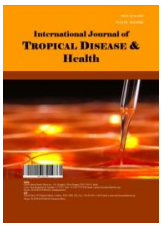

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Detection of Extended Spectrum Beta-lactamase Gene (CTX-M) among Representative Multidrug-Resistant Gram-negative Bacterial Isolates from Patients with Urinary Tract Infections

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Urinary tract infection (UTI) is a huge public health problem and the emergence of extended spectrum-beta-lactamase producing bacterial pathogens increases the burden of infectious diseases in Nigeria. This study determined the current prevalence of cephalosporin resistance among Gram-negative bacteria isolated from patients with urinary tract infections between February 2018 and June 2018. This study was aimed to determine cephalosporin resistance prevalence among Gram-negative bacteria isolated from patients with urinary tract infections between February 2018 and June 2018. A total number of forty representative Gram-negative bacterial isolates namely *Escherichia coli* (n=14), *Klebsiella pneumonia* (n=9), *Proteus mirabilis* (n=12), and *Klebsiella oxytoca* (n=5) were subjected to polymerase chain reaction (PCR) to detect extended spectrum beta-lactamase (ESBL) genes using primers specific for *bla*TEM, *bla*SHV and *bla*CTX-M.

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The molecular evaluation indicated the presence of *blaCTX-M* gene in 20.0% of the tested organisms, while other ESBL genes variants were not detected. The organisms carrying the *blaCTX-M* gene included *E. coli* (n=3, 37.5%), *K. pneumoniae* (n=1, 12.5%), *P. mirabilis* (n=1, 12.5%),) and *K. oxytoca* (n=3, 37.5%). The presence of cephalosporin resistant Gram-negative bacteria among patients with UTI may constitute a serious threat to public health and efforts must be intensified to regulate the clinical use of the cephalosporins.

Keywords: UTI; E. coli; ESBL; antibiotics.

1. INTRODUCTION

Urinary tract infections are the most common infectious disease globally in community and healthcare settings, with significant morbidity, mortality and economic burden [1-3]. Nigeria also has its share of the global burden of UTIs [4]. Some studies have confirmed high prevalence of UTI in clinical settings while community acquisition of UTI among individuals is increasing in different epidemiological settings [5,6]. Despite the variation in epidemiology of UTI across different countries and locations, there is scientific consensus that UTIs are more common among females, elderly individuals, and children. In addition, specialized procedures in clinical settings, most especially urinary catheterization, increase the risk of UTI [7].

The common aetiologies for UTI include both Gram-positive bacteria, Gram-negative bacteria and some *Candida* spp. [8]. It has been reported that *Escherichia coli* is the most common cause of UTIwhile other gram negative bacteria also remain a leading cause of UTI [9]. The high frequency of multidrug resistant Gram-negative bacteria commonly recovered from individuals with confirmed cases of UTI further complicates the problem [10]. It has been recommended that continuous surveillance of multiple resistant bacteria in clinical and community setting is essential to know the current prevalence of multidrug resistant organisms among UTI patients in order to enforce mitigation strategies to tackle the problem. The third generation cephalosporins are frequently used drugs of choice for treatment of urinary tract infections [11].

We earlier reported a high prevalence of UTIs at a tertiary health center in Ekiti-State Nigeria [12]. Gram-negative bacteria were recovered from patients confirmed with UTI and the bacteria were subjected to antibiotic susceptibility tests against common antibiotics. The bacteria showed resistance to multiple antibiotics, including the third generation cephalosporins. The objective of this present study was to determine the presence of genes that code for extended spectrum beta-lactamases among forty representative Gram-negative bacteria that were selected on the basis of their antibiotic resistance phenotypes.

2. METHODOLOGY

Forty representative Gram-negative bacteria isolates that showed resistance to third generation cephalosporins were pooled out of 106 Gram-negative isolates earlier recovered from patients with UTI at two tertiary healthcare facilities. The representative isolates comprise of *E. coli* 13 isolates, *K. pneumonia* 9 isolates, *P. mirabilis* 12 isolates and *K. oxytoca 5* isolates. For genomic DNA extraction, all selected bacterial cells were previously sub-cultured onto nutrient agar plates and incubated at 37^{O}C for 24 hours. Distinct colonies for each organism were subsequently sub-cultured onto sterile nutrient broth and further incubated overnight. For each organism in broth, about 1000µL was aseptically transferred into sterile Eppendorf tubes and centrifuged at 1000 rev/min for 5 minutes. Sterile molecular grade water was added and the cells were washed 3 times by vortexing. The vortexed cells were subjected to boiling at 100°C. After heating, cells were heat-shocked by placing on ice at -4°C after which the cells were centrifuged at 1000 rev/min. The supernatant were kept for PCR [13].

