International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Phenotypic Characterization of Extended Spectrum Beta-Lactamase (ESBL) - Producing Clinical Isolates of *Escherichia Coli* And *Klebsiella* Spp from Mile Four General Hospital, Abakaliki

[1]Iroha I. R., [2]Igwe O. F., [1]Moses I. B., [3]Iroha C. S., [1]Nwakaeze E. A., [1]Ejikeugwu P. C. [4]Ajah M. I., [1]Nwuzo A. C., [1]Afiukwa F. N., [5]Eluu S. C.

[1]Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, P.M.B. 053, Abakaliki, Ebonyi State, Nigeria.
[2]Department of Microbiology, Federal Polytechnic Uwana, Afikpo.
[3]Pharmacy Department, Federal Teaching Hospital, Abakaliki.
[4]Cancer Screening Unit, Well Women Centre, Federal Teaching Hospital, Abakaliki.
[5]Department of Biotechnology, Faculty of Science, Ebonyi State University, P. M. B. 053 Abakaliki, Ebonyi State, Nigeria.

Corresponding Author: Moses I. B.

Received: 11/10/2016 *Revised: 05/11/2016* *Accepted: 28/11/2016*

ABSTRACT

The objective of this study was to phenotypically characterize extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates obtained from Mile Four General Hospital, Abakaliki. One hundred and three (103) clinical Gram-negative bacteria isolates were obtained from 657 clinical samples (urine, sputum, pus, cerebrospinal fluid, ear swab, high vagina swab, stool, wound swab and semen). Twenty of the clinical isolates were identified as *Escherichia coli* while 83 were *Klebsiella*s pp based on cultural, morphological and biochemical techniques. The phenotypic screening of the 103 bacterial isolates for ESBL production was done by disc diffusion method using second and third generation cephalosporins. The resistant strains were further tested for ESBL production using ceftazidime, cefotaxine and amoxicillin/clavulanic acid, a method called double disc diffusion. Susceptibility of the ESBL-producing bacterial isolates to antibiotics was done on Muller-Hinton agar by Kirby-Bauer disc diffusion methods using the following antibiotics; sulfamethoxazole/trimethoprim, nitrofurantion, Nalidixic acid, tobramycin, ofloxacin, cefoxitin, ertapenem, ciprofloxacin and gentamycin. ESBL production was observed in 8 (7.76 %) *E. coli* and 13 (12.6 %) *Klebsiella* spp. isolates. *E. coli* and *Klebsiella* spp. were highly resistant to sulfamethoxazole and nitrofurantion with a resistance frequency ranging from 60 % to 82 % while gentamicin was the most active antibiotic against the bacterial isolates as they were 100 % susceptible to this antibiotic. This was closely followed by ertapenem (85 %) and ciprofloxacin (82 %). ESBL production is still one of the major mechanisms of drug resistance among Enterobacteriaceae in hospitals. Hence, there is need for more prevalence/surveillance studies to curtail its spread.

Keywords: Extended Spectrum beta-lactamases, bacterial isolates, clinical samples, Mile four, *E. coli* and *Klebsiella* spp.

INTRODUCTION

The increasing prevalence of antibiotic resistant microorganisms, especially those with multidrug resistance mechanisms such as extended spectrum beta-lactamases is of global concern as they are known to make the treatment of bacteria-related infections difficult (Jacoby

