Original Article

Molecular Assessment of Extended-Spectrum Beta Lactamases among Gram-Negative Bacilli Bacteria Causing Community Acquired Urinary Tract Infection among Females in Southwestern Nigeria

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ABSTRACT

Background: Urinary tract infections (UTI) is commoner in females than males and mainly caused by Gram Negative bacilli. The emergence of Extended – spectrum beta lactamases (ESBL) has made its treatment difficult especially in the community because of limited therapeutic options. This study was undertaken to determine the prevalence of ESBL-producing Gram negative bacilli causing UTI among females in Babcock University Community; evaluate the associated risk factors and to determine the prevalence of the associated genes among the ESBL isolates.

Methods: A descriptive cross-sectional study of which 200 female participants with clinical UTI were recruited in Babcock University Community. Pretested interviewer–based structured questionnaire was used to obtain the socio-demographic and risk factors. Mid-stream urine was also collected for evaluation of ESBL- producing Gram-negative bacilli. DNA extraction was done for ESBL positive isolates and SHV, TEM, CTX-M and OXA ESBL genes were detected by PCR. SPSS Version 23.0 was used for data analysis.

Results: The 200-urine samples tested from the participants yielded 33-Gram negative bacilli isolates. Out of these 33 isolates, 17(51.5%) were ESBL-producers giving prevalence rate of 8.5% among the participants. The distribution of the ESBL genes among the ESBL isolates was as follows: SHV 15/17(88.2%), TEM 4/17(23.5%), CTX-M 3/17(17.6%) and OXA 5/17(29.4%). No associated risk factors were found.

Conclusion: All the four types of ESBLgenes evaluated were present among the participants. Routine screening for ESBL for Community acquired UTI is necessary to curb treatment failure.

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INTRODUCTION

Urinary tract infection(UTI)is among the common clinical bacterial infections that are frequent among women.[1] They can be acquired both in the hospital and community settings.[1,2]An estimated 150 million cases of UTI was documented worldwide.[2,3]The high prevalence of UTI in women than men is related to the female anatomy and physiology.[4,5,6]A higher percentage of women will acquire one or more episodes of UTI during their lifetime and this has been corroborated by studies from Nigeria.[7,8,9,10,11] UTI is mainly caused by Gram Negative bacilli such as the Enterobacteriaceae group and Pseudomonas.[12] Beta-Lactams are the most widely used antibiotics for the treatment of the infection because they have reduced toxicity.[2]However, the emergence of Extended – spectrum beta lactamases (ESBL) offer limited therapeutic options and therefore made the treatment of UTI difficult especially in the community setting.[13,14]

ESBL are enzymes that can hydrolyze penicillin extended-spectrum cephalosporins, and monobactams but can be inhibited by clavulanic acid.[15,16] Theenzymes are derivatives of some genes such as TEM, SHV, CTX-M and OXA.[12]These ESBL coding genes are located on the bacteria Chromosomes and can be acquired via plasmid which can be transferred from one bacterium to the other.[12,17]These plasmids are also known to carry resistance genes that encode for other antibiotics resistancesuch as, fluoroquinolones, aminoglycosides, tetracyclines, sulfamethoxazole–trimethoprim and chloramphenicol.[2,14] Therefore, infection with ESBL gene positive bacilli will be associated with increased morbidity which will lead to increased health care costs.[12]

The burden of ESBL is increasing in developing countries because of limited studies on ESBL and drug availability.[18]However, the prevalence of ESBL associated infections, in this case, UTI depends upon local epidemiology and probably antibiotics prescribing pattern.[15]Therefore, an evaluation of local prevalence of ESBL-associated UTI becomes essential for effective therapy and reduction of the burden of UTI. Therefore, this study also aimed to determine the prevalence of TEM, SHV, CTX-M and OXA ESBL Genes among the ESBL positive isolates as well as associated risk factors.

