Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2020; 1(1): 50-57
© 2020 JAMR
www.microbiojournal.com
Received: 12-12-2019
Accepted: 19-01-2020

Dr. Uyanga Florence Zion
Senior lecturer, Department of Microbiology, Akwa Ibom State University, Mkpat Enin, Nigeria

Nwankwo, Emmanuel Onwubiko
Senior lecturer, Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Owowo, Etanguno Effiong
Senior lecturer, Department of Microbiology, Akwa Ibom State University, Mkpat Enin, Nigeria

Correspondence
Dr. Uyanga, Florence Zion
Senior lecturer, Department of Microbiology, Akwa Ibom State University, Mkpat Enin, Nigeria

Molecular characterization of antibiotic resistance genes in extended spectrum beta-lactamase producing gram-negative bacteria isolated from urine specimens of pregnant women in Akwa Ibom state, South Nigeria

Dr. Uyanga Florence Zion, Nwankwo Emmanuel Onwubiko and Owowo, Etanguno Effiong

Abstract
Extended spectrum beta-lactamases (ESBL) are enzymes produced by members of the *Enterobacteriaceae* which can hydrolyze the beta-lactam antibiotics like penicillins and cephalosporins and thereby confer antibiotic resistance on strains producing them. Bacterial isolates producing ESBLs have spread to different parts of the world. The ESBLs are encoded by several different genetic elements borne on the chromosome and plasmids. This study was carried out to characterize the genes responsible for antibiotic resistance in ESBL producing Gram -negative bacteria isolated from urine specimens of pregnant women attending antenatal care at three General Hospitals in Akwa Ibom State. A total of 660 urine specimens were collected from the women between July and December 2018. The specimens were inoculated on MacConkey agar and incubated at 37ºC for 24h. The biochemical characterization of the isolates was done using the Microbact 24E (Oxoid Ltd, UK). Antibiotic susceptibility testing was done by Kirby-Bauer disc agar diffusion method. The isolates were tested for the production of ESBL using Double Disk Synergy test and CHROMagar ESBL. Genomic and plasmid DNA from ESBL producing strains was extracted and amplified using the Polymerase Chain Reaction (PCR) with primers for bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M\text{-}15}$genes. A total of 252 isolates of which 231(92%) were ESBL producers were recorded. In this study, *Enterobacter cloacae* was the most frequently isolated ESBL producer (25.7%, followed by *Escherichiacoli* (20.2%) and *Klebsiella pneumoniae* (16.3%). Genotypic characterization of the ESBL producing isolates showed $bla_{CTX\text{-}M\text{-}15}$ to be the most prevalent (26%). The prevalence of $bla_{TEM}$and bla_{SHV} was 20%, respectively. The incidence of carriage of multiple *bla* genes was low, ranging from 2-6% of different combinations. This study has shown the existence of multiple *bla* genes in the Gram-negative bacterial isolates from pregnant women from the community in the study areas. This calls for urgent public health measures to implement antimicrobial resistance stewardship to mitigate against the potential adverse effects of the spread of resistant bacteria carrying genes for resistance to extended spectrum beta-lactam antibiotics which are vital for management of serious bacterial infections.

Keywords: Extended spectrum beta-lactamase (ESBL), blaCTX-M-15, blaTEM, blaSHV, PCR

Introduction
The key mechanism of resistance to oxyimino-cephalosporins developed by members of the Enterobacteriaceae family is the production of extended-spectrum beta -lactamases (ESBLs) (Garcia-Fernandez, 2015) [11] ESBLs are enzymes that cleave the beta-lactam ring of antibiotics belonging to the beta-lactam families, such as penicillins (e.g. amoxicillin), first, second, and third generation broad-spectrum oxyimino, cephalosporins (e.g. ceftriaxone, ceftazidime, cefotaxime), and monobactams (aztreonam), making them inactive against bacteria . They are often distinguished by the following characteristics: (i) they are usually inhibited by clavulanic acid, tazobactam, and sulbactam; (ii) they are unable to hydrolyze cephamycins (e.g. cefoxitin) and carbapenems (e.g. imipenem, meropenem); and (iii) they are encoded by genes that are strongly compatible between bacteria (Shaikh *et al.*, 2015 and Pitout) [35, 29] [The TEM and SVH type beta-lactamases, the CTX-M type beta-lactamases, and the OXA type beta-lactamases are the most common ESBLs. They can be categorized based on molecular homology (amino acid similarity) or functional properties of the enzymes, with the latter being more clinically important because it considers substrate enzyme specificity (Rawat and Nair 2010) [32].

The resistance to beta -lactamase is either plasmid-mediated or chromosomally expressed. Despite this, plasmid-mediated ESBLs, especially the CTX-M family, have been connected to the spread of beta -lactamases on a regular basis (Pitout, 2010; WHO, 2017) [29] CTX-M enzymes are currently the most widespread and have spread exponentially around the world. Six groups of CTX-M enzymes have been identified (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25, and KLUC, named after the first member of the group that was described). CTX-M-15 and CTX-M-14, all group 1 and 9 enzymes, are commonly present in Gram negative bacteria around the world (Edward *et al.*, 2017.) [8]. Since 2000, the prevalence of CTX-M ESBLs has increased, posing a serious threat to healthcare, with insufficient treatment choices for infections caused by CTX-M-producing bacteria. The blaCTXM-15 and blaCTX-M-14 genotypes have spread globally and are now the most widespread. The global prevalence of CTX-M variants is complicated, although it is apparent that blaCTX-M-15 has risen over time in most countries and is now dominant in most regions. The exceptions are China, Southeast Asia, South Korea, Japan, and Spain, where group 9 variants (especially CTX-M-14) are dominant, and South America, where blaCTX-M-2 remains important (Woerther *et al*., 2017; Edward *et al.*, 2017) [48, 8]. It is important to consider the prevalence of ESBL-positive strains in hospitals in order to establish an appropriate empirical therapy strategy (CDC, 2009). The aim of this study was to isolate and identify the types of extended spectrum beta-lactamases genes (ESBL) produced by *E. cloacae* and *E. coli* and *K. pneumoniae.*

