

*Full Length Research Paper*

# **Isolation and molecular characteristics of extended spectrum beta-lactamase-producing uropathogenic *Escherichia coli* isolated from hospital attendees in Ebonyi State, Abakaliki**

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**This present study was designed to determine the occurrence frequency of blaCTX-M and blaTEM genes in extended-spectrum beta-lactamase (ESBL)-producing uropathogenic *Escherichia coli*, and their antibiotic resistance patterns among hospital attendees in Abakaliki, Nigeria. Out of the 73 uropathogenic *E.coli* isolates analyzed, 52 were identified to be ESBL producers using double disk synergy technique. The frequency of beta-lactamase (bla) genes (CTX-M and TEM) among *E. coli* was molecularly determined by polymerase chain reaction (PCR). Out of 52 (71.2%) ESBL-producing uropathogenic *E. coli*, 17 (32.7%) were positive for blaTEM, 35 (67.3%) were positive for blaCTX-M while 8 (15.3%) harboured both blaTEM and blaCTX-M genes. CTX-M gene was the most prevalent gene. Isolates evaluated in our study were resistant to cefotaxime (83.6%), ceftazidime (79.5%), amoxicillin (72.6%), cefpodoxime (68.5%), aztreonam (61.6%), ceftriaxone (57.5%), and cefepime (37%). This study demonstrated the occurrence frequency of CTX-M and TEM genes in uropathogenic *E. coli* strains in Abakaliki. Even though molecular techniques are more reliable in the detection of ESBL production, routine clinical screening for ESBL-producing uropathogens using phenotypic method should be introduced and encouraged in clinical settings as they are less expensive. This will go a long way in checkmating drug resistance.**

**Key words:** *Escherichia coli*, uropathogens, extended-spectrum beta-lactamase (ESBL), resistance genes, antibiotics.

## INTRODUCTION

Microbial resistance by pathogenic *Escherichia coli* is a major worldwide concern. Antibacterial agents, especially beta-lactams are becoming less useful against Enterobacteriaceae (Dia et al., 2015). Urinary tract infections (UTIs), a common nosocomial and community-acquired bacterial infection, occur in all genders and age groups (Abdulaziz et al., 2018). Antibiotic resistance by *E. coli* to numerous antibiotics is now developing and evolving (Raju et al., 2019). *E. coli* implicated in UTIs are becoming multidrug-resistant as a result of their extended-spectrum beta-lactamase (ESBL)-producing ability. Beta-lactam resistance is mediated by ESBL genes that are mostly encoded by plasmid (Topaloglu et al., 2010). ESBLs are a branch of beta-lactamases that have the ability of hydrolyzing the  $\beta$ -lactam ring of penicillins, aztreonam, and cephalosporins. However, they often remain susceptible to cephamycins and carbapenems (imipenem and ertapenems) (Shehani and Sui, 2013). *E. coli* could acquire some resistance factors from environmental bacteria of surroundings; this could transmit its resistance genes to other bacterial pathogens in arrays of habitats. Diagnosing of UTIs accurately and proper usage of antimicrobials for treatment and prevention are paramount in reducing drug resistance (Roshan et al., 2020). CTX-M, TEM, SHV, and AmpC beta-lactamase genes have been identified in *E. coli* isolates from UTI patients over the years (Koshesh et al., 2017). TEM (Temoneira), SHV (sulfhydryl variable), and CTX-M (cefotaximase) belong to class A ESBLs. Shahid et al. (2011) and Iroha et al. (2010) observed different frequencies of ESBLs among Gram-negative bacteria to range between 6 and 88% in different health institution settings. Sima et al. (2016) also reported CTX-M (74%), SHV (45%), and TEM (67%) genes in *E. coli* isolates. CTX-M have been increasingly recorded in various clinical specimens and *E. coli* remains the major organism implicated (Mohammed et al., 2011). This study was therefore designed to molecularly characterize ESBL-producing uropathogenic *Escherichia coli* from hospital attendees in Ebonyi State, Nigeria.

## METHODOLOGY

### Collection of samples

Seventy three (73) *E. coli* were obtained from mid-stream urine samples of 133 patients attending Federal Teaching Hospital, Abakaliki metropolis, Nigeria between February, 2018 and November, 2018. Sterile universal container was used to collect urine samples from patients suspected of UTIs. Every patient was properly instructed on self-collection of urine samples. The samples

were immediately transported to the laboratory of Applied Microbiology Department, Faculty of Sciences, Ebonyi State University, Abakaliki for bacteriological analysis.