et al., 2005). In addition to this, antibiotic resistant bacteria may also lead to increase in the length of hospitalization of a patient, severity of illness and the overall cost of treatment. The emergence and spread of extended-spectrum beta lactamases (ESBLs) which initially looked benign has become one of the major resistance problems that now bedevil our health sector around the world, putting the available antibiotics for treatment of bacteria-related infections into risk (SCIEH, 2004). Since beta-lactam antibiotics came into clinical use after their discovery some decades ago, enzymes that destroy and cause resistance to the beta-lactam drugs have also co-evolved with them (Jacoby *et al*., 2005). Early in the 1980s, expanded-spectrum antibiotics including cephalosporins with an oxyimino side chain, carbapenems and the monobactams were introduced into clinical practice for the treatment of bacterial related infections caused by bacteria that were resistant to the penicillins because these organisms produce beta-lactamase enzymes which destroy/inactivate and cause resistance to the penicillins. The introduction of these broad-spectrum antibiotics into clinical practice was largely heralded as a major breakthrough in the fight against bacteria resistance to drugs owing to the fact that these antibiotics are stable to the earlier beta-lactamases and are also an important tool for the treatment of severe bacterial infections (Cindy, 2011). Extended spectrum beta-lactamases (ESBLs) are plasmid-mediated beta-lactamases capable of hydrolyzing many beta-lactam antibiotics including third-generation cephalosporins and monobactams (Abhilash *et al*., 2010), but are inhibited by clavulanic acid, a beta-lactamase inhibitor (Bonnet, 2004). Since the discovery of the ESBLs in the 1980's, over100 different enzymes have been described, and they have become a worldwide health problem affecting many countries of the world with varying prevalence (SCIEH, 2004). ESBLs arise by mutation in genes for common plasmid-mediated beta-lactamases (especially TEM and SHV enzymes) that alter the amino acid configuration of the enzyme near its active site to increase the affinity and hydrolytic ability of the beta-lactamases for oxyiminocephalosporins (Jac oby *et al*., 1996). ESBLs are detected mostl y in Enterobacteriaceae especially *Klebsiella pneumoniae*, *Escherichia coli*, and *Klebsiella oxytoca*. They often contain resistance determinants for other classes of antibiotics such as the aminoglycosides, sulfonamides and fluoroquinolones which are readily transmissible from one strain of organism to another and between different species of Gram-negative bacteria (Mehrgan *et al*., 2010). Resistance of Gram-negative bacteria to third-generation cephalosporins is mediated by ESBLs and they are an important cause of treatment failure in patients receiving cephalosporins (IDSA, 2006) because these agents are hydrolyzed *in vivo* by ESBLs when used for therapy especially in patients harbouring ESBL-producing bacteria. Infections caused by ESBL-producing bacteria have continued to be associated with high rates of mortality, and high health cost. They are some of the most important pathogenic bacteria in clinical medicine, and it has been reported that they acquire a transmissible form of antibiotic resistance genes including ESBLs and via genetic transfer routes such as conjugation (STRAMA, 2007). This implies that some groups of powerful antibiotics including penicillins, carbapenems, cephalosporins to mention but a few, which have been used to treat infections such as urinary tract infections (UTIs), post-operative infections and blood infections (bacteremia and septicemia) are no longer effective to a large extent as they used to be during their first introduction into clinical medicine some decades ago. This study evaluates the prevalence of extended spectrum beta-lactamases in clinical isolates of *E. coli* and *Klebsiella* spp. from Mile Four General Hospital Abakaliki.

MATERIALS AND METHODS

Sample collection

One hundred and three (103) Gram negative bacteria were isolated from six hundred and fifty seven (657) clinical samples collected over a period of twelve months (April, 2015 to March 2016). The 103 Gram negative bacteria were isolated from urine (98), sputum (168), pus (49), CSF (63), ear swab (31), HVS (87), stool (72), wound swab (35) and semen (54) from out-patients in Mile 4 general hospital and immediately transported to the Department of Applied Microbiology laboratory unit of Ebonyi State University for bacteriological analysis. The samples were aseptically inoculated on the surface of agar (nutrient, CLED and MacConkey) by collecting a loopful of the homogenized samples and carefully streaked using sterile inoculating loop. This was incubated for 18-24 hours at 37 °C and observed for microbial growth.

Isolation, characterization and identification of the isolates

The isolates from clinical specimens were characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining, catalase test, motility test and other biochemical tests which include oxidase test, indole test, coagulase test, simmon's citrate test, H_2S production test, voges proskauer test, methyl red test and sugar fermentation test. (Alten *et al*., 2009). The isolates were further confirmed using API kits.

Screening for ESBL production by clinical isolates of *E. coli* and *Klebsiella* spp.

All standardized clinical isolates of *E. coli* and *Klebsiella* spp. isolated from various clinical samples were screened for ESBL production by aseptically placing antibiotic disks namely cefotaxime (CTX, 30 µg), cefuroxime (CXM, 30 µg), aztreonam (ATM, 30 µg), cefepime (FEP, 30 µg) and ceftazidime (CAZ, 30 µg) on the surface of Mueller-Hinton (MH) agar plates (Oxoid, UK). The plates were allowed to stand for about 30 minutes for pre-diffusion of the antibiotics; and were then incubated for 18-24 hours at 37 °C. After incubation, the zones of inhibition were measured in millimeter using a metre rule. ESBL production was suspected if any of the test bacteria showed reduced susceptibility or i s resistant to any one of the third generation cephalosporins (cefotaxime 30 µg, ceftazidime 30 ug, cefuroxime 30 µg, aztreonam 30 µg and cefepime 30 µg) used for the screening studies according to the CLSI guidelines (CLSI, 2009; Iroha *et al*., 2008).

