Phenotypic detection of Extended-spectrum β-lactamases (ESBLs) among Enterobacteriaceae and *Pseudomonas aeruginosa* from Mubi, Adamawa State, Nigeria.

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Abstract

Background: Extended-Spectrum β-Lactamases (ESBLs) are group of enzymes produced by bacterial species which enable them to withstand the effect of cephalosporin antibiotics. They are mostly reported among Enterobacteriaceae and other Gram-negative bacteria especially *Pseudomonas aeruginosa*.
Method: One hundred clinical samples comprising of urine, high vaginal swab (HVS), sputum, stool, semen and wound swab were analysed for bacterial growth. Bacterial species isolated were subjected to antibiotic susceptibility testing and phenotypic extended-spectrum β-lactamase production (ESBL).
Result: Only 62(62.0%) of the clinical samples yielded bacterial growth which belonged to eight (8) genera. *Escherichia coli* (35.5%) was the most predominant, followed by *P. aeruginosa* (16.1%), while the least was *P. mirabilis* (1.6%). Extended-spectrum β-lactamases (ESBLs) was observed in 27/62 isolates, an overall prevalence of 43.5% with a predominance of *E. coli* (40.7%), followed by *P. aeruginosa* (22.2%). Although the number of non-ESBL isolates (35) was more than that of ESBL isolates (27%) but with no statistical difference (P=0.703). The antimicrobial susceptibility profile of all the isolates showed that 61(98.4%) and 56(90.3%) of the isolates were resistant to the β-lactam antibiotics, ceftriaxone and cefpodoxime respectively and were variable to other antibiotics. All the ESBL producers were found resistant (100%) to ceftriaxone and cefpodoxime (all cephalosporins). Also, all ESBL producing *P. mirabilis, K. pneumoniae* and *P. agglomerans* were found resistant (100%) not only to cephalosporins antibiotics, but also to other classes of antibiotics. However, resistance to all fluoroquinolones (sparfloxacin, ciprofloxacin, perfloxacin and ofloxacin) and gentamycin was moderately low especially among ESBL producing *E. coli* and *P. aeruginosa*, while ESBL producing *K. pneumoniae* was susceptible to the afore mentioned antibiotics.
Conclusion: The finding of this study is worrisome considering the fact that most of the ESBLs strains exhibit MDR phenotype. This constitutes a threat to therapy not only in the hospital environment but also in the community.

Keywords: Phenotypic, ESBLs, Enterobacteriaceae, Mubi

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Introduction

Beta-Lactam antibiotics are commonly used to treat bacterial infections caused by multi-drug resistant (MDR) organisms especially Enterobacteriaceae which constitutes a major threat to human health. This is because this class of antibiotics has high ability to produce the desired result with low side effect.[1,2] However, continuous contact of bacteria to a variety of β-lactams probably due to excessive or misuse of such drugs both in hospitals and communities has initiated the production of β-lactamase enzymes especially extended-spectrum β-lactamases (ESBLs) which many bacterial species employed and used as an important mechanism of β-lactam resistance.[3]

Extended-spectrum β-lactamases (ESBLs) are enzymes which are produced by variety of bacteria especially Enterobacteriaceae and *Pseudomonas aeruginosa* [4] and other Gram-negative bacilli.[5] These enzymes are mostly plasmid mediated-lactamase that efficiently hydrolyze penicillins, cephalosporins and monobactams, yet are inhibited by β-lactamase inhibitors, cephamycins and carbapenems.[6,7] Plasmids coding for ESBLs may also carry genes conferring resistance to other classes of antibiotics.[8] This can limit the chemotherapeutic options for ESBL-producing pathogens and facilitate the dissemination of ESBLs among organisms of the same or different species.[7] thereby promoting the spread of MDR among bacteria species globally. Therefore, phenotypic detection of ESBLs among *Enterobacteriaceae* and *Pseudomonas* species is important for epidemiological purposes as well as for limiting the spread of resistance mechanisms.

Therefore, the present study was undertaken to phenotypically detect ESBL in clinical isolates of Enterobacteriaceae and *P. aeruginosa*.

