptococcus* sp | **15** | **0** | **0** | **0** | **0** | **0** | **26** | **18** | **0** | **0** |
| **G07** | *Streptococcus* sp | **25** | **15** | **0** | **0** | **0** | **21** | **14** | **22** | **20** | **23** |
| **G08** | *Staphylococcus* sp | **22** | **18** | **13** | **15** | **18** | **24** | **22** | **26** | **23** | **26** |
| **G09** | *Streptococcus* sp | **31** | **16** | **0** | **0** | **0** | **18** | **30** | **22** | **25** | **15** |
| **G10** | *Streptococcus* sp | **25** | **0** | **0** | **0** | **0** | **25** | **32** | **14** | **0** | **8** |
| **G11** | *Streptococcus* sp | **18** | **10** | **0** | **0** | **0** | **19** | **24** | **25** | **14** | **0** |
| **G12** | *Corynebacterium*sp | **21** | **21** | **8** | **0** | **0** | **20** | **32** | **28** | **21** | **24** |
| **B13** | *Corynebacterium*sp | **29** | **19** | **0** | **0** | **8** | **22** | **34** | **25** | **0** | **19** |
| **B14** | *Streptococcus* sp | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **8** | **0** |
| **B15** | *Streptococcus* sp | **24** | **20** | **0** | **0** | **0** | **20** | **26** | **16** | **20** | **22** |
| **B16** | *Corynebacterium*sp | **22** | **16** | **12** | **0** | **13** | **21** | **15** | **20** | **15** | **16** |
| **B17** | *Staphylococcus* sp | **32** | **19** | **18** | **0** | **20** | **21** | **32** | **0** | **20** | **22** |
| **B18** | *Streptococcus* sp | **25** | **0** | **0** | **0** | **0** | **17** | **35** | **16** | **14** | **13** |

**KEY:** PEF – Pefloxacin, GN – Gentamicin, APX – Ampiclox, Z – Zinnacef, AM –Amoxacilln, R – Rocophin, CPX – Ciprofloxacin, S – Streptomycin, SXT – Septrin, and E –Erythromycin.

**TABLE 5: PHENOTYPIC CONFIRMATION OF ESBL PRODUCTION IN DOUBLE DISC SYNERGY TEST (DDST).**(Inhibition zone diameter measured in mm)

**S/N PROBABLE ORGANISMS CAZ AMZ CTX**

G01 *Staphylococcus* sp 0 0 0

G02 *Staphylococcus* sp 0 0 0

G03 *Staphylococcus* sp 22 25 36

G04 *Escherichia coliI* 9 10 8

G05 *Streptococcus* sp 0 12 0

G06 *Streptococcus* sp 9 15 18

G07 *Streptococcus* sp 0 10 0

G08 *Staphylococcus* sp 0 0 0

G09 *Streptococcus* sp 12 16 20

G10 *Streptococcus* sp 13 0 0

G11 *Streptococcus* sp 0 0 0

G12 *Corynebacterium*sp 0 0 0

B13 *Corynebacterium*sp 16 0 13

B14 *Streptococcus* sp 0 0 0

B15 *Streptococcus* sp 0 10 0

B16 *Corynebacterium*sp 0 0 0

B17 *Staphylococcus* sp 15 0 0

B18 *Streptococcus* sp 0 0 0

**KEY:** CAZ –Ceftazidime; AMC - Amoxycillin - clavulanic acid; CTX - Cefotaxime

****

**FIG 2: CAZ\_\_\_\_\_\_\_\_\_\_\_\_\_\_AUG\_\_\_\_\_\_\_\_\_\_\_\_\_CPX**

There was no visible increased susceptibility to both antibiotics disc used.

**KEY:** CAZ – Cefotaxime

AMC – Amoxycillin – clavulanic acid

CTX –Ceftazidime

**CHAPTER FIVE**

**DISCUSSION AND CONCLUSION**

**5.1 DISCUSSION**

Antimicrobial resistance has been declared a global threat to public health, as a massive increase in this problem has been observed in different parts of the world (Kang and Song 2013).The alarming increase in the prevalence of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae has serious consequences for treatment outcomes (Pitout 2010).The ESBL enzymes produced by these bacteria make them resistant to the first-choice antibiotic therapies that are commonly used.This research was aimed at phenotipically identifying the presence of ESBL producing organism among a few (60) students of Godfrey Okoye University. Of thesixty (60)mid-stream catch urine samples collected, eighteen (18) isolates were identified with only one Gram negative organism. The antimicrobial sensitivity tests carried out showed an important level of sensitivity.The Double Disc Synergy Test was then carried outto phenotypically confirm the presence of ESBL by placing ceftaxidime (CAZ), amoxicillin-clavulanic acid disc (AMX) and cefotaxime (CTX) 15mm apart. As shown in the result, none of the isolates had an increase in inhibition diameter suggesting the absence of ESBL, including the Gram negative *E. coli*. This result may require a molecular analysis to confirm the absence of these class of resistant organisms.

The findings of this study confirms that most ESBL producing organism are of Gram negative origin which may explain the absence of positive outcomes in the study.

**5.2 CONCLUSION**

High increase of Gram positive organisms suggests normal flora of the urinary tract and poor personal hygiene.

This findings shows the absence of ESBL producing organism among the tested students of Godfrey Okoye University. This may be due to controlled use of antibiotics and level of education.

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