METHODS

This was a descriptive cross-sectional study conducted between June 2018 till November 2019 in Babcock University Community. We enrolled 200 female residents in the community with clinical symptoms of UTI such as nocturia, suprapubic pain, dysuria and frequency by simple random technique. Other inclusion criteria were females of childbearing age, consent to fill the questionnaire and to give mid- stream urine while exclusion criteria were females not willing to give consent and those who took antibiotics in the last six weeks. The sample size was calculated based on 13.5% prevalence of community acquired UTIcaused by ESBL producing organism in a previous study.[19]

Semi- structured questionnaire was used to obtain sociodemographic and behavioural characteristics of the respondents.

The mid-stream urine samples collected from the participants were inoculated into blood and Cysteine lactose electrolyte deficient (CLED) media by standard wire loop of 0.001ml. The culture media were incubated aerobically at 37˚C for 24 hours and colonies greater than 10^5cfu/ml were considered significant and were processed further. The bacterial isolate was identified by Gram staining and biochemical tests such as Oxidase, Citrate, urease,Indole, Kriggler Iron agar and Motility.[20]

The antibiotic susceptibility test was done by modified Kirby Bauer method while the Clinical and laboratory standard institute (CLSI) chart was used for interpretation.[21,22,23]Isolates that were resistant to third generation cephalosporins were subjected to phenotypic ESBL screening by using the double disc synergy test(CLSI criteria).[23] The positive control strain used was *Klebsiellapneumoniae* ATCC

700603 while Escherichia coli ATCC 25922 was used as negative control.[23]

DNA extraction was done by using the quick- DNA fungal/ bacteria miniprep DNA extraction kit (Zymo research, USA) for all the isolates that were phenotypically confirmed as ESBL positive isolate. The manufacturers instruction was followed for the DNA extraction. Primers were from previously published articles and were synthesized by Inqaba Biotechnical Industries (Pty) Ltd Hatfield 0028, South Africa. [24,25] These primers were used for the identification of ESBL genes TEM, SHV, CTX-M and OXA. Table 1 shows the list of primers used for amplification of TEM, SHV, CTX-M and OXA.

The PCR mixture consisted of 2.5ul of 10x PCR buffer,2ul of 25mM MgCl2, 1ul each of forward primer and reverse primer, 1ul of DMSO, 2µl of 2.5m MDNTPs, 0.1ul of 5u/ulTaq DNA polymerase, and 3ul of genomic DNA. The total reaction volume was made up to 25ul using 12.4ul Nuclease free water.

The ready to use Mastermix (Biolabs, New England) cocktail consisted of 3ul of genomic DNA, 12.5ul of mastermix, 2ul each of 10uM forward and reverse primer and 5.5ul of Nuclease free water. The reaction was amplified in GeneAmp PCR system 9700 (USA) by 9 cycles of initial denaturation at 94^0 C for 5 minutes, denaturation at 94^0C for 15 seconds, annealing temperature of 65^0 C for 20 seconds, extension at 72^0C for 30 seconds and holding temperature at 10^0C. This was followed by 35 cycles of denaturation at 94^0C for 15 seconds, annealing temperature of 55^0 C for 20 seconds, extension at 72^0C for 30 seconds and final extension of 72^0C for 7 minutes.

The information from the participants' questionnaire and data from the study were analyzed by the IBM SPSS Statistics (version 23; IBM Corporation, Armonk, New York). Variables of interest included age, family type, housing type, antibiotics misuse, visit to health care facility in the last one month, surgery in the last one month and being sexually active. The Chi-square test was used to determine the association between categorical variables and was set at statistical significance level of 5%.

Written informed consent was obtainedfrom eligible participants. Ethical approval was obtained from Babcock University ethical review committee (BUHREC 582/18)before commencement of the study.