Materials and Methods

Study area

Mubi metropolis is a geopolitical area consisting of two local government areas: Mubi North and Mubi South. The metropolis is located between latitudes 10^0 05' and 10^0 30'N of the equator and between longitude 13^0 12' and 13^0 19'E of the Greenwich meridian. The two Local government areas occupy a land area of 192,307 Km^2 and support a total population 260,009 people (National

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Population Census 2006). The area shares boundary with Maiha LGA in the South, Hong LGA in the West, Michika LGA and Cameroon Republic in the East. The major ethnic group in Mubi includes; Fali, Gude, Kilba, Higgi, Margi and Njanyi [9].

Isolation and Identification of Bacterial isolates

This study was conducted over a period of three months (May – July, 2018) at a secondary care hospital. A total of 100 clinical samples (comprising of urine, high vaginal swab, sputum, wound swab, semen and stool) were processed for significant bacterial growth in Microbiology Laboratory of the Department of Biological Science Technology, Federal Polytechnic Mubi. The samples received were directly inoculated on MacConkey agar and after 24 h of aerobic incubation at 37°C; isolates were identified using standard biochemical tests [10].

Antibiotic susceptibility testing

Susceptibility to various antimicrobial agents was determined by disc diffusion method of Kirby Bauer on Mueller Hinton Agar (Himedia) as described by Clinical Laboratory and Standard Institute guidelines.[11] The following antibiotic discs (drug concentration in μg) were used: amoxicillin-clavulanic acid (30) gentamycin (30), streptomycin (30), cefotaxime (30), amoxacillin (30), co-trimoxazole (30), ciprofloxacin (10), perfloxacin (30), ofloxacin (30) and chloramphenicol (30).

Phenotypic Detection of ESBL

Presumptive test

In the presumptive test to detect potential ESBL producers, all the isolates were screened for susceptibility to cefpodoxime (10 μg), ceftazidime (30 μg) and ceftriaxone (30 μg) antibiotic discs (Oxoid, UK). Isolates with zones of inhibition of ≤ 17 mm for cefpodoxime; ≤ 22 mm for ceftazidime and ≤ 25 mm ceftriaxone are considered potential ESBLs producers and were subjected to confirmatory test using the double discs synergy test as described in the CLSI guidelines.

Confirmatory test

The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin/clavulanic acid (20/10 μg) and cefotaxime (30 μg) were placed at a distance of 15 mm apart and incubated. Organism that showed a clear extension of cefotaxime inhibition zone towards the disc containing clavulanate was considered as ESBL producer [11].

Result

The result in Table 1 showed the distribution of bacterial species based on clinical samples screened. The result revealed that only 62(62.0%) of the clinical samples yielded bacterial growth. Eight (8) bacterial genera were isolated out of which *E. coli* (35.5%) was the most predominant, followed by *P. aeruginosa* (16.1%), while the least was *P. mirabilis* (1.6%). Majority of the *E. coli* (72.7%), *Salmonella* spp (66.7%), *P. vulgaris* (66.7%) and all *K. pneumoniae* (100%), were isolated from urine samples. However, *P. aeruginosa* was mostly isolated from HVS (40%) and wound swab (30%) samples.

Table1: Distribution of bacterial isolates according to specimen

Bacterial isolates	HVS n=21	Urine n=41	Semen n=6	Stool n=5	Sputum n=8	Wound swab n=19	Total (%) n=100
E. coli	-	16	-	-	3	3	22(35.5)
C. freundii	2	2	-	1	-	1	6(9.7)
C. diversus	2	2	-	-	-	-	4(6.5)
P. rettgeri	2	1	-	-	-	2	5(8.1)
P. aeruginosa	4	2	1	-	-	3	10(16.1)
Salmonella spp	-	2	-	1	-	-	3(4.8)
P. agglomerans	2	1	-	-	1	2	6(9.7)
K. pneumoniae	-	2	-	-	-	-	2(3.2)
P. vulgaris	-	2	-	-	-	1	3(4.8)
P. mirabilis	-	-	-	-	-	1	1(1.6)
Total (%)	12(57.1)	30(73.2)	1(16.7)	2(40.0)	4(50.0)	13(68.4)	62(62.0)

The results confirmed by DDST showed that ESBL was observed in 27/62 isolates, an overall prevalence of 43.5% with a predominance of *E. coli* (40.7%), followed by *P. aeruginosa* (22.2%). Extended-spectrum beta-lactamase production was not observed in *P. rettgeri* and *P. mirabilis*. Although the number of non-ESBL isolates (35) was more than that of ESBL isolates (27), but with no statistical difference (P=0.703) (Table 2).