The multiplex PCR was used to target ESBL genes: b*laTEM*, *blaSHV* and *blaCTX-M*. This was carried out using the Solis Biodyne 5X HOT FIRE Pol Blend Master mix. PCR was performed in a 20 µL reaction mixture, and the reaction concentration was brought down from 5X concentration to 1X concentration containing 1X Blend Master mix buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200µM of each deoxynucleoside triphosphates (dNTP)(Solis Biodyne), 20pMol of each primer (Jena Bioscience, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 5µl of the extracted DNA,

Table 1. Sequences of primers used in this study for Polymerase Chain Reaction

S/N	Primer Name	Sequence (5^1-3^1)	Base Pair	Annealing temperature	
1	Bla SHV F	TGGTTATGCGTTATATTCGCC	868	56	[14]
	Bla SHV R	GGTTAGCGTTGCCAGTGCT			
2	Bla TEM F	TCCGTCATGAGACAATAACC	972	56	
	Bla TEM R	TTGGTCTGACAGTTACCAATGC			[15]
3	Bla CTX-M1	AAAAATCACTGCGCCAGTTC	415	56	[16-17]
	Bla CTX-M2	AGCTTATTCATCGCCACGTT			

and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in an Pielter thermal cycler (MJ Research Series) for an initial denaturation of 95°C for 5 minutes followed by 30 amplification cycles of 30 seconds at 95°C; 1 minute at 56°C and 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker. The primer sequences used for the reactions are shown in Table 1.

3. RESULTS AND DISCUSSION

Among the forty isolates selected for PCR, 8 (20.0%) organisms were found to carry at least 1 ESBL gene. In the multiplex PCR, amplicons were found only for the *blaCTX-M* gene. The eight organisms found to carry the ESBL gene were distributed across two different tertiary health care centers in Ekiti-State, Nigeria. The distribution of the bacteria that carried the CTX-M gene across the different healthcare facilities are shown (Figs. 1 and 2). Five bacterial isolates carried the gene among representative bacteria from the first healthcare facility while the remaining 3 isolates that carried the gene were at the second healthcare facility.

Phenotypically, the organisms under study that were isolated from the patients showed high level of multidrug resistance (Table 2). Notably, the organisms also showed resistance to third generation cephalosporins. Therefore, the detection of the *blaCTX-M* gene among some of the selected isolates buttresses the high incidence of cephalosporin resistance among the organisms during the period under study at the respective hospitals. This finding agrees with other reports that have confirmed the high prevalence of cephalosporin resistance in clinical settings [18]. The third generation cephalosporins are still a drug of choice for treatment of various infections within the clinical setting, and these drugs are reputed for the treatment of infections caused by Gram-negative bacteria. Therefore the presence of multiple antibiotic resistant Gram-negative bacteria among patients with UTI in the study locations is a serious cause for concern. High level of multidrug resistance, including resistance to third generation cephalosporins, severely limit the drugs of choice for the treatment of UTI and other infections in the clinical setting [19]. This will also increase the reliance to other drugs considered the drugs of last resort.

The multiplex PCR protocol was used in this study to detect *blaCTX-M*, *blaTEM* and *blaSHV*. It should be noted that only the *blaCTM-X* was the variant that was detected among the bacteria confirmed to carry the ESBL gene [20]. Previous studies have confirmed the presence of the *bla*CTX-M gene among clinical isolates causing UTIs, while other variants of the ESBL genes are also common among clinical infections caused by Gram-negative bacteria [20-22]. The potential for the ESBL genes, including the *blaCTX-M* gene detected in this study, to be transferred to other bacteria remains another serious concern in clinical management of infections caused by bacteria carrying them. In conclusion, the *blaCTX-M* gene was confirmed among multi-drug resistant gram negative bacteria recovered from patients with UTIs and this gene appears to be a common variant of the ESBL gene within the clinical environments studied.