RESULTS

The 200-urine samples tested from the participants yielded 33-Gram negative bacilli isolates. The remaining 167 samples yielded insignificant growth. Out of these 33 isolates, only 17(51.5%) were ESBL producers thus giving a prevalence of 8.5% among the participants. The distribution of the ESBL-producing genes among the ESBL-producers were:SHV 15/17(88.2%, Fig. 1), TEM 4/17(23.5%, Fig. 2), CTX-M 3/17(17.6%, Fig. 3) and OXA 5/17(29.4%, Fig. 4). Some of the isolates harbored more than one gene such as 4/17(23.5%) isolates harbored both SHV and OXA, 4/17(23.5%) SHV and TEM, 2/17(11.8%)SHV and CTX-M, 1/17(5.9%) OXA and TEM, 1/17(5.9%) CTX-M and OXA while no isolate harbored both TEM and CTX-M.(Fig. 5). The prevalence of ESBL among

Table1: List of Primers used for Amplification of TEM, SHV, CTX-M and OXA

Target gene	Nucleotide Sequences	Amplicon size (bp)	References
SHV-F	CGCCTGTGTATTATCTCCCT	293	24
SHV-R	CGAGTAGTCCACCAGATCCT		24
TEM-F	TTTCGTGTCGCCCTTATTCC	403	24
TEM-R	ATCGTTGTCAGAAGTAAGTTGG		24
CTX-M-F	CGCTGTTGTTAGGAAGTGTG	569	24
CTX-M-R	GGCTGGGTGAAGTAAGTGAC		24
OXA-1-F	GGATAAAACCCCCAAAGGAA	369	25
OXA-1-R	TGCACCAGTTTTCCCATACA		25

Table 2: Prevalence of ESBLamong the Gram-Negative Bacilli isolated from the Participants

Variables	ESBL		Total(N=33)
	Positive (%)	Negative (%)	
Escherichia coli	7(53.8)	6(46.2)	13(39.4)
Klebsiellapneumoniae	4(57.1)	3(42.9)	7 (21.2)
Klebsiellaoxytoca	1(100)	0(0.0)	1(3.0)
Proteus vulgaris	0(0.0)	1(0.0)	1(3.0)
Proteus mirabilis	4(44.4)	5(55.6)	9(27.3)
Pseudomonas aeruginosa	1(50.0)	1(50.0)	2

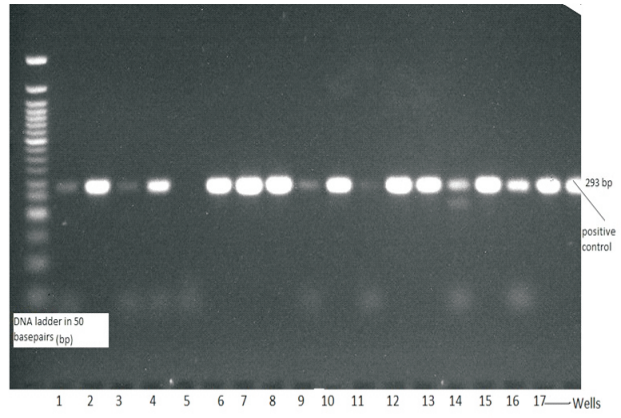

Figure1: Electrophoretic gel picture of SHVAmplicons

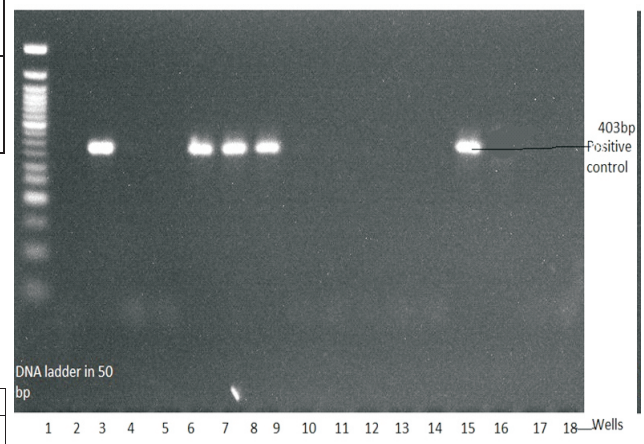

Figure2: Electrophoretic gel picture of TEMAmplicons

Table 3: Socio-Behavioural Factors associated with UTIamong the Participants (N-200)