Double disk synergy test (DDST)

ESBL production was confirmed in clinical isolates of *E. coli* and *Klebsiella* spp. that were resistant to any of the cephalosporins used in the preliminary testing by double disc synergy test (Iroha *et al*., 2008a and Aibinu *et al*., 2007). DDST was performed as a standard disk diffusion assay on Mueller-Hinton (MH) agar (Oxoid, UK) plates in line with CLSI criteria (CLSI, 2009). Sterile swab sticks were dipped into bacterial suspension(s) standardized to 0.5 McFarland turbidity standards, and were spread on prepared Mueller-Hinton (MH) agar plates. Antibiotic discs of amoxicillin/clavulanic acid (20/10 µg) was placed at the center of the MH agar plate and antibiotic disks containing cefotaxime (30 ug) and ceftazidime(30 µg) were each placed at a distance of 15 mm (centre to centre) from the central disc, amoxicillin/clavulanic acid 20/10 µg. The plates were incubated at 37 °C for 18-24 hours. ESBL production was inferred phenotypically when the zones of inhibition of the cephalosporins; cefotaxime(30 ug) and ceftazidime(30 µg) were expanded by the amoxicillin/clavulanic acid disk (20/10 µg). A 5 mm increase in the inhibition zone diameter for either of the cephalosporins (ceftazidime and cefotaxime) tested in combination with amoxicillin/clavulanic acid versus its zone of inhibition when tested alone confirms ESBL production phenotypically (Iroha *et al*., 2008a; Bradford, 2001).

ANTIBIOTICS SUSCEPTIBILITY STUDIES

The susceptibility patterns of isolated ESBL-producing *E. coli* and *Klebsiella* spp. were determined by the Kirby and Bauer susceptibility test method as recommended by CLSI (CLSI, 2009). Each of the isolate was standardized to 0.5 Macfarland equivalent and aseptically inoculated on prepared Muller-Hinton agar plates using sterile swab stick. The inoculated plates were allowed to stand for 10-15 minutes. Antibiotic impregnated discs namely cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), cefuroxime (CXM, 30 µg), aztreonam (ATM, 30 µg), cefepime (FEP, 30 µg), ampicillin (AMP, 10 µg), tetracycline (TE, 30 µg), ciprofloxacin (CIP, 10 µg), gentamicin (CN, 10 µg), ofloxacin (OFX, 5 µg), amikacin (AK, 10 µg), sulfamethoxazole/trimethoprim (SXT, 25 µg), nitrofurantoin (F, 300 µg), amoxicillin clavulanic acid(AMC, 30 µg), cefoxitin (FOX, 10 µg), ertapenem (ETP, 30 µg) and nalidixic acid (NA, 30 µg) (Oxoid, UK) were placed on the inoculated plates using sterile forceps. The plates were incubated at 37 ^{0}C for 24 hours after which the zones of inhibition around each disc were measured to the nearest mm with a metre rule, recorded and interpreted according to the Clinical Laboratory Standard Institutes (CLSI, 2009).

Determination of multiple antibiotic resistance index (MARI)

Multi-drug resistance index was done to ascertain the resistance level of the isolates; that is, the numbers of antibiotics the isolates were resistant to.

MARI = a/b where; a = number of antibiotics to which the isolates were resistant; b = total number of antibiotics to which the isolates were subjected.

RESULTS

Table 1: Clinical samples positive for Gram-negative bacteria pathogens

Isolate Number	Clinical Source	Number of clinical sample collected	Number and type of organism isolated from each specimen		Percentage (%)
			E. coli	*Klebsiella* spp.	
1	Urine	98	3	16	18.4
2	Sputum	168	2	34	34.9
3	Pus	49	2	2	3.88
4	CSF	63	2	6	7.77
5	Ear Swab	31	0	1	1.0
6	HVS	87	5	12	16.5
7	Stool	72	5	7	11.7
8	Wound swab	35	1	1	1.94
9	Semen	54	0	4	3.88
Total		**657**	**20**	**83**	**100**

Key: HVS = High vaginal swab, CSF= Cerebrospinal flui

	MALE								FEMALE							
Age range	**Urine**	**Stool**	**Wound swab**	**Sputum**	**Ear Swab**	**CSF**	**Semen**	**Pus**	**Urine**	**Stool**	**Wound swab**	**Sputum**	**Ear Swab**	**CSF**	**Pus**	**HVS**
1-20 yrs.	*E. coli* (0) *K.*spp. (0)	*E. coli* (4) *K.*spp. (2)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (2)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (1)	*E. coli* (1) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (2)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (2)
21-40yrs	*E. coli* (1) *K.*spp. (2)	*E. coli* (2) *K.*spp. (0)	*E. coli* (0) *K.*spp. (1)	*E. coli* (1) *K.*spp. (7)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (3)	*E. coli* (0) *K.*spp. (3)	*E. coli* (0) *K.*spp. (0)	*E. coli* (2) *K.*spp. (10)	*E. coli* (0) *K.*spp. (2)	*E. coli* (0) *K.*spp. (1)	*E. coli* (0) *K.*spp. (9)	*E. coli* (0) *K.*spp. (1)	*E. coli* (0) *K.*spp. (2)	*E. coli* (0) *K.*spp. (0)	*E. coli* (3) *K.*spp. (7)
41-60yrs	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (1)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (4)	*E. coli* (0) *K.*spp. (0)	*E. coli* (1) *K.*spp. (0)	*E. coli* (0) *K.*spp. (1)	*E. coli* (0) *K.*spp. (2)	*E. coli* (0) *K.*spp. (1)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (2) *K.*spp. (6)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (2) *K.*spp. (3)
61-80yrs	*E. coli* (1) *K.*spp. (1)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (2)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (2)	*E. coli* (0) *K.*spp. (0)	*E. coli* (1) *K.*spp. (1)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (1)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)	*E. coli* (0) *K.*spp. (0)
Total	5	9	1	16	0	6	4	4	14	3	1	20	1	2	0	17