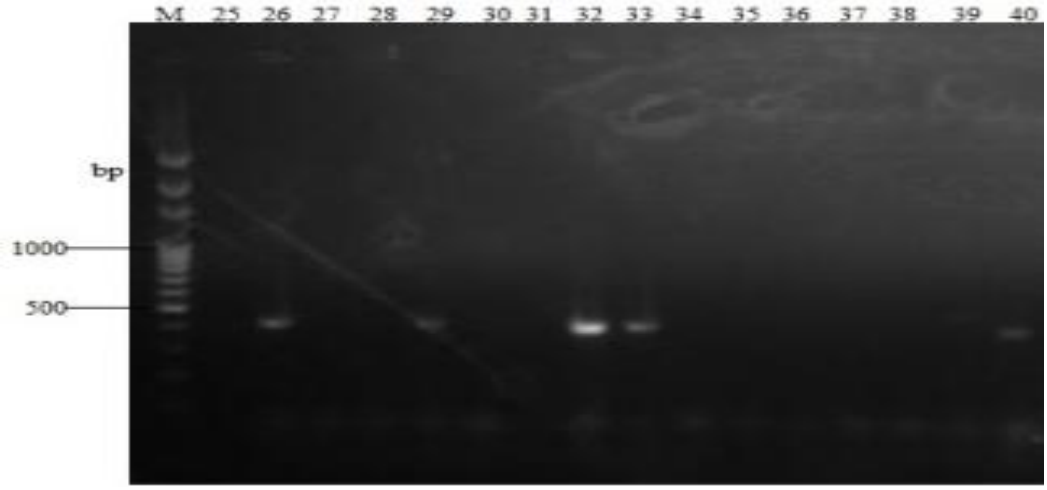

Fig. 1. *blaCTX-M* genes among some selected bacteria from the first teaching hospital
Keys: M= 1 kb marker lane; Lane 25=F118; Lane 26=F94(1); Lane 27=F58; Lane 28=F86; Lane 29=F3; Lane 30=F100; Lane 31=F107; Lane 32=F52; Lane 33=F122; Lane 34=F1.3; Lane 35=F92(1); lane 36=F47; lane 37=F115; lane 38=F65; Lane 39=F106; Lane 40=F15

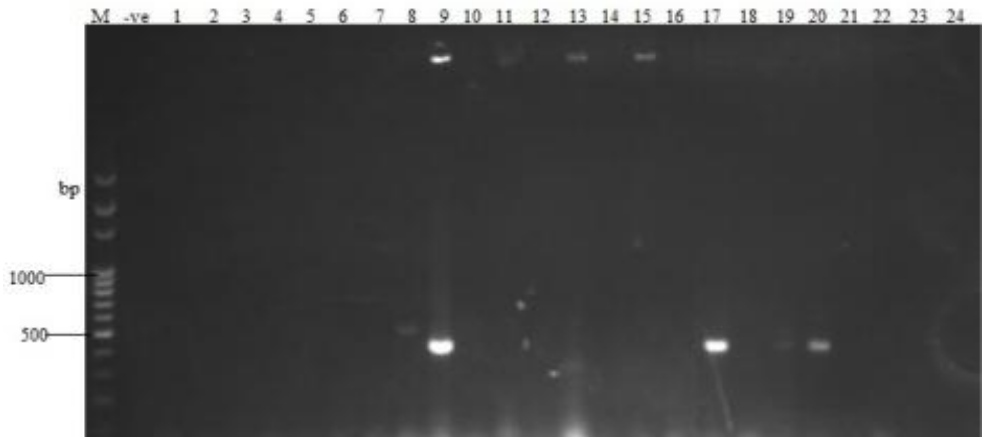

Fig. 2. *blaCTX-M* genes among some selected bacteria from the second teaching hospital
Keys: M=1 kb marker lane; bp=base pairs; lane 1= 115; lane 2=E111; lane 3=-E01; lane 4=E21; lane 5= E47; lane 6=E19 lane 7=E75; lane 8= E5(2); lane 9= E03; lane 10=E119; lane 11=E94; lane 12=E51; lane 13=E71 lane 14=E86; lane 15=E95; lane 16=E112; lane 17= E103; lane 18= Ex95 lane 19=E93; lane 20=E74; lane 21=E79 lane 22=E07 lane 23=E44; lane 24=E26.

Table 2. Antibiotic resistance profiles of isolates carrying *bla*CTX-M gene

S/N	ESBL-producing isolates	Organism	Antibiotics resistance patterns
1	E74	*Escherichia coli*	NOR/TET/PEF/CAZ/CRO/GEN
2	E03	*Klebsiella pneumoniae*	NOR/TET/PEF/CAZ/CRO/AMP
3	E103	*Proteus mirabilis*	NOR/TET/PEF/CAZ/CRO/AMP/GEN/ETP/MER
4	F94(1)	*Klebsiella oxytoca*	NOR/TET/PEF/GEN/AMP
5	F3	*Klebsiella oxytoca*	NOR/TET/PEF/CAZ/CRO/AMP/GEN
6	F52	*Escherichia coli*	NOR/TET/PEF/CAZ/CRO/AMP/GEN
7	F122	*Escherichia coli*	NOR/TET/PEF/CAZ/CRO/AMP/GEN
8	F1(5)	*Klebsiella oxytoca*	TET/PEF/CAZ/AMP/GEN

4. CONCLUSION

This study has shown that bacterial strains that cause UTI and are resistant to cephalosporins are present at the healthcare facilities chosen for this study. It further confirmed that some of the bacteria carried genes that code for some extended spectrum beta-lactamases. It is necessary that continuous surveillance be prioritized in the respective clinical settings as one of the measures to mitigate spread of antibiotic resistant bacteria within the health care facilities and prevent dissemination into the community. These findings in this study further reinforce the need for proper antimicrobial stewardship to preserve the efficacy of clinically important antibiotics.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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