Variables	Sub - variables	ESBL		STATISTICS	
		YES (%)	NO (%)	P-value	X^2
Age (years)	15- 20	14(7.8)	165(92.2)	0.32	1.01
	21- 25	3(14.3)	18 (85.7)		
Family type	Monogamous	15(8.2)	169(91.9)	0.55	0.36
	Polygamous	2(12.5)	14(87.5)		
Housing type	Personal	15(8.0)	173(92.0)	0.30	1.20
	Rented	2(16.7)	10(83.3)		
Antibiotic misuse	Yes	1(16.7)	5(83.3)	0.47	0.53
	No	16(8.2)	178(91.8)		
Visit to health care facility in the last one month	Yes	0(0.0)	11(100.0)	0.30	1.08
	No	17(9.0)	172(91.0)		
Surgery in the last one month	Yes	0(0.0)	6(100.0)	0.45	0.58
	No	17(8.8)	177(91.2)		
Sexually active	Yes	4(9.3)	39(90.7)	0.83	0.05
	No	13(8.3)	144(91.7)		
P-value less than 0.05 is considered significant					

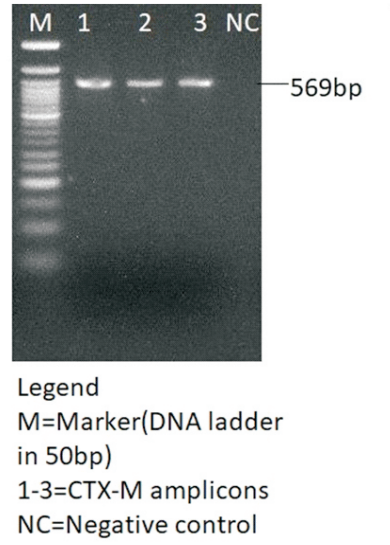

Figure3: Electrophoretic gel picture of positive CTX-MAmplicons

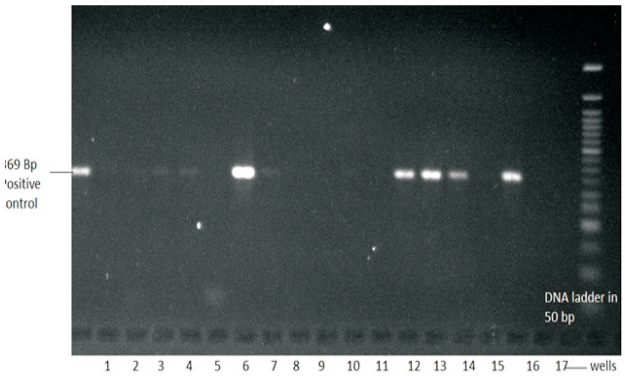

Figure4: Electrophoretic gel picture of OXAsAmplicons

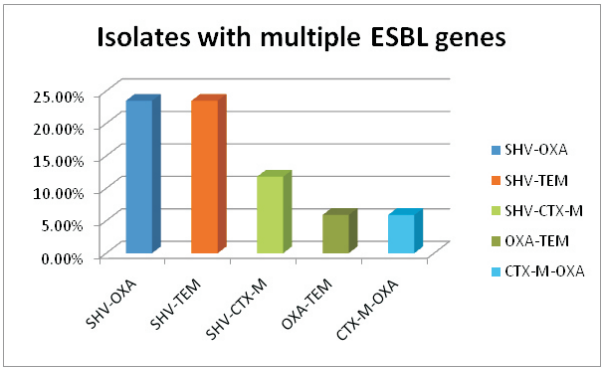

Figure 5: Distribution of isolates with more than one ESBL genes

the organisms is seen in Table 2. However, there were no risk factors found to be associated with ESBL producing Gram negative bacilli responsible for UTI among the participants (Table 3).