Materials and Method

Specimen collection

A total of 660 urine samples were collected from pregnant women attending antenatal at the three general hospitals between July to December, 2018. All pregnant women who were not on any antibiotics and willing to participate were included in the research, while those on any antibiotic therapy were excluded from the research. Mid-stream clean-catch urine samples were collected and inoculated on MacConkey and incubated at 37 °C for 24 hours. The presence of Extended-Spectrum Beta-lactamase (ESBL) was also detected by ESBL Chromogenic Culture Medium (France, Paris). A prepared suspension of the test organism to turbidity equivalent to 0.5 McFarland standards was inoculated on CHROMagar ESBL culture agar plate. Inoculated plates were incubated at 37 °C aerobically for 24 hours and 48 hours; change in color of colonies was observed and interpreted as per guidelines.

Study area

This was a hospital-based study conducted at three General Hospitals, in Akwa Ibom state, south-south Nigeria.

Antimicrobial Susceptibility Testing

Isolates prepared in suspension equivalent to 0.5 McFarland standards were used for antibiotic susceptibility testing with ceftazidime (30µg), cefotaxime (30 µg), azetronam (30 µg), and cefodoxime (10 µg) (Oxoid Ltd, United Kingdom). The test was conducted in accordance with Kirby-Bauer Disc diffusion method. Zones of inhibition were interpreted as concurring to Clinical Laboratory Standard Institute (CLSI). *E. coli* ATCC 25922 and *S. aureus* 6571 were used as quality control strains.

Detection of Extended-Spectrum Beta-lactamase

Isolates showing inhibition zone size of <22mm with ceftazidime (30µg), <25mm with cefotaxime (30 µg), <27mm with azetronam (30 µg) and <22mm with cefodoxime (10 µg) were suspicious for producing ESBL and thus subjected to screening.

Double Disc Synergy Test (DDST)

Extended-Spectrum Beta-lactamase (ESBL) was detected by the Double Disc Synergy Test (DDST) (CLSI, 2016; Jarlier *et al.*, 1988). The prepared suspension of the isolates to turbidity equivalent to 0.5 McFarland standards was inoculated on Mueller-Hinton agar plate. Clavulanic-amoxicillin (30 µg) disc was placed at the center of the Mueller-Hinton agar plate. Ceftazidime (30 µg) and cefotaxime (30 µg) discs were placed 20 mm out from the edge of clavulanic-amoxicillin disc. Inoculated plates were incubated at 37 °C aerobically for 24h. Observation of cephalosporin/ clavulanate synergy was interpreted as positive for ESBL production (Chaudhary and Aggarawal, 2004; Ho *et al.*, 2000) [6, 14].

Ethical Consideration

Ethics committee of Akwa Ibom State Ministry of Health provided ethical clearance for the study. Participants' privacy and confidentiality were assured and all data and results were handled and treated confidently.

Statistical Analysis

Chi square was used for statistical analysis. A p-value of <0.05 was considered as statistically significant.

Plasmid DNA Analysis

Plasmid extraction was carried out using ZR Plasmid Miniprep-Classic extraction kit. A modified alkaline lysis protocol together with Zymo-Spin technology to yield high quality plasmid DNA in minutes was followed, the buffers were color-coded (red, green, yellow) for easy visualization of complete cell lysis and neutralization. Extracted DNA was stored initially at-22 °C for further use.

Detection of ESBL genes types by PCR

ESBL producing isolates were amplified using blaTEM /SHV/CTX-M-15 specific primers listed in Table 1. The reaction was performed in Gene Amp PCR system Px2thermocycler (Thermo Electron Corporation, USA) under the following conditions: Initial denaturation at 94 °C for 5 minutes followed by 35 cycles of 30 seconds denaturation at 94 °C, 30 seconds annealing at 58 °C, 60 seconds extension at 72 °C and a final extension at 72 °C for 7 minutes. Polymerase chain reaction (PCR) products was separated by electrophoresis in 1.5% agarose gel and stained with ethidium bromide. A molecular marker (DNA ladder size range: 1kb) was used to assess PCR product size.

Results

During a six-month period, a total of 252 uropathogens from pregnant women attending antenatal at Government General Hospitals, Eket, Ikot Ekpene and Oron were identified. Two hundred and thirty-one isolates were confirmed as potential ESBL producers. Occurrence of ESBL isolates were as follows: *E. cloacae* (25%) followed by *E. coli* (19%) and *K. pneumoniae* (15%). Comparison methods employed for detection of ESBLs showed that DDST (50%) was less

sensitive than CHROMagar ESBL method (87.8%). Out of the 50 antibiotic resistant ESBLs producing strains comprising of *E. cloacae* (18), *E. coli* (12) and *K. pneumoniae* (20). The individual incidence of bla CTX-M-15 among our *E. cloacae*, *E. coli* and *K. pneumoniae* isolates are in the order of 17%, 42% and 25% respectively. BlaTEM had an incidence of 0%, 25% and 35% respectively for *E. cloacae*, *E. coli* and *K. pneumoniae* isolates while the incidence of blaSHV among *E. cloacae*, *E. coli* and *K. pneumoniae* isolates was 0%, 25% and 35%. The incidence of carriage of multiple *bla* genes was low, ranging from 2-6% of different combinations.