Table 2: prevalence of ESBL's producing bacteria spp from clinical sources

Bacterial spp	Prevalence	
	ESBL (%)	Non-ESBL (%)
E. coli	11(40.7)	11(31.4)
C. freundii	2(7.4)	4(11.4)
C. diversus	2(7.4)	2(5.7)
P. rettgeri	0	5(14.3)
P. aeruginosa	6(22.2)	4(11.4)
Salmonella spp	2(7.4)	1(2.9)
P. agglomerans	2(7.4)	4(11.4)
K. pneumoniae	1(3.7)	2(5.7)
P. vulgaris	1(3.7)	1(2.9)
P. mirabilis	0	1(2.9)
Total	27(43.5)	35(56.5)

The antimicrobial susceptibility profile of all the isolates showed that 61(98.4%) and 56(90.3%) of the isolates are resistant to the β-lactam antibiotics, ceftriaxone and cefpodoxime respectively. Resistance to other classes of antibiotics were variable (Table 3).

Table 3: Resistance profiles of isolates

Bacterial Isolates	Resistance profile	No. of antibiotics	No. of isolates	ESBL +ve	ESBL -ve
E. coli	S,SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX, CRO,CPD	11	5	2	3
	SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX, CRO,CPD	10	2	1	1
	S,SXT,CHL,SPX,CIP,AMX,CN,PEF,CRO,CPD	10	1	-	1
	S,SXT,SPX,CIP,AMX,PEF,OFXCRO,CPD	9	1	1	-
	S,SXT,CHL,SPX,CIP,AMX,PEF,CRO,CPD	9	1	1	-
	S,SXT,CIP,AMX,CN,CRO,CPD	7	2	1	1
	SXT,CHL,AMX,OFX,CRO	5	1	-	1
	S,SXT,CHL,AMX, CRO	5	2	-	2
	SXT,CHL,AMX,CRO,CPD	5	2	1	1
	S,SXT,AMX,CRO,CPD	5	1	1	-
	S,CN,AMX,CRO,CPD	5	1	1	-
	S,SXT,CHL,CRO,CPD	5	1	1	-
	S,AMX,CRO,CPD	4	1	1	-
	SXT,AMX	2	1	-	1
C. freundii	S,SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX,CRO,CPD	11	3	1	2
	SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX,CRO,CPD	10	1	-	1
	S,SXT,CHL,CN,AMX,OFX,CRO,CPD	8	1	-	1
	S,SXT,CHL,SPX,AMX,CRO,CPD	7	1	1	-
C. diversus	S,SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX,CRO,CPD	11	1	1	-
	S,SXT,CHL,SPX,AMX,CN,PEF,CRO,CPD	9	1	1	-
	SXT,CHL,AMX,CRO,CPD	5	1	-	1
	SXT,AMX,CRO,CPD	4	1	-	1
P. rettgeri	S,SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX,CRO,CPD	11	3	-	3
	S,SXT,CHL,AMX,CN,PEF,OFX,CRO,CPD	9	1	-	1
	SXT,AMX,CN,PEF,CRO,CPD	6	1	-	1
P. aeruginosa	S,SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX,CRO,CPD	11	2	1	1
	S,SXT,CHL,SPX,AMX,CN,PEF,CRO,CPD	9	1	1	-
	S,SXT,CHL,AMX,CN,OFX,CRO,CPD	8	1	1	-
	S,SXT,AMX,CN,CRO,CPD	6	1	1	-
	SXT,AM,AMX,CN,CRO,CPD	6	1	-	1
	SXT,CHL,AMX,CRO,CPD	5	1	1	-
	SXT,SPX,CHL,CRO,CPD	5	1	-	1
	SXT,AMX,CRO.CPD	4	2	1	1
Salmonella spp	S,SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX,CRO,CPD	11	1	1	-
	S,SXT,SP,CIP,AMX,CN,PEF,OFX,CRO,CPD	10	1	-	1
	SXT,SPX,AMX,CN,PEF,CRO,CPD	7	1	1	-
P. agglomerans	S,SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX,CRO,CPD	11	2	1	1
	S,SXT,SPX,CIP,AMX,CN,PEF,OFX,CRO	9	1	1	-
	SXT,SPX,AMX,CN,PEF,CRO,CPD	7	1	-	1
	S,AMX,PEF,CRO,CPD	5	1	-	1
	SXT,SPX,CPX,CRO,CPD	5	1	-	1
K. pneumoniae	S,SXT,CHL,SPX,AMX,CN,PEF,OFX,CRO,CPD	10	1	-	1
	S,SXT,CHL,AMX,CRO,CPD	6	1	1	-
P. vulgaris	S,SXT,CHL,SPX,CIP,AMX,CN,PEF,OFX,CRO,CPD	11	1	1	-
	S,SXT,CHL,SPX,AMX,CN,PEF,OFX,CRO,CPD	10	1	-	1
	S,SXT,AMX,CN,CRO,CPD	6	1	-	1
P. mirabilis	SXT,AMX,PEF,CRO	4	1	-	1