DISCUSSION

In this present study, the prevalence of ESBL producers among the Gram-negative urinary isolate was 17/33 (51.5%) while the prevalence of ESBL responsible for UTI among the study participants was 17/200 (8.5%). Most studies done in Nigeria to detect ESBL among urinary isolate based their prevalence on the clinical Isolate and not human participants, which made comparison of UTI ESBL producer among the study participants difficult. However, the prevalence of ESBL producers among the urinary Isolate in this study is similar to that of a previous study in Abia state in Nigeria by Nwosu*et al* but slightly higher than those of two other studies in Nigeria by Ogefere*et al* and Olonitola*et al* respectively that detected the prevalence of ESBL producers among Urinary Gram negative bacilli pathogens.[26,27,28] Across the world, the prevalence recorded varies, 44.4% in Sudan, 46% in Jordan, 12% in Lisbon, 23% in Ethiopia, 20,7% in Taiwan, 42.38% in Saudi and 45.2% in Tanzania.[6,17,29-34]These variances might be due to different behavioral and socio-cultural characteristics of the participants across the world. Moreover, differences in study designs make comparison of prevalence rates a difficult task.[24]Nonetheless; the prevalence of ESBL from our study indicated the presence of ESBL producers in our community. Generally, community acquired UTI are treated with short courses of empirical antibiotic but with the presence of resistant organism, treatment failures will eventually lead to chronicity, relapse, recurrence and complications.[34]

Among the ESBL producers from our study, SHV has the highest percentage and this finding is similar to that of two previous studies in Nigeria although their isolates were not only from urine [22,33]. CTX-M had the lowest frequency in this study and this finding is similar to that of a previous study in Nigeria and another study in Turkey.[35,36]However, the CTX-M gene is now reported to be increasing in some parts of the world and this is an important public health concern because CTX-M is noted to be associated with outbreaks of infection worldwide[37,38,39] Some authors have also reported CTX-M as a cause of several nosocomial infections in immunodeficient patients.[40,41,42] Thus the existence of this gene in some of the isolates from our study indicates the presence of CTX-M in the community and this is of major concern to the clinicians because it will make the management of UTI difficult.Some isolates from our study also had multiple occurrences of genes. This finding is similar to that of a study by Goyal*et al* and it

indicates that isolates with multiple genes are likely to be more multi drug resistant.[43] Another finding observed from this study was that *Escherichia coli* was commonest cause of UTI and also the commonest organism producing ESBL among the participants. This finding is similar to a previous study in Kano, Nigeria but was in contrast with another study by Meeta and colleagues in Nigeria.[44,45] These variances might be due to those arising from Study designs as well as participants faecal carriage of bacteria. There were no associated risk factors for ESBL detected among the participants most probably because the prevalence of ESBL was low among them while majority of our study population were students and might not be exposed to the associated risk factors yet. The limitation of this study was that the number of significant isolates from the participants was low and this is because of poor specimen collection method. Majority of the participants did not clean their genital area very well before sample collection and this led to isolation of a lot of skin contaminants which were insignificant.

CONCLUSION

The prevalence of ESBL was high among the total Gram-negative bacilli isolated with majority producing SHV genes and CTX-M has the lowest percentage. All the four types of ESBL (SHV, TEM,CTX-M and OXA genes) evaluated were present in Ogun State, Nigeria. *Escherichia coli* was the commonest organism producing ESBL and commonest cause of UTI among the participants. Therefore, proper microbiology evaluation of urine sample is advocated in the case of community acquired UTI and antibiotic stewardship programs are necessary to curb treatment failure in UTI patients.

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Conflict of interest

None declared

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