### Culturing of samples, isolation, and biochemical characterization of bacterial isolates

Mid-stream urine samples were streaked on MacConkey agar aseptically and then incubated for 24 h at exactly 37°C. Plates were then observed for *E. coli* growth (red or pink colonies) on MacConkey agar. These suspected bacterial isolates were thereafter characterized by standard microbiology techniques such as motility test, Gram-stain, and other biochemical tests such as methyl red, indole, urease test, Voges-Proskauer, and citrate (Cheesbrough, 2010, Moses et al., 2018). Pure colonies of isolates were then inoculated on nutrient agar slants, incubated for 24 h at 37°C and kept in a refrigerator for future use at 4°C (Moses et al., 2018).

### Ethical clearance

Ethical approval was given by the Federal Teaching Hospital, Abakaliki (FETHA) research and ethical committee

### Antimicrobial susceptibility test

Isolates were tested to evaluate their antimicrobial sensitivity patterns by the Kirby-Bauer disk diffusion method. Test organisms were adjusted to McFarland equivalent standards and inoculated using sterile swab stick on Mueller-Hinton (MH) agar plates. Cefpodoxime (10  $\mu$ g), amoxicillin (20  $\mu$ g), ceftriaxone (30  $\mu$ g), ceftazidime (30  $\mu$ g), cefepime (30  $\mu$ g), cefotaxime (30  $\mu$ g), and aztreonam (30  $\mu$ g) antibiotic discs were carefully placed on the MH agar using sterile forceps. Antibiotics were allowed to properly diffuse for 10 min and plates were then incubated for 18 h at 37°C. The inhibition zone diameters (IZDs) were measured and results were interpreted as resistant or susceptible as per the CLSI guidelines after incubation (CLSI, 2018; Moses et al., 2020). The confirmed uropathogenic *E. coli* were stored in agar slant at -70°C and were subjected to further ESBL phenotypic and molecular identification.

### Phenotypic test for ESBL detection

The turbidity of suspected potential ESBL-producers were properly adjusted to 0.5 McFarland standards. Sterile swab sticks were then used to make lawn culture of the standardized isolates on the surface of MH agar plates (CLSI, 2018). Cefotaxime and ceftazidime antibiotic discs were placed at a distance of 15 mm centre-to-centre from the central disc containing amoxicillin/clavulanic acid (20  $\mu$ g/10  $\mu$ g). Plate was incubated overnight at 37°C. An increase of  $\geq 5$  mm in the IZD for either of the cephalosporins (cefotaxime and ceftazidime) tested in combination with amoxicillin/clavulanic acid versus its zone when tested alone confirms ESBL production (Sima et al., 2016). *E. coli* ATCC 25922 was used as quality control.

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**Table 1.** PCR primers of 16S rRNA and ESBL gene.

Primer name	Targeted gene	Primer sequence (5'-3') <sup>1</sup>	Amplicon product size (bp)
27 805	16S rRNA	F-AGT TTG ATC MTG GCT CAG R-GGA CTA CHA GGG TAT CTA AT	797, Gudjónsdóttir (2015)
TEM	blaTEM	F-ATGAGTATTCAACATTTCCGTGT R-TTACCAATGCTTAATCAGTGAGG	861, Azam et al. (2016)
CTX-M	blaCTX-M	F-SCS ATG TGC AGY ACC AGT AA R-ACC AGA AYV AGC GGB GC	585, Gudjónsdóttir (2015)

### DNA extraction and polymerase chain reaction (PCR) detection of blaCTX-M and blaTEM genes

Genomic DNA was extracted from pure colonies of an overnight growth of *E. coli* on Luria-Bertani agar using QIAamp DNA isolation columns (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purity and concentration of extracted DNA was determined using NanoDrop-Spectrophotometer at an absorbance of A260/280. Quality control of extracted DNA was done by testing all extracted genomic DNA for 16S rRNA gene. Table 1 shows the oligonucleotide primer sequences and the targeted genes (blaCTX-M and blaTEM) sizes. The PCR reactions for detection of blaCTX-M and blaTEM genes were done in a total reaction volume of 25 µl which contains 12.5 µl of Master Mix Red, 10 µl of Sigma water, 0.25 µl of forward primer, 0.25 µl of reverse primer, and 2 µl of the isolated genomic DNA. PCR was done with a C1000 Touch™ Thermo Cycler (Bio-Red) (Azam et al., 2016; Gudjónsdóttir, 2015).