Legend: S=Streptomycin, SXT=Cotrimozaxole, CHL=Chloramphenicol, SPX=Sparfloxacin, CIP=Ciprofloxacin, AMX=Amoxacillin-Clavulanic acid, CN=Gentamycin, PEF=Perfloxacin, OFX=Ofloxacin, CRO=Ceftriaxone, CPD=Cefpodoxime

Table 4: Resistance patterns of ESBL isolates

Organism	S	SXT	CHL	SPX	CIP	AMX	CN	PEF	OFX	CRO	CPD
E. coli	9(81.8)	9(81.8)	6(54.5)	5(45.5)	6(54.5)	10(90.9)	5(45.5)	5(45.5)	4(36.4)	11(100)	11(100)
C. freundii	2(100)	2(100)	2(100)	1(50)	1(50)	2(100)	1(50)	1(50)	1(50)	2(100)	2(100)
C. diversus	2(100)	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)
P. aeruginosa	4(66.7)	6(100)	4(66.7)	2(33.3)	1(16.7)	6(100)	4(66.7)	2(33.3)	2(33.3)	6(100)	6(100)
Salmonella spp	1(50)	2(100)	1(50)	2(100)	1(50)	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)
P. agglomerans	2(100)	2(100)	1(50)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)
K. pneumoniae	1(100)	1(100)	1(100)	-	-	-	1(100)	-	-	1(100)	1(100)
P. vulgaris	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)

Legend: S=Streptomycin, SXT=Cotrimozaxole, CHL=Chloramphenicol, SPX=Sparfloxacin, CIP=Ciprofloxacin, AMX=Amoxacillin-Clavulanic acid, CN=Gentamycin, PEF=Perfloxacin, OFX=Ofloxacin, CRO=Ceftriaxone, CPD=Cefpodoxime

All the ESBL producers were found resistant (100%) to ceftriaxone and cefpodoxime (all cephalosporins). Amazingly, almost all the ESBL isolates were also found resistant to amoxicillin-clavulanic acid. Also, all ESBL producing *P. mirabilis, K. pneumoniae* and *P. agglomerans* were found resistant (100%) not only to cephalosporins antibiotics, but also to other classes of antibiotics.

However, resistance to all fluoroquinolones (sparfloxacin, ciprofloxacin, perfloxacin and ofloxacin) and gentamycin was relatively low especially among ESBL producing *E. coli* and *P. aeruginosa*, while ESBL producing *K. pneumoniae* was susceptible to the afore mentioned antibiotics (Table 4).

Discussion

The emergence and prevalence of ESBLs producing bacteria have been on the increase globally and constitute a major threat to therapy especially in developing countries. This is because ESBLs hydrolysed β-lactam antibiotics which are often used as first line agents to treat many severely ill patients. [12, 13] It may also be due to the fact that ESBLs can be carried on mobile genetic element such as plasmid which enhances transmission of the trait to other organisms.[14] Report also showed that untimely detection and therapy of diseases caused by ESBL producing organisms was responsible for high rate of morbidity and mortality.[12] This may be because in developing countries, detection of ESBLs is mostly limited to research centres or institutions of higher learning; whereas routine tests for detection of ESBLs in secondary and tertiary health care facilities are rarely provided or carried out.