Table 1: Primer sequence for Beta-lactamase gene Detection

Gene	Target	Primer	Product size (bp)	Reference
bla TEM	β-lactam	F: ATAAAATTCTTGAAGACGAAAR: ACAGTTACCAATGCTTAATC	1080	Sharma *et al.*, 2010
bla SHV	β-lactam	F: CACTCAAGGATGTATTGTG R: TTAGCGTTGCCAGTGCTCG	928	Sharma *et al.*,2010
bla CTX-M-15	β-lactam	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550	Sharma *et al.*,2010

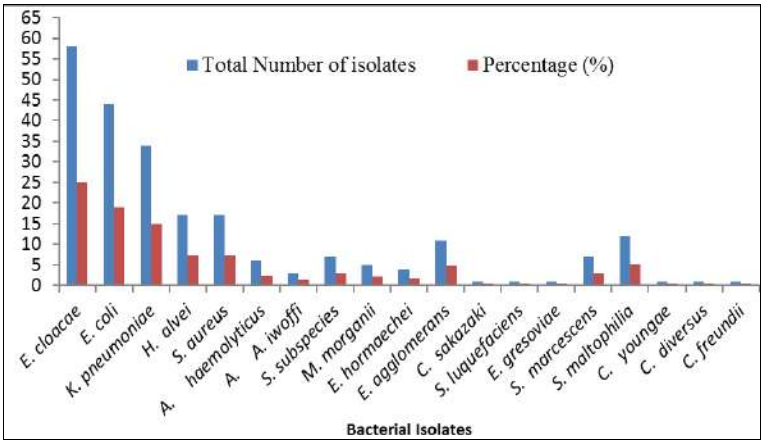

Fig 1: Percentage occurrence of ESBL producing isolates among pregnant women across the three study areas

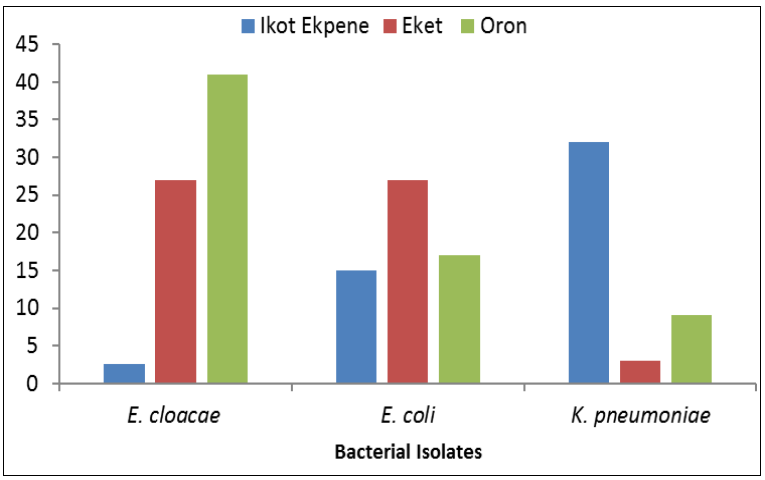

Fig 2: Distribution of ESBL producing *E. cloacae*, *E. coli* and *K. pneumoniae* in the study area

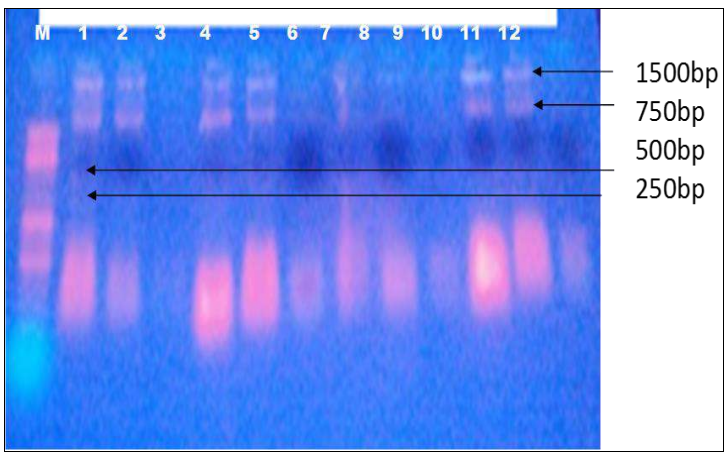

Fig 3: Agarose gel electrophoresis of plasmids recovered from the ESBL isolates laneM: 1kb DNA ladder lanes 2 – 8, *K. pneumoniae*, lanes 9 -10 *Enterobacter cloacae*, lanes 10 – 12 = *E. coli.*

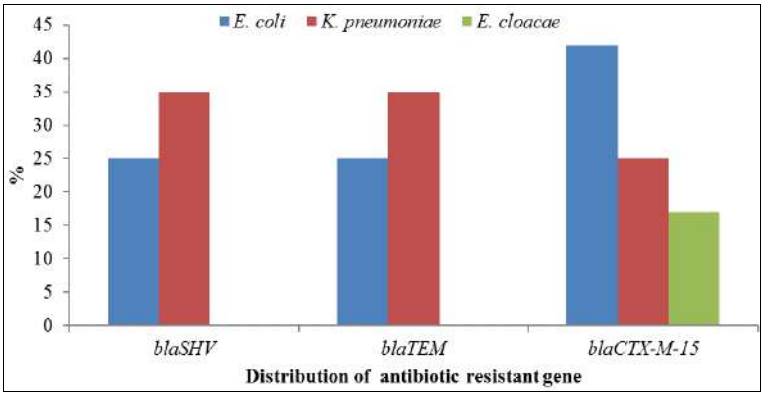

Fig 4: Distribution of gene encoding *bla TEM*, *blaSHV* and *blaCTX-M* in ESBL producing *E. cloacae*, *E. coli* and *Klebsiella pneumonia*e.