Table 2: Distribution of clinical bacteria isolates from male and female patients

Key: HVS = High vaginal swab, CSF = Cerebrospinal fluid, *E. coli* = *Escherichia coli*, *K.* spp. = *Klebsiella* species

Table 3: Prevalence of ESBL among clinical bacterial isolates

Organism	Number of isolates screened for ESBL potential	ESBL positive (%)	ESBL Negative (%)
E. coli	20	8(7.76)	12(11.6)
*Klebsiella*spp.	83	13(12.6)	70(67.96)
Total	103	21(20.4)	82 (79.6)

Table 4: Distribution of ESBL-producing bacterial isolates based on clinical source

Clinical source	Sample size	ESBL positive phenotype	*E. coli*	*Klebsiella spp.*
Sputum	168	5	2	3
Stool	72	7	4	3
HVS	87	5	1	4
Urine	98	4	1	3
Total	**425**	**21**	**8**	**13**

Table 5: Antibiotic susceptibility patterns of ESBL-producing *E. coli* isolates

S/N	Organism	Resistant	Susceptible	Intermediate
1	*Escherichia coli*	NA, ETP, CIP, F, SXT, TOB, OFX, AMP, FOX	CN	
2	*Escherichia coli*	CIP, F, SXT, OFX, AMP, FOX		CN
3	*Escherichia coli*	ETP, CIP, F, SXT, OFX, AMP, FOX	NA, ETP, CN TOB, CN	
4	*Escherichia coli*	NA, ETP, CIP, F, SXT, TOB, OFX, AMP	CN, FOX	
5	*Escherichia coli*	NA, ETP, CIP, F, SXT, TOB, OFX, AMP, FOX	CN	
6	*Escherichia coli*	NA, ETP, F, SXT, AMP, FOX	CN, CIP, TOB, OFX	

Key: SXT = Sulfamethoxazole - trimethoprim, NA = Nalidixic acid, F = nitrofurantion AMP = Ampicilin, FOX = cefoxitin, CIP = Ciprofloxacin, TOB = Tobramycin, ETP = Ertapenem, CN = Gentamicin

Table 6: Antibiotic susceptibility patterns of ESBL-producing *Klebsiella* spp

S/N	Organism	Resistant	Susceptible	Intermediate
1	*Klebsiella* spp	NA, F, SXT, TOB, OFX, AMP, FOX	CN, ETP, CIP	
2	*Klebsiella* spp	SXT, OFX, AMP	CN, NA, ETP, CIP, F, TOB, FOX	
3	*Klebsiella* spp	CIP, F, SXT, AMP	CN, NA, ETP, TOB, OFX	FOX
4	*Klebsiella* spp	F	CN, NA, CIP, SXT, TOB, OFX, AMP,	FOX, ETP
5	*Klebsiella* spp	F, TOB, AMP	CN, NA, ETP, CIP, SXT, OFX, FOX	
6	*Klebsiella* spp	NA, F, SXT, OFX, AMP,	CN, ETP, CIP, FOX	TOB
7	*Klebsiella* spp	NA, F, SXT, TOB, AMP	ETP, CIP, OFX, FOX	CN
8	*Klebsiella* spp	NA, SXT, TOB, OFX, AMP, FOX	CN, CIP, F	ETP
9	*Klebsiella* spp	NA, F, SXT, TOB, AMP,	CN, ETP, CIP, OFX.	FOX
10	*Klebsiella* spp	NA, ETP, CIP, CIP, F, SXT, TOB, OFX, AMP, FOX	CN	
11	*Klebsiella* spp	NA, ETP, F, TOB, AMP	CN, SXT, OFX	FOX
12	*Klebsiella* spp	NA, ETP, SXT, AMP, FOX	CN, CIP, F, TOB, OFX	
13	*Klebsiella* spp	NA, SXT	CN, ETP, CIP, F, OFX	NA, SXT, TOB, FOX
14	*Klebsiella* spp	FOX, AMP, OFX, TOB, SXT, NA	CN, ETP, F, CIP	

Key: SXT = Sulfamethoxazole - trimethoprim, NA = Nalidixic acid, F = nitrofurantion AMP = Ampicilin, FOX = cefoxitin, CIP = Ciprofloxacin, TOB = Tobramycin, ETP = Ertapenem, CN = Gentamicin

Table 7: Multiple antibiotic resistance index (MARI) of ESBL-producing *E. coli and Klebsiella* spp.