The majority of the isolates resistant to either of the β-lactam antibiotics used suggest ESBL producers. However, only 27 (43.5%) of these were ESBL producers. The reason may be attributed to the presence of factors (resistant enzymes) other than ESBL which could have been present in these ESBL-non-producing but β-lactam resistant isolates. Some strains have also been reported as producing both ESBL and AmpC which prevents the visible expression of ESBL.[15]

The 43.5% ESBL prevalence rate confirmed by DDST was higher than previous report which indicated that Nigeria and other African countries have prevalence rates of ESBL-producing organisms varying from 3.8% to 22.8% from both clinical or community sources.[16] However, the ESBL prevalence rate of 43.5% as shown in our study was comparable to 44.3 %, 45.6%, and 47.1% reported in Benin City, Owerri and Calabar respectively.[4,17,18] Prevalence rates lower than that of our study was also reported in different parts of Nigeria. These include 7.5%, 8.1%, 16%, 23.6%, 24.5% and 34.3% reported in Ogun State, Anambra State, Nnewi, Maiduguri, Ilorin and Zaria respectively.[15,19-23]

Higher ESBL prevalence rate of 58.0%, 61%, and 87% have been reported in various quarters.[24-26] These observations confirmed the variability and changing pattern in ESBL prevalence among clinical bacterial isolates globally and in different geographical areas as previously reported.[25]

The variation in the ESBL prevalence could be due to the excessive use of cephalosporin's and differences in the risk factors.[24] It may also be attributed to patient selection, differences in study design and geographical differences.[23] Other reasons for the discordance could be due to precise placement of discs, correct storage of the clavulanic discs and performance of appropriate control tests.[13, 27]

The finding of this study showed that *E. coli* has the highest number of ESBL-producing isolates with 11(40.7%), followed by *Pseudomonas aeruginosa* with 6(22.2%). This observation collaborates with previous finding from Owerri which reported that out of the 114(45.6%) ESBL positive isolates, 66(26.4%) were *E. coli* and 48(19.2%) were *P. aeruginosa.*[4] However, other studies noted that ESBL prevalence rate was higher in *E. coli* followed by *K. pneumoniae.*[15, 28] This was contrary to our finding with respect to *K. pneumoniae*. Several other studies reported that *E. coli* possessed ESBL than most clinical isolates.[20, 29, 30]

High level of resistance to amoxicillin-clavulanic acid by ESBL producing isolates in our study concurred with previous report.[14] This may be attributed to the ability of the organisms under study to produce multiple ESBLs which may reduce the effectiveness of β-lactam/ β-lactamase inhibitor combinations.[31,32]. This type of resistance has been reported to be on the increase.[32]

Our results also showed that majority of the ESBL isolates exhibits multi-drug resistant phenotype. This conforms to previous findings that reported that all ESBL-producing organisms are multi-drug resistant.[33] This might be attributed to the presence of plasmids which not only responsible for ESBL production but at the same time also carry multiple resistant genes to other antimicrobial classes giving rise to the development of multi-drug resistant phenotype.[34, 35]

Moderately low resistance to aminoglycosides and fluoroquinolones exhibited by ESBL producing *E. coli* and *P. aeruginosa* in this study can be collaborated with previous studies which reported that fluoroquinolones and aminoglycosides have antimicrobial activity against ESBL organisms than other non-beta lactam drugs.[36,37] Consequently, these drugs gave a good indication and may be useful as drug of choice in treating infections caused by ESBL producing organisms' particularly *E. coli and P. aeruginosa* in the study area.

Conclusion

To the best of our knowledge, this is the first report of ESBLs among clinical bacterial isolates in the study area. The study revealed high prevalence of ESBL producing Enterobacteriaceae and *P. aeruginosa*. Almost all the

ESBL producing isolates exhibit multi-drug resistant phenotype which constitutes major threat to human health. Consequently, detection of ESBL producing organisms in an early stage is paramount to prevent or limit their spread and establish appropriate antimicrobial stewardship.