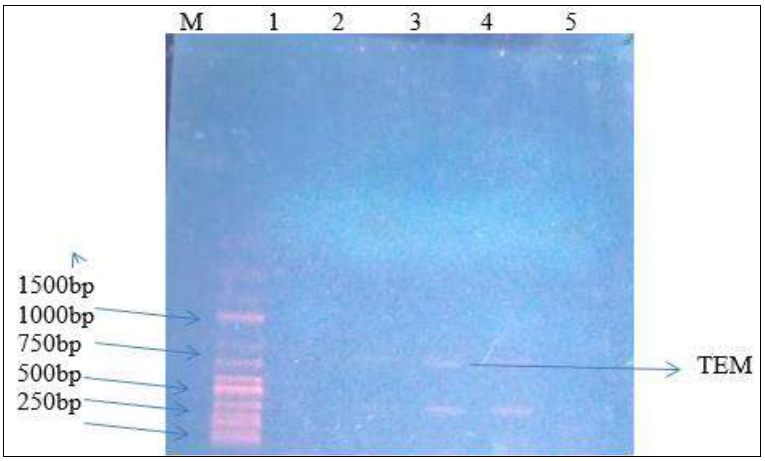

Fig 5: Agarose gel showing PCR products of amplified blaTEM gene among the bacteria isolates. L. Lane M: 1kb DNA ladder, Lane 2-control, lane 3 and 4 *E. coli* isolates.

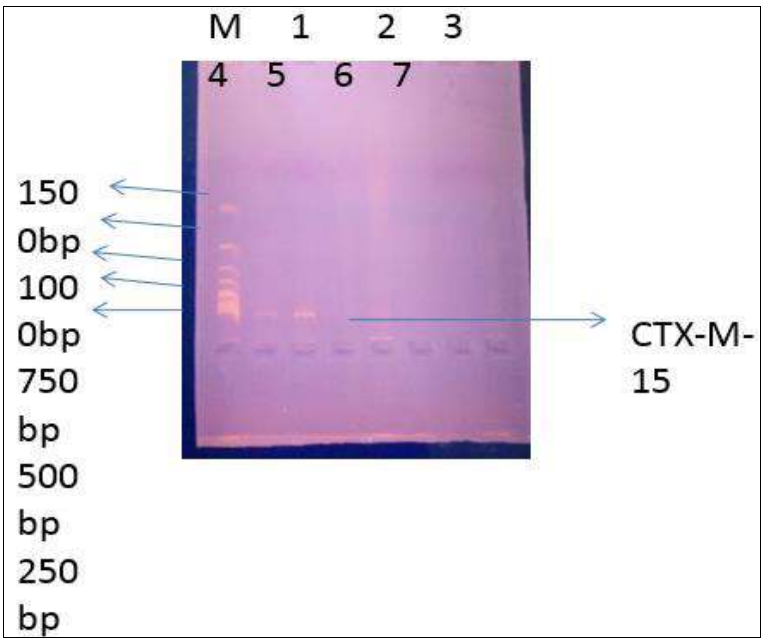

Fig 6: Agarose gel showing PCR products of amplified blaCTX-M-15 gene among the bacteria isolates. L. Lane M: 1kb DNA ladder, lane 1, 2 and 4 *E cloacae* isolates

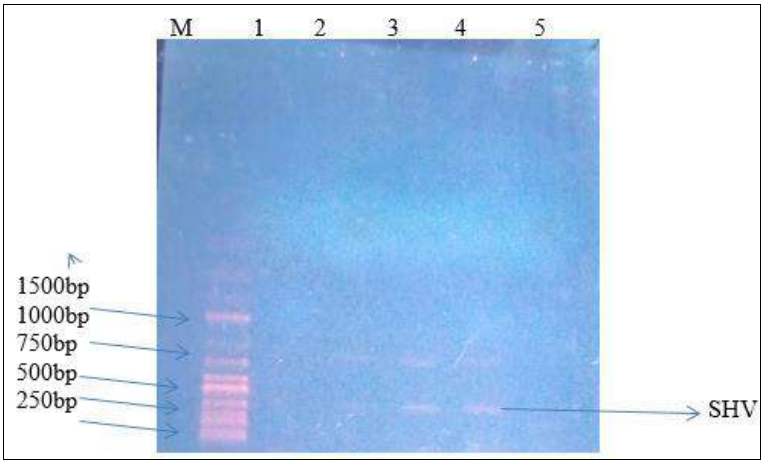

Fig 7: Agarose gel showing PCR products of amplified blaSHV gene among the bacteria isolates. L. Lane M: 1kb DNA ladder, lane 1 control, lane 2 and 3 *E coli* isolates.

Discussion

The emergence of extended-spectrum cephalosporin resistance among bacterial isolates is becoming a major problem across the world, complicating antibiotic treatment and limiting therapeutic options (Walsh *et al.,* 2005) [45]. The 660 urine samples from different pregnant women yielded 252 clinical isolates. The isolates were screened for ESBL production and 92% (231) were found to be ESBL producers including *E. cloacae* (25%), *E. coli* (19%), *K. pneumoniae* (15%), *H. alvei* (7.4%) and *A. haemolyticus* (2.5%), as shown in fig. 1.

Comparison methods employed for detection of ESBLs showed that DDST (50%) was less sensitive than CHROMagar ESBL method (87.8%). Out of the 50 antibiotic resistant ESBLs producing strains comprising of *E. cloacae* (18), *E. coli* (12) and *K. pneumoniae* (20). It was observed that 26% of the strains had blaCTX-M-15, 20% had bla TEM while 20% had blaSHV. A incidence rate of 0% blaSHV, 0% blaTEM and 3 (17%) blaCTX-M-15 was observed for *E. cloacae*, while 3 (25%) blaSHV, 3 (25%) blaTEM and 5 (42%) bla CTX-M-15 was observed for *E. coli*. *Klebsiella pneumoniae* possessed 7 (35%) blaSHV, 7 (35%) bla TEM and 5 (25%) bla CTX-M-15 (Fig. 4). The incidence of carriage of multiple *bla* genes was low, ranging from 2-6% of different combinations. The agarose gel electrophoresis of the bla genes after PCR amplification is shown in figures 5, 6 and 7. The agarose gel electrophoresis of plasmids recovered from the ESBL isolates is shown in figure 2.