S/N	ORGANISM *Klebsiella* spp.	(MARI)	ORGANISM *(E. coli)*	(MARI)
1	*Klebsiella* spp.	0.5	*E. coli* (13)	0.6
2	*Klebsiella* spp.	0.6	*E. coli* (14)	0.6
3	*Klebsiella* spp.	0.4	*E. coli* (8i)	0.5
4	*Klebsiella* spp.	0.5	*E. coli* (8ii)	0.5
5	*Klebsiella* spp.	0.3		
6	*Klebsiella* spp.	0.6		
7	*Klebsiella* spp.	0.3		
8	*Klebsiella* spp.	0.4		
9	*Klebsiella* spp.	0.3		
10	*Klebsiella* spp.	0.7		

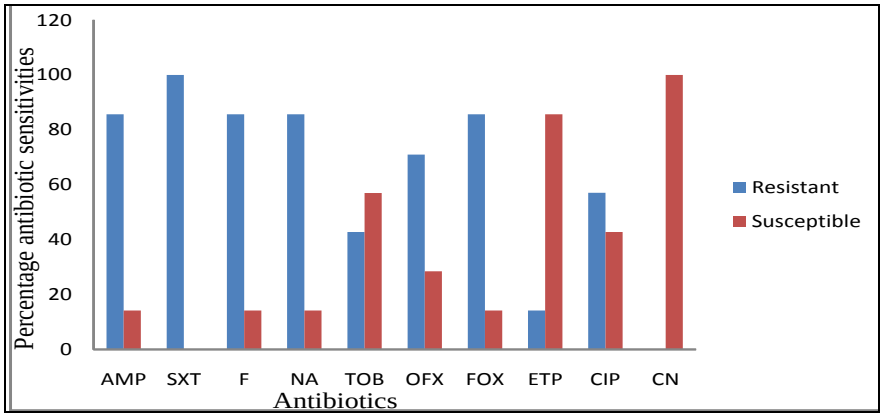

Figure 1: Percentage sensitivity of ESBL-positive *E. coli* isolates to antibiotics

Key: SXT = Sulfamethoxazole - trimethoprim, NA = Nalidixic acid, F = nitrofurantion AMP = Ampicilin, FOX = cefoxitin, CIP = Ciprofloxacin, TOB = Tobramycin, ETP = Ertapenem, CN = Gentamicin

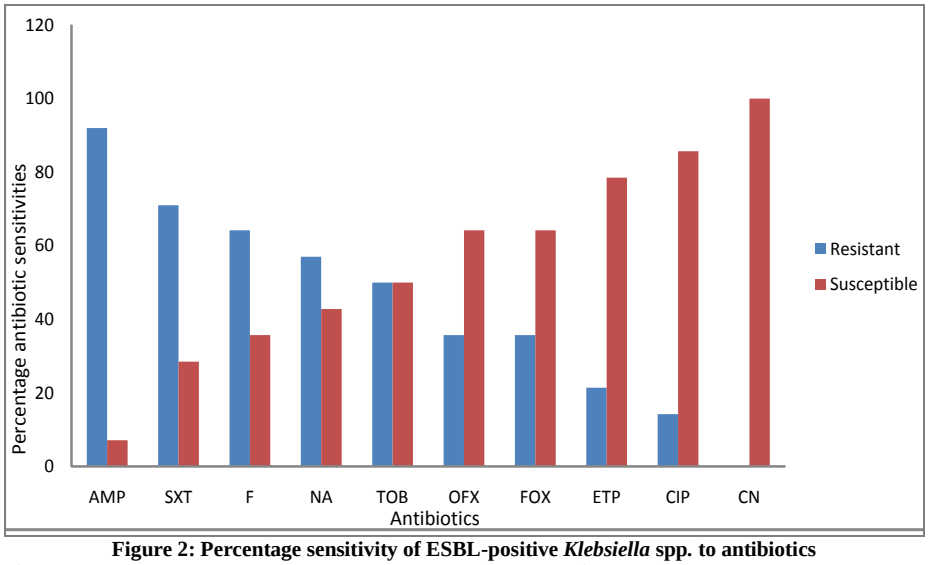

Figure 2: Percentage sensitivity of ESBL-positive *Klebsiella* spp. to antibiotics

Key: SXT = Sulfamethoxazole - trimethoprim, NA = Nalidixic acid, F = nitrofurantion AMP = Ampicilin, FOX = cefoxitin, CIP = Ciprofloxacin, TOB = Tobramycin, ETP = Ertapenem, CN = Gentamicin

DISCUSSION

Antibiotic resistance among Gram-negative rods of Enterobacteriaceae such as *Escherichia coli* and *Klebsiella* spp is on the increase. This has made treatment of infections related to these organisms difficult in our hospitals and has also led to an increase in health care cost, increase in mortality, morbidity and pressure on both social and economic conditions of patients and communities. One of the major causes of these antibiotic resistances is the emergence of new beta-lactamase enzymes known as extended spectrum beta-lactamase (ESBL) produced mostly by *Klebsiella pneumoniae* and *Escherichia coli*. *Klebsiella pneumoniae* and *Escherichia coli* can acquire these enzymes through its gene mutation or horizontal gene transfer from other strains via its plasmids. The incidences of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* have been reported worldwide.