References

1. Drawz SM, Bonomo RA. Three decades of b-lactamase inhibitors. Clin Microbiol Rev. 2010; 23:160–201.
2. TangdenT, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. J Intern Med. 2015: 277: 501–512.
3. Teethaisong Y, Eumkeb G, Chumnarnsilpa S, Autarkool N, Hobson J, Nakouti I, Hobbs G, Evans K. Phenotypic detection of AmpC β-lactamases, extended-spectrum β - lactamases and metallo-β-lactamases in Enterobacteriaceae using a resazurin microtitre assay with inhibitor-based methods. J Med Microbiol. 2016; 65:1079–1087.
4. Braide W, Madu LC, Adeleye SA, Korie MC, Akobondu CI. Prevalence of Extended Spectrum Beta Lactamase Producing *Escherichia coli* and *Pseudomonas aeruginosa* Isolated from Clinical Samples. Intl J Sci. 2018; 7(2): 89-93.
5. Nwosu IL, Amadi ES, Nwanyanwu CE, Chikwendu IC, Madu CL. The prevalence of extended spectrum beta-lactamase (ESBLs) among *Escherichia coli* and *Klebsiella* species urinary isolates from Abia State University Teaching Hospital (ABSUTH) Aba, Abia State. Intl J Microbiol Mycol. 2014; 2(3):20-28.
6. Bush K, Fisher JF. Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from Gram-negative bacteria. Annu Rev Microbiol. 2011; 65:455–478.
7. Poulou A, Grivakou E, Vrioni G, Koumaki V, Pittaras T, Pournaras S, Tsakris A. Modified CLSI Extended-Spectrum β-Lactamase (ESBL) Confirmatory Test for Phenotypic Detection of ESBLs among *Enterobacteriaceae* Producing various β-Lactamases. J Clin Microbiol. 2014; 52(5): 1483–1489
8. Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. Lancet Infect Dis. 2008; 8:159–166.
9. Adebayo AA. Mubi Region. A Geographic Synthesis; Paraclete Publishers, Yola. 2004; 17 – 37.
10. Washington CW, Stephen DA, Williams MJ, Elomer WK, Gary WP, Paul CS, Gail LW. Koneman's Colour Atlas and Textbook of Diagnostic Microbiology, 6th edn. Lippincott Williams and Wilkins, Philadelphia, USA. 2006.
11. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
12. Villanueva FD, Tupasi TE, Abiad HG, Baella BQ, Candano RC. Extended-spectrum β-lactamase production among *Escherichia Coli* and *Klebsiella spp.* at the Makat Medical Centre: Tentative Solutions. Phil J Microbiol Infect Dis. 2003; 32(3):103-08.
13. Ahmed K, Thakur M, Fomda B, Bashir G, Maroof P, Kadri SM. Extended spectrum β-lactamase in clinical isolates of *Escherichia coli* in a tertiary care centre. Arch Clin Microbiol. 2010;1(41): 1-5
14. Rani S, Jahnavi I, Nagamani K. Phenotypic and Molecular Characterization of ESBLs producing Enterobacteriaceae in A Tertiary Care Hospital. IOSR J Dent Med Sci. 2016; 15(8): 27-34
15. Akujobi C N, Ewuru CP. Detection of extended spectrum beta-lactamases in gram negative bacilli from clinical specimens in a teaching hospital in South-eastern Nigeria. Niger Med J. 2010; 51:141-146
16. Tansarli GS, Poulikakos P, Kapaskelis A, Falagas ME. Proportion of extended-spectrum β-lactamase (ESBL)-producing isolates among Enterobacteriaceae in Africa: evaluation of the evidencesystematic review. J Antimicrob Chemother. 2014; 69(5): 1177-1184.
17. Azekhueme I, Moses AE, Abbey SD. Extended spectrum beta-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from University of Uyo Teaching Hospital, Uyo, Nigeria. J Adv Med Pharm Sci. 2005; 2:117-25.
18. Ogefere HO, Aigbiremwen PA, Omoregie R. Extended-spectrum beta-lactamase (ESBL) producing Gram-negative isolates from urine and wound specimens in a tertiary health facility in Southern Nigeria. Trop J Pharm Res. 2015; 14:1089-1094.
19. Chukwunwejim CR, Eze PM, Ujam NT, Abonyi IC, Ejikeugwu CP, Abonyi, DO, Esimone CO. Incidence of community acquired ESBL-producing bacteria among asymptomatic University students in Anambra State, Nigeria. Euro J Biol Res. 2018; 8 (3): 138-147
20. Mohammed Y, Gadzama GB, Zailani SB, Aboderin AO. Characterization of extended-spectrum beta-lactamase from *Escherichia coli* and *Klebsiella* species from North Eastern Nigeria. J Clin Diagn Res. 2016; 10: 07-10.
21. Faari BU, Akanbi AA, Fadeyi A, Wahab KW, Nwabuisi C. Prevalence of extended spectrum beta-lactamase-producing *Klebsiella* species at the University of Ilorin Teaching Hospital. J Med Investig Pract. 2015; 10:20-3.
22. Giwa FJ, Ige OT, Haruna DM, Yaqub Y, Lamido TZ, Usman SY. Extended-Spectrum beta-lactamase production and antimicrobial susceptibility pattern of uropathogens in a Tertiary Hospital in Northwestern Nigeria. Ann Trop Pathol. 2018; 9:11-16.
23. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum β–lactamase producing gram negative bacteria in a tertiary care hospital. Indian J Med Res. 2002; 115:153-157.
24. Siddiqui N, Bhakre J, Damle A, Bajaj J. Prevalence of ESBL producing Gram-negative bacilli. IOSR J Med Sci. 2014; 13(9): 8-11.
25. Machanda V, Singh NP, Goyal R, Kumar A, Thukral SS. Phenotypic characteristics of Klebsiella pneumoniae and evaluation of available techniques for detection of ESBLs. Indian J Med Res. 2005; 122: 330-337.
26. Duttaroy B, Mehta S. Extended spectrum β-lactamases (ESBL) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. Indian J Pathol Microbiol. 2005; 48(1):45-48.
27. Ampaire L, Nduhura E, Wewedru I. Phenotypic prevalence of extended spectrum beta-lactamases among Enterobacteriaceae isolated at Mulago National Referral Hospital: Uganda. BMC Res Notes, 2017; 10:448
28. Kumar M. S., Lakshmi V., Rajagopalan R.: Occurrence of extended-spectrum beta-lactamases among Enterobacteriaceae species isolated at a tertiary care institute. Indian J. Med. Microbiol. 2006; 24: 208-11.
29. Wadekar MD, Anuradha K and Venkatesha D. Phenotypic detection of ESBL and MBL in clinical isolates of