The prevalence of ESBL-producing bacteria was 92% in our study. This is akin to previous researches in which a prevalence rate of 84% and 82.3% was reported (Muller-schuite *et al*., 2019: Tanko *et al.,* 2020) [25, 37]. In contrast, lower ESBL prevalence rates of 17.6%, 32%, 29%, 15.8% and 47% have also been reported (Chinyere *et al.* 2020, Mohamoud *et al.,* 2021, Johnson *et al.,* 2020; Beleta, 2020: Viet *et al*. 2021) [7, 24, 17, 4, 44]. It's conceivable that there's a lot of antibiotic misuse going on, which explains the high degree of resistance seen in the isolates utilized in this study (Bradford, 2001). This differences in prevalence rate could be due to the geographical area, time, and the diagnostic technique used (Nanoty *et al.,* 2018) [26].

Among the 231 ESBL producing isolates, *E. cloacae* was the predominant species (25%), followed by *E. coli* (19%), *K. pneumoniae* (15%). Although, *E. cloacae* was found to be the commonest ESBL producing isolate, a study carried out by Bebe *et al*., (2020) [2] showed similar prevalent rate of 17% for ESBL producing *E. cloacae.* In contrast, other researchers reported a higher prevalent rate of 53% and 50% for ESBL producing *E. cloacae* (Sangare *et al.,* 2017: Teklu *et al*., 2019) [34, 39]. Lower rates of ESBL producing *E. cloacae* are also reported by Tayh *et al*. (3.5%) and Omnia *et al*. (1.9%) (Tayh *et al*., 2019: Omnia *et al*., 2019) [38, 28]. Due to the over prescription of antibiotics in recent years, ESBL production among *Enterobacter cloacae* isolates has become a serious clinical issue. *Enterobacter cloacae* is an opportunistic pathogen that has been linked to human infections both locally and systemically (Hoffmann and Roggenkamp, 2003) [15].

Comparison methods employed for detection of ESBLs in our study showed that confirmed ESBL producers by DDST was (50%) while confirmed ESBL producers by CHROMagar ESBL method was (87.8%) with a significant P-value at 0.05. This is akin to previous research in which confirmed ESBL producers by Chromagar ESBL was 75% (Groh *et al.,* 2013) [12]. This is not in accordance with the findings of Prabha *et al*. who reported that comparison methods employed for detection of ESBLs in their study showed that DDST confirmed 29% of the isolates while CHROMagar ESBL method confirmed 35% ESBL producers (Prabha *et al.,* 2016) [31]. Elsewhere in the eastern part of Nigeria, Ezeanya *et al*. reported that confirmed ESBL producers by DDST method was 91.3% while 97.8% ESBL producers was confirmed by Brilliance ESBL agar (Ezeanya *et al*., 2017) [10].

The detection of ESBL producers among isolates as observed in this study is not in line with the work of Ugbo *et al*. where they stated that confirmed ESBL producers by brilliance agar and DDST was 100% respectively (Ugbo *et al.,* 2020) [15]. The subjective interpretation of the results could be the primary drawback of the double disk synergy test and also the distance between antibiotics discs affects sensitivity (Behera *et al.,* 2008) [15]. The use of chromogenic medium for the quick and accurate identification of ESBL producing bacteria has grown in popularity in clinical laboratories, but it has drawbacks in that there are frequent discrepancies between the responses stated by the manufacturer and the actual findings obtained in the lab. Differences in colonial morphology may arise as a result of variations in the laboratory circumstances in which the tests are carried out (Messeir *et al.,* 2012) [16].

The result of genotype analyses in the present study showed

the prevalence of 26% blaCTX-M-15, 20% blaTEM and 20% blaSHV. Molecular test using PCR identified the presence of bla genes in 23 (46%) isolates out of the 50 isolates characterized for the presence of ESBL genes. Worthy to note, *Escherichia coli* had the highest number of ESBL genes as 35% of the isolates harboured the bla genes. This agreed with work done in Egypt, where the prevalence of ESBL gene among *E. coli* was 38.9% respectively (Saedii *et al.,* 2017) [25]. The individual incidence of bla CTX-M-15 among our *E. cloacae, E. coli* and *K. pneumoniae* isolates are in the order of 17%, 42% and 25% respectively. BlaTEM had an incidence of 0%, 25% and 35% respectively for *E. cloacae, E. coli* and *K. pneumoniae* isolates while the incidence of blaSHV among *E. cloacae, E. coli* and *K. pneumoniae* isolates was 0%, 35% and 37% (Fig. 4). A study by Tayh *et al*. (2019) [13] from Palestine reported a similar incidence for blaTEM gene among *E. cloacae, E. coli*, and *K. pneumoniae* in the order of 33%, 20% and 20% respectively. Their report also observed an incidence rate of 33% *E. cloacae*, 55% *E. coli* and 20% for bla CTX-M-15. While the blaSHV were in the order of 0%, 0% and 20%.