In our study, *Klebsiella* spp (80.6 %) were more predominant than *E. coli* (19.4 %) in the 103 clinical isolates of Gram-negative bacteria obtained from Mile Four General Hospital, Abakaliki, Nigeria (Tables 1 and 2). Out of the 103 clinical isolates, 21 (20.4 %) were positive for ESBL production while 82 (79.6 %) were negative (Table 3). Eight (7.76 %) *E. coli* isolates were ESBL-positive while 13 (12.6 %) *Klebsiella* spp. were ESBL-positive out of the 103 clinical bacterial isolates (Table 3). This study is in agreement with the work of Iroha *et al.* (2011), who reported that *Klebsiella* spp. has the highest percentage of ESBL production among the clinical isolates obtained in bacteraemic patients in Federal Teaching Hospital, Abakaliki (FETHA). This present study disagrees with the work of Narayanaswamy *et al.* (2011) who reported that 54.43 % of the *E. coli* isolates in their study were positive for ESBL production.

ESBL production was phenotypically confirmed in 20.4 % of all the Gram-negative bacilli employed in this study (Table 3). This is in agreement with the work of Iroha *et al.* (2010) who reported that ESBL production was phenotypically confirmed in 16.2 % of all the 99 Gram-negative rods employed in their study. Our study which showed that 7.76 % of the *E. coli* isolates were ESBL-positive also agrees with the work of Iroha *et al.* (2010) who reported that 7.5 % of *E. coli* isolates were positive for ESBL production. The prevalence frequency value of 20.3 % for ESBL production among the tested Enterobacteriaceae is in tandem to similar studies carried out in a medical center in Iran where ESBL production was detected in 21 % of *E. coli* isolates (Behroozi *et al.*, 2010). This study is in agreement with the

work of Iroha *et al*. (2008a) were 25.2 % of *E. coli* isolates out of 123 screened isolates were positive for ESBL production.

It was observed from this study that 7 ESBL-positive isolates were obtained from 12 stool samples (Table 4). Stool samples had the highest number of ESBL-positive isolates despite having the lowest sample size (Table 4). It was also observed that 5 ESBL-positive bacterial isolates were recovered from sputum samples despite having the highest sample size of 168 (Table 4). Five ESBL-positive bacterial isolates were also recovered from 17 HVS samples. Four ESBL-positive bacterial isolates were recovered from 98 urine samples (Table 4). These results show that ESBL-positive bacterial isolates are more prevalent in the stool samples collected than in sputum, HVS and urine samples. The prevalence of ESBL-positive bacterial isolates in the clinical samples collected for this study does not completely corroborate the report of Iroha *et al*. (2008b) where all the *E. coli* isolates (5.4 %) that were positive for ESBL production were obtained from urine samples while other samples produced no ESBL-positive *E. coli* isolate. Our study revealed that *Klebsiella* spp. had prevalence frequency value of 12.6 % for ESBL production than *E. coli* with a prevalence frequency value of 7.76 % (Table 4). This result agrees with the work of Iroha *et al.* (2010) who reported that *Klebsiella* spp. had a prevalence frequency value of 59.4 % for ESBL production than *E. coli* with a value of 56.6 %. These results showed that the prevalence of ESBL production is higher in *Klebsiella* spp. than *E. coli* and this may be due to the fact that ESBL was first detected in *Klebsiella pneumoniae* strain (bearing SHV-2 gene) from Germany in 1983 (Jacoby *et al*., 2000 and Gniadkowski, 2011) and have more exposure to the enzyme.

ESBL-producing bacteria pose a serious threat to antimicrobial therapy and infection control practices as they are resistant to different classes of antibiotics. ESBL-producing *E. coli* and *Klebsiella pneumoniae* which are plasmid-mediated drug resistant organisms, also carry other resistance genes that make them resistant to different classes of antibiotics. This is the major danger of ESBL prevalence in Gram-negative rods. The antibiotic sensitivity results revealed that the *E. coli* isolates were resistant to sulfamethoxazole-trimethoprim, nitrofurantion, ofloxacin, cefoxitin, ciprofloxacin and ertapenem (Table 5). This is in line with the work done in South-west Nigeria and Iran where a greater percentage of the *E. coli* and *Klebsiella pneumoniae* were resistant to ofloxacin and ciprofloxacin (Oyinloye *et al*., 2011; Jain *et al*., 2007 and Babypadmini *et al*., 2004). The high resistance of ESBL-positive *E. coli* to sulfamethoxazole-trimethoprim in this study is comparable to previous study done in Bosnia where a large percentage (81 %) of ESBL producers was resistant to sulfamethoxazole-trimethoprim (Uzunovic – Kamberovic *et al*., 2006).