Enterobacteriaceae. Intl J Curr Res Acad Rev. 2013; 1(3): 89-95.
30. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 2005; 18(4):657-86.
31. Chauhan S, Mahawal BS, Ramola DC. Extended spectrum β-lactamases in urinary isolates of *Escherichia coli* - prevalence and susceptibility pattern at a tertiary care hospital. Intl J Res Med Sci. 2015; 3(7):1622-1626.
32. Aibinu IE, Ohaegbulam VC, Adenipekun EO, Ogunsola FT, Odugbemi TO, Mee BJ. Extended-Spectrum Beta-Lactamase Enzymes in clinical isolates of *Enterobacter species* from Lagos, Nigeria. J. Clin. Microbiol. 2003; 41: 2197-2200.
33. Jacoby GA, Sutton L. Properties of plasmids responsible for production of extended spectrum β-lactamases. Antimicrob Agents Chemother. 1991; 35:164-169.
34. Nathisuwan S, Burgess DS, Lewis JS. Extended spectrum β-lactamases: Epidemiology, detection and treatment. Pharmacother. 2001; 21:920-928.
35. Jean S, Teng L, Hauch P. HosLuh K. Antimicrobial susceptibility among clinical isolates of extended spectrum cephalosporin-resistant gram-negative in a Taiwanese University. J. Antimicrob. Chemother. 2002; 49:69-79.
36. Quale JM, Landman D, Bradford PA. Molecular epidemiology of a citywide out breaks of extended spectrum bet-lactamase producing *Klebsiella pneumoniae* infection. Clin Infect Dis. 2002*;* 35:834-841.
37. Ruth AA, Damian CO, Romanus II, Charles OE. Antimicrobial resistant status and prevalence rates of extended spectrum beta-lactamase producers isolated from a mixed human population. Bosnian J Basic Med Sci. 2011; 11(2): 91-96.