This research observed that 35% of ESBL producing *K. pneumoniae* harboured blaTEM and blaSHV respectively. A study from northern India and southern India revealed a higher incidence rate of 72% and 77.58% for blaSHV for *K. pneumoniae* which is not consistent with our findings (Vaida *et al*., 2010; Kotekani and Kotigadde, 2018) [18, 43] Our study, revealed an incidence of 25% for blaCTX-M-15 gene in *K. pneumoniae*. Kotekani and Kotigadde reported a significantly high occurrence of 75.51% for blaCTX-M-15 genes among *K. pneumoniae* isolates from India (Kotekani and Kotigadde, 2018) [43]. The blaCTX-M-15gene has been reported in *Klebsiella pneumoniae* isolated from various hospitals in Iran (Hashemi, 2014). To further buttress the findings from this study, Ahmad and Khali reported a prevalence of 64.7% for blaTEM gene in *K. pneumoniae* from Iraq which is significantly higher than what we reported (Ahmad and Khali, 2019) [01].

E. coli isolates carried blaTEM, blaSHV and blaCTX-M-15 type ESBL gene. In our study, CTX-M-15 type ESBLs was confirmed in 42% of *E. coli* isolates. This result emphasized that this enzyme is now one of the commonest CTX-M beta-lactamase in Nigeria. A significantly lower incidence rate of 18.2% was obtained from China where 1168 ESBL-*E. coli* isolates from various clinical specimens harboured blaCTX-M-15 type ESBL gene (Xia *et al.,* 2014) [49]. Similar to our findings blaCTX-M-15 was observed at a frequency of (50%) in *E. coli* from India (Shivakumara *et al*., 2018) [36]. *K. pneumoniae*, *E. coli*, and most Gram-negative bacteria posses the ability to acquire plasmid encoding for ESBL genes such as bla-TEM, blaSHV and bla-CTX-M and become highly resistant to different antibiotics and wide spectrum of 3rd generation cephalosporins in hospitals and in community (Mazzariol *et al.*, 2017) [20, 42].

The incidence of carriage of multiple *bla* genes was low, ranging from 2-6% of different combinations which was lower than the report from India, Saudi Arabia and Iran where an incidence rate of 60%, 32.2% and 50% respectively was observed from ESBL producing isolates that harboured all the three ESBL genes (Ponnusamy and Nagappan 2015; Yazdansetad *et al*., 2019 and Salah and Elsafi, 2020) [30, 50, 15]. Several investigators reported the co-existence of various β-lactamase genes within the same isolates (Mirkalantari *et al.,* 2020; Miaad *et al.,* 2019; Jena *et al.,* 2017) [23, 22, 16]. Co-existence of all the three bla genes was observed in (6%) of the isolates which agrees with the report of Jena *et al*. and Tahy *et al*. who reported incidence rate of 4.34% and 10% respectively for isolates that harboured all the three genes (Jena *et al*., 2017; Tahy *et al*., 2019) [16, 38]. The major co-existence of both genes was CTX-M -15 and SHV (4%) in our isolates. This result is also in agreement with others Miaad *et al*. (2019) [22] and Mirkalantari *et al*. (2018) [23] who reported both CTX-M-15 + SHV as the most common type. The co-existence of all three genes could be due to the carriage of multiple plasmids (Salah and Elsafi, 2020). The coexistence of *CTX-M* ESBL and *SHV* type β-lactamases in these isolates may have also contributed to the observed high rate of antimicrobial drug resistance (Wang *et al.*, 2013) [46].

Conclusion

Overall, this study provided genomic insight into ESBL-positive clinical isolates of *E. cloacae*, *E. coli* and *K. pneumoniae* from three secondary healthcare facilities in Akwa Ibom State, Nigeria. We identified the genes encoding antimicrobial resistance to clinically important antibiotics used to treat infections due to Gram-negative bacteria. We observed that the major groups of ESBL genes, TEM, SHV and CTX-M-15 were present in some strains of *E. cloacae*, *E. coli* and *K. pneumoniae*. BlaCTX-M-15 ESBL gene was the highest occurring gene. This study provides an insight on the currently rapid dissemination of multidrug resistance, strong selection pressure for the maintenance and dispersal of the blaCTX-M-15 genotype among uropathogenic *E. coli*, *K. pneumoniae* and *E. cloacae* in Nigerian hospitals. As a result, medical laboratories should screen clinical isolates of *E. coli*, *E. cloacae*, and *K. pneumoniae* for blaCTX-M-15 on a regular basis, and hospitals should establish and incorporate an antibiotic stewardship policy to halt the trend.

Acknowledgement
Not available

Author's Contribution
Not available

Conflict of Interest
Not available

Financial Support
Not available

References

1. Ahmad HP, Khalil MK. Prevalence of blaTEM, blaSHV, and blaCTX-M Genes among ESBL Producing *Klebsiella pneumoniae* and *Escherichia coli* Isolated from Thalassemia Patients in Erbil, Iraq, Mediterr J Hematol Infect Dis. 2019;11(1):e2019041.
2. Bebe T, Odetoyin B, Rahman B. Occurrence of Multidrug-resistant Uropathogens Implicated in Asymptomatic Bacteriuria in Adults with Sickle Cell Disease in Ile-Ife, Southwest Nigeria, Oman Medical Journal. 2020;35(2):e109.
3. Behera B, Mathur P, Das A, Kapil A, Sharma V. An Evaluation of Four Different Phenotypic Technique for Detection of Metallo-Beta lactamase producing P.