These *E. coli* isolates were resistant to Ertapenem, a carbapenem that is usually the drug of choice for bacterial infections caused by ESBL-producing bacteria. This new and emerging antibiotic resistance to carbapenems is a serious public health problem. However, all the *E. coli* and *Klebsiella pneumoniae* isolates were susceptible to gentamicin, an aminoglycoside. This report suggests that gentamicin is still effective against bacterial infections associated with *E. coli* and *Klebsiella pneumoniae* strains. In this study, the resistance of some ESBL-positive *Klebsiella pneumoniae* strains to ciprofloxacin is in agreement with the work of Iroha *et al.* (2011) whose report showed that 31.3 % of *Klebsiella pneumoniae* were resistant to ciprofloxacin. All the strains of the ESBL-positive *Klebsiella* spp were resistant to ampicillin; most were resistant to sufamethoxazole/trimethoprim, cefoxitin, nitrofurantion and tobramycin (Table 6). This shows the multiple drug resistance nature of the ESBL strains as they were resistant to at least two different classes of antibiotics. However, most of the strains

were susceptible to gentamicin, ertapanem ciprofloxacin, nitrofuration, cefoxitin. This indicated that these drugs could be the last drug of choice against bacterial infections associated with these bacterial strains.

In this study, the multiple antibiotic resistance index (MARI) of *E. coli* and *Klebsiella* spp. were very high (Table 7) and this calls for urgent action. It was also observed that a bacterial strain was resistant to a member of a class of antibiotic and also susceptible to another member of the same class of antibiotic. This report calls for routine antibiotic susceptibility studies in our hospitals to determine the appropriate drug of choice in the treatment of any bacterial infection.

E. coli and *Klebsiella* spp. were resis tance sulfamethoxazole/trimethoprim (99) a nd nitrofurantion (60%) while gentamicin has the highest activity (100 %) on the bacterial isolates followed by ertapenem (85%) and ciprofloxacin which confirms the fact that gentamicin or ertapenem could be a last drug of choice in the treatment of bacterial infections caused by these bacterial strains (Figures 1 and 2). This calls for regular ESBL screening and detection tests in our hospitals and microbiology laboratories to serve as a formidable epidemiologic strategy in checking the spread of these ESBL-producing organisms.

CONCLUSION

Twenty-one (21) bacterial isolates (*Klebsiella* spp. and *E. coli*) were confirmed to be ESBL-positive while 82 isolates were ESBL-negative out of 103 bacterial isolates recovered from 657 clinical samples collected from Mile Four General Hospital, Abakaliki, Nigeria. This high prevalence of ESBL-positive bacterial isolates in our hospital today calls for synergistic effort among health practitioners to prevent wide and indiscriminate use and further abuse of limited drug of choice for the treatment of bacterial diseases. Gentamicin, ertapenem and sometimes, ciprofloxacin are still effective in the treatment of bacterial infections. Microbiologists and epidemiologists should intensify surveillance on the prevalence of ESBL in our hospitals by carrying out more screening and detection studies in hospitals. Also, Health administrators should create more suitable and strict antibiotic-use policies towards the fight against the spread of ESBL producing bacteria.

REFERENCES

- Abhilash, K. P.P., Veeraraghavan, B. and Abraham, O. C. (2010). Epidemiology and outcome of Bacteremia caused by Extended Spectrum Beta - Lactamase (ESBL) -producing *Escherichia coli* and *Klebsiella* spp. in a Tertiary Care Teaching Hospital in South India. *Supplement to Jap*., 58:13-17.
- Aibinu, I., Nwanneka, T. and Odugbemi,T. (2007). Occurrence of ESBL and MBL in clinical isolates of *Pseudomonas aeruginosa* from Lagos, Nigeria. *Journal of American Sciences*, 3(4):81-85.
- Alten, H. K. L. A., Moc, J. Rodbumerer, A., Gaarder and Aanddsman, J. (2009). Fundamental Metagenomics reveals diverse beta – lactamases in a remote Alsaskan soil. *ISME J*.3: 245 – 251.
- Babypadmini, S. and Appalaraju, B. (2004). Extended spectrum β-lactamases in urinary isolates of *Esherichia coli* and *Klebsiella* pneumonia – prevalence and susceptibility patten in tertiary care hospital. *Indian Journal Microbiology*, 22(3): 172 – 174.
- Behroozi, A., Rahbar, M. and Yousefi, J. V. (2010). Frequency of extended –spectrum beta-lactamases (ESBLs) producing *Escherichia coli* and *Klebsiella pneumonia* isolated from urine in an Iranian 100-0-bed tertiary care hospital. *African Journal of Microbiology Research*, 4(9): 881 – 884.
- Bonnet, R. (2004). Growing group of Extended – spectrum β – Lactamases: the CTX –M Enzymes. *Antimicrobial Agents Chemotherapy*, 48(1): 1-14.
- Bradford, P. A. (2001). Extended – Spectrum β – Lactamases in the 21st century; Characterization, Epidemiology, and Detection of this Important Resistance Threat. *Clinical Microbiology Reviews*, 14(4): 933 – 951.
- Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. 2nd edition. Cambridge University Press. UK. Pp. 178-187.
- Cindy, W. S. TSE (2011). Emergence of Extended - Spectrum Beta - Lactamases in