aeruginosa. Indian Journal of Medical Microbiology. 2008;26 (3):233-37.
4. Belete MA. Bacterial Profile and ESBL Screening of Urinary Tract Infection Among Asymptomatic and Symptomatic Pregnant Women Attending Antenatal Care of Northeastern Ethiopia Region. Infect Drug Resist. 2020;13:2579-2592.
5. Bradford PA. Extended – spectrum β – lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. 2001;14(4):933-951.
6. Chaudhary U, Aggarwal R. Extended Spectrum B-Lactamases (Esbl) – An Emerging Threat To Clinical Therapeutics, Indian Journal of Medical Microbiology. 2004;22 (2):75-80
7. Chinyere EL, Nura SM, Ahmad GM, Kemi AF, Sani MN. Prevalence and molecular analyses of extended spectrum β-lactamase producing uropathogens among pregnant women, Jigawa state, Nigeria. J Clin Microbiol Biochem Technol. 2020;6(1):033-038.
8. Edward RB, Annie MJ, Peter MH.Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype, Journal of Antimicrobial Chemotherapy. 2017;72(8): 2145-2155.
9. Elsaf HS. Occurrence and Characteristics of the Extended-spectrum Beta-lactamase producing Enterobacterale. The Open Microbiology Journal. 2020(14):191-195.
10. Ezeanya CC, Agbakoba NR,Ejike CE,Okwelogu SI. Evaluation of a Chromogenic Medium for the Detection of ESBL with Comparison to Double Disk Synergy Test, British Journal of Medicine and Medical Research. 2017;21(12):1-11.
11. Garcia-Fernandez S, Morosini MI, Marco F, Gijon D, Vergara A, Vila J, et al., Evaluation of the eazyplex® SuperBug CRE system for rapid detection of carbapenemases and ESBLs in clinical Enterobacteriaceae isolates recovered at two Spanish hospitals. Journal of Antimicrobial Chemotherapy. 2015;70(4):1047-1050.
12. Grohs P, Tillecovidin B, Caumont-Prim AE, Carbonnelle N, Day I Podglajen, Gutmann L. Comparison of Five Media for Detection of Extended-Spectrum BetaLactamase by Use of the Wasp Instrument for Automated Specimen Processing, Journal of Clinical Microbiology. 2013;51(8):2713.
13. Hashemi A. Detection of blactx-m, blatem, blashv genes in *Klebsiella pneumoniae* strains isolated from two Hospitals of Tehran, Iran. Iran, Journal of Public Health. 2014;43(2):98.
14. Ho PL, Chow KH, Yuen KY, Ng WS, Chau PY. Comparison of novel, inhibitor potentiated disc diffusion test with other methods for the detection of ESBL in *E. coli* and *K. pneumoniae*. J Antimicrob Chemther. 1998;42:49-54.
15. Hoffmann H, Roggenkamp A. Population genetics of the nomen species Enterobacter cloacae. Appl Environ Microbiol. 2003 Sep;69(9):5306-18.
16. Jena J, Sahoo RK, Debata NK, Subudhi E. Prevalence of TEM, SHV, and CTX-M genes of extended-spectrum β-lactamase-producing *Escherichia coli* strains isolated from urinary tract infections in adults. 3 Biotechnology. 2017;7(4):244.
17. Johnson B, Stephen BM, Joseph N, *et al.* Prevalence and bacteriology of culture-positive urinary tract infection among pregnant women with suspected urinary tract infection at Mbarara regional referral hospital, South-Western Uganda. BMC Pregnancy Childbirth. 2021;21:159.
18. Kotekani L, Kotigadde S. Virulence determinant and extended spectrum beta-lactamase production in *Klebsiella pneumoniae* isolated from a Tertiary care Hospital, South India. Journal of Laboratory Physicians. 2018;10:155-61.
19. Mariam M, Youssef HAR, Noha AH. Phenotypic and Genotypic Characterization of Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Asymptomatic Bacteriuria in Pregnancy, Microbial Drug Resistance. 2019;25:5.
20. Mazzariol A, Alda B, Giuseppe C.Multidrug-resistant Gram-negative bacteria causing urinary tract infections: a review, Journal of Chemotherapy. 2017;29:2-9.
21. Messeir L, Pedro MDS Abrantes, Charlene WJ Africa. Strengths and Limitations of different Chromogenic Media for the Identification of Candida Species, Journal of Microbiology Research. 2012;2(5):133-140.
22. Miaad K, Alkhudhairy MM, Meteab A. Extended spectrum β-lactamase-producing *Escherichia coli* isolated from pregnant women with asymptomatic UTI in Iraq, Eur Asian Journal of BioSciences Eurasia J Biosci. 2019;13:1881-1889.
23. Mirkalantari S, Faramarz Masjedian, Gholamreza Irajian, Emmanuel Edwar Siddig, Azam Fattahi. Determination of the frequency of β-lactamase genes (bla SHV, bla TEM, bla CTX-M) and phylogenetic groups among ESBL-producing uropathogenic Escherichia coli isolated from outpatients J Lab Med. 2020;44(1):27-33
24. Mohamoud H, Senait T, Awoke D. Antimicrobial Resistance and ESBL Profile of Uropathogens among Pregnant Women at Edna Adan Hospital, Hargeisa, Somaliland. Ethiop J Health Science. 2021;31(3):645.
25. Müller-Schultea E, Marie Nonfra Tuoc, Chantal Akoua-Koffic, Frieder Schaumburge, Sören L Beckerb. High prevalence of ESBL-producing Klebsiella pneumoniae in clinical samples from central Côte d'Ivoire. International Journal of Infectious Diseases. 2019;91:207-209.
26. Nanoty VV, Agrawal GN, Tankhiwale SS. Evaluation ofAntibiotic Resistance and β-lactamase production in Clinical Isolates from a Tertiary Care Hospital in Central India. Journal of Clinical and Basic Research. 2018;2:1-5. Not available
27. Nuhu T, Rebecca O, Adebola TO, Busayo OO. A Systematic Review on the prevalence of Extended-Spectrum Beta Lactamase-producing Gram-negative bacteria in Nigeria, Journal of Global Antimicrobial Resistance. 2020;22:488-496.
28. Omnia HM, Gumaa SA, Ibrahim AA, *et al*. Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae: Hospital Acquired Urinary Tract Infections, Khartoum-Sudan. Annals of Microbiology and Research. 2019;3(1):76-82.
29. Pitou JD. Infections with extended-spectrum beta-lactamase-producing enterobacteriaceae: changing

epidemiology and drug treatment choices. Drugs. 2010;70(3):313-33.
30. Ponnusamy P, Nagappan R. Molecular Characterization of bla CTX-M, bla TEM, bla SHV- beta lactamase produced by uropathogenic *Escherichia coli* isolates. Int J Microbiol Res. 2015;6:67-73.
31. Prabha R, Joshy ME, Swapna M. Phenotypic detection of Extended Spectrum Beta- Lactamase producing uropathogens using DDST, PCT, Chrom agar and E-test –A comparative study. International Journal of Current Microbiology and Applied Science. 2016;5(4):565-577.
32. Rawat D, Nair D. Extended-spectrum β-lactamases in Gram Negative Bacteria. J Glob Infect Dis. 2010;2(3):263-74.
33. Saedii AAF, Abdelraheim AR, Abdel-Aziz AA, Swelam SH. ESBL-producing *E. coli* and Klebsiella among patients treated at Minia University hospitals. Journal of Infectious Diseases and Preventive Medicine. 2017;5:156-162.
34. Sangare SA, Rondinaud E, Maataoui N, Maiga AI, Guindo I, Maiga A, *et al*. Very high prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in bacteriemic patients hospitalized in teaching hospitals in Bamako, Mali. PLoS ONE. 2017;12(2):e0172652.
35. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal M. A. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci. 2015;22(1):90-101.
36. Shivakumara S, Karthik P, Bhagya T, Ranjan D, Rani B, Bhavani M, *et al*. Characterization of blaCTX-M Sequences of Indian origin and thirteen uropathogenic *Escherichia coli* isolates Resistant to multiple antibiotics BMC Res Notes. 2018;11:630.
37. Tanko N, Bolaji RO, Adebola TO, Busayo O. A systematic review on the prevalence of extended spectrum beta lactamase producing Gram-negative bacteria in Nigeria, Journal of Global Antimicrobial Resistance. 2021;S2213-7165(20)30104-1
Tayh G, Nahed Al Laham, Houssem Ben Yahia, Rym Ben Sallem, Abed Elkader Elottol, Karim Ben Slama, Extended-Spectrum β-Lactamases among Enterobacteriaceae Isolated from Urinary Tract Infections in Gaza Strip, Palestine", BioMed Research International; c2019. Article ID 4041801, 11 pages, 2019.
38. Teklu DS, Negeri AA, Legese MH, *et al*. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. Antimicrob Resist Infect Control. 2019;8:39.
39. Thapa R, Lamichhane P, Banjara M, Acharya GP. Prevalence of Extended Spectrum Beta lactamase producing Uropathogens in Pregnant women. Asian Journal of Pharmaceutical and Clinical Research. 20158(1):207-210.
40. Ugbo EN, Anyamene CO, Moses IB, Iroha IR, Babalola, OO, Ukpai, EG, *et al*. Prevalence of *bla*TEM, *bla*SHV, and bla CTX-M genes among Extended Spectrum Beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* of Cinical Origin. Elsevier. 2020;21(2):56-31.
41. Uyanga FZ, Ekundayo EO, Nwankwo EO, Ibanga IA. Molecular Characterization of Extended Spectrum Beta-lactamase from *Enterobacter cloacae*, *E. coli* and *Klebsiella pneumoniae* from Pregnant Women in South-south Nigeria. International Journal of Microbiology and Biotechnology. 2020;5(2):48-54.
42. Vaida S, Marius L, Aurelija B, Justas P, Rita P. and Agne G. Molecular characterization of extended-spectrum b-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from hospitals in Lithuania. Journal of Medical Microbiology. 2010;59(10):1263-65.
43. Viet NT, Van Du V, Thuan ND, Van Tong H, Toan NL, Van Mao C, *et al*. Maternal Vaginal Colonization and Extended-Spectrum Beta-Lactamase-Producing Bacteria in Vietnamese Pregnant Women. Antibiotics. 2021;10:572.
44. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo β – lactamases: the quiet before the storm? Clinical Microbiology Review. 2005;18(2):306–325.
45. Wang G, Huang T, Surendraiah PK, Wang K, Komal R, Zhuge J, *et al* CTX-M β-lactamase-producing *Klebsiella pneumoniae* in suburban New York city, New York, USA. Emergence of Infectious Disease.2013;9:1803-1808.
46. WHO, (2017) https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.
47. Woerther PL, Andremont A, Kantele A. Travel-acquired ESBL-producing Enterobacteriaceae: impact of colonization at individual and community level. J Travel Med. 2017 Apr 1;24(1):S29-S34.
48. Xia S, Fan X, Huang Z, Xia L, Xiao M, Chen R, *et al*. Dominance of CTX-M-type extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* isolated from patients with community-onset and hospital-onset infection in China. PLoS One. 2014;9(7):e100707.
49. Yazdansetadab S, Miaad K, Alkhudhairyc R, Najafpourd E, Farajtabrizid RM, Al-Mosawie MS, *et al*. Preliminary Survey of Extended-Spectrum β-lactamases (ESBLs) In nosocomial Uropathogen *Klebsiella pneumoniae* in north-central Iran Heliyon. 2019;5:e0234.

How to Cite This Article
Uyanga FZ, Nwankwo EO, Owowo EO. Molecular characterization of antibiotic resistance genes in extended spectrum beta-lactamase producing gram-negative bacteria isolated from urine specimens of pregnant women in Akwa Ibom state, South Nigeria. Journal of Advances in Microbiology Research 2020; 1(1): 50-57.

Creative Commons (CC) License
This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.