our Community - what does it mean for clinicians? *Medical Bulletin*, 16(4):23-25.

- Clinical and Laboratory Standards Institute (CLSI). (2009). Performance standards for antimicrobial susceptibility testing. Nineteenth Information supplement. Vol 29, No. 3. CLSI/NCCLS document M100-S19, Wayne, Pennsylvania.
- Ginadkowski, M. (2001). Evolution and epidemiology of extended –spectrum β – lactamases (ESBLs) and ESBL – producing microorganisms. *Clin. Microbial Infect.*7: 597 – 608.
- Iroha I.R.,Amadi S.E., Adikwu M.U. and Esimone C.O (2008a). Detection ofPlasmid Borne Extended Spectrum Beta - Lactamase enzymes in Clinical Isolates of *Escherichia coli* from a Community General Hospital. *International Journal of Molecular Medicine and Advance Sciences*, 4(2):46-49.
- Iroha I.R., Oji A.E. and Esimone C.O. (2008b). Antimicrobial resistance pattern of plasmid - mediated extended - spectrum p - lactamase producing strains of *Escherichia coli. Scientific Research and Essay*, 3(6):215-218.
- Iroha, I.R., Amadi, E.S., Oji, A.E., Nwuzo, A.C. and Ejikeugwu, P.C. (2010). Detection of Plasmid Borne Extended - Spectrum Beta - Lactamase Enzymes from Blood and Urine Isolates of Gram - Negative Bacteria from a University Teaching Hospital in Nigeria. *Current Research in Bacteriology*, 3(2):77-83.
- Iroha, I.R., Oji, A.E., Nwakaeze, A.E.,Ayogu, T.E., Afiukwa, F.N.,Ejikeugwu, P.C. and Esimone, C.O. (2011). Strains of *Klebsiella pneumonia* from Intensive Care Unit Producing CTX-MM5 Extended Spectrum Beta Lactamases. *American Journal of Microbiology*, 2(2):35-39.
- Jacoby, G.A. and Munoz-Price, L.S. (2005). Mechanisms of Disease: The
- Jain, A. and Modal R. (2007). Prevalence and antimicrobial resistance pattern of extended spectrum β – lactamase producing *Klebsiella* spp. Isolated from cases of neonatal speticaemia. *Indian J. Med. Res.* 88 – 94.
- Mehrgan, H., Rahbar, M and Arab-Hal,vali, Z. (2010). High prevalence of extendedspectrum beta - lactamase - producing *Klebsiella pneumonia* in a tertiary care hospital in Tehran. Iran. *J Inject Devectries,* 4(3):132-138.
- Narayanaswamy, A. and Mallika, M. (2011). Prevalence and susceptibility of extended spectrum beta-lactamases in urinary isolates of *Escherichia coli* in a tertiary care hospital, Chenuai, south India. *Internet Journal of Medical Update*, 6(1):39-43.
- Oyinloye, J.M.A. and Ezekiel, C.N. (2011). Extended Spectrum Beta - Lactamase (ESBL) -Producing multidrug Resistant *Enlerobacteriaceae* from commercial Poultry Feeds in Nigeria. *Annals of Biological Research*, 2(2):250-254.
- Scottish center for infection and Environmental Health. SCIEH (2004). Extended spectrum p - lactamases - are we prepared to face the threat *Supplement to SCIEH*, 38:50.
- The Swedish Strategic Programme against Antibiotic Resistance, STRAMA (2007). ESBL Resistance in Enteric Bacteria Proposed Action Plan. November 2007 Pp. 1 – 24.
- Uzunovic-Kambeerovic, S., Saric, D. and Sestic, S. (2006). Community – acquired urinary tract infections by extended – Spectrum beta – lactamase – producing *Enterobacteriaceae*in Zenica – Doboj Canton, Bosina and Herzegovina. *Medicinskiglasnik*, 3(2): 46 – 52.

How to cite this article: Iroha IR, Igwe OF, Moses IB et al. Phenotypic characterization of extended spectrum beta-lactamase (ESBL) - producing clinical isolates of *escherichia coli* and *klebsiella* spp from mile four general hospital, Abakaliki. Int J Health Sci Res. 2016; 6(12):65-73.

****