### Gel Electrophoresis

Exactly 1.0 g of agarose and 100 ml of 1X Tris-acetate-ethylenediaminetetraacetate (TAE; pH 8.0) buffer (Bio-Rad) was used to prepare 1% (w/v) agarose gel. The mixture was then heated up for about 3 min in a microwave for total dissolution of agarose. It was then cooled to about 50°C and ethidium bromide (1 µl/ml) was added to stain the prepared agarose gel. The molten agarose gel was then cast into a gel casting tray containing combs and allowed to solidify. After about 30 min of agarose gel solidification, gel combs were carefully removed and gel casting tray containing the gel was placed into a gel electrophoresis chamber filled with TAE buffer (40 mM Tris, 20 mM acetic acid, and 100 mM EDTA pH 8.0). For each run, 5 µl of Extend Quick-Load DNA Ladder (1 kb; New England, Bio Labs) was added to one of the wells to estimate the band sizes and 5 µl of negative control, comprising Sigma water, was added to another well. Exactly 5 µl of each PCR product was carefully and properly loaded in the remaining wells. Electrophoresis was run at 80 V and 400 mA (mini Ampere) for exactly 1 h. Gels were then visualized and photographed by a gel documentation system (Bio-Rad) (Gudjónsdóttir, 2015).

## RESULTS AND DISCUSSION

This work provided insights into the antibiotic resistance profiles and molecular characteristics of uropathogenic *E. coli* with the ability to produce ESBLs. Out of the 130

samples analyzed between February and November, 2018, 73 uropathogenic *E. coli* isolates were obtained and fifty 52 isolates were ESBL producers as investigated phenotypically using double disk diffusion techniques (Tables 2 and 3). The isolates that demonstrated resistance or reduced susceptibility to ceftazidime, cefotaxime, and cefpodoxime were subjected to ESBL phenotypic detection. The keyhole pattern exhibited by uropathogenic *E. coli* isolates expressing ESBL production is as shown in Plate 1. This property is an important characteristic of ESBL-producing bacteria as a result of the synergistic effect between amoxicillin-clavulanic acid (a beta-lactamase inhibitor) and third generation cephalosporins (ceftazidime and cefotaxime). Three different sets of primers were used to amplify 16S rRNA, blaCTX-M, and blaTEM genes (Table 1). Agarose gel showed PCR product of the amplified 16S rRNA gene among the isolates to be 797 bp (Figure 1). Out of the 52 ESBL positive uropathogenic *E. coli* isolates, 17 (32.7%) harboured blaTEM gene, 35 (67.3%) harboured blaCTX-M gene, while 8 (15.3%) harboured both blaTEM and blaCTX-M genes (Table 4). PCR product band sizes of blaCTX-M and blaTEM genes were estimated to be 861 and 585 bp, respectively (Figure 2). Uropathogenic *E. coli* evaluated in this study exhibited varying frequencies of resistance to antibiotics tested. Isolates exhibited resistance to cefotaxime (83.6%), ceftazidime (79.5%), amoxicillin (72.6%), cefpodoxime (68.5%), aztreonam (61.6%), ceftriaxone (57.5%), and cefepime (37%) (Table 2). Indiscriminate use and abuse of beta-lactam antibiotics by individuals have caused problems in the treatment of microbial infections and diseases caused by these antibiotic-resistant organisms as a result of ESBL production. Some ESBL-producing bacteria failed to be detected using disk diffusion technique, thus resulting in serious treatment failures among infected patients/individuals (Umadevi et al., 2011). The lack of routine screening and detection of bacteria among clinical isolates in hospitals with the ability to produce ESBL was evident in this present study. These types of discrepancies between susceptibility data and disc diffusion call for improved ESBL detection and incorporation into routine susceptibility techniques in

**Table 2.** Susceptibility patterns of uropathogenic *E. coli*.

Antibiotics (concentrations in µg)	<i>Escherichia coli</i> , No. (%)	
	S	R
Cefepime (30)	46 (63.0)	27 (37.0)
Ceftazidime (30)	15 (20.5)	58 (79.5)
Ceftriaxone (30)	31 (42.5)	42 (57.5)
Cefpodoxime (10)	23 (31.5)	50 (68.5)
Aztreonam (30)	28 (38.4)	45 (61.6)
Cefotaxime (30)	12 (16.4)	61 (83.6)
Amoxycillin (20)	20 (27.4)	53 (72.6)

S-Susceptible; R-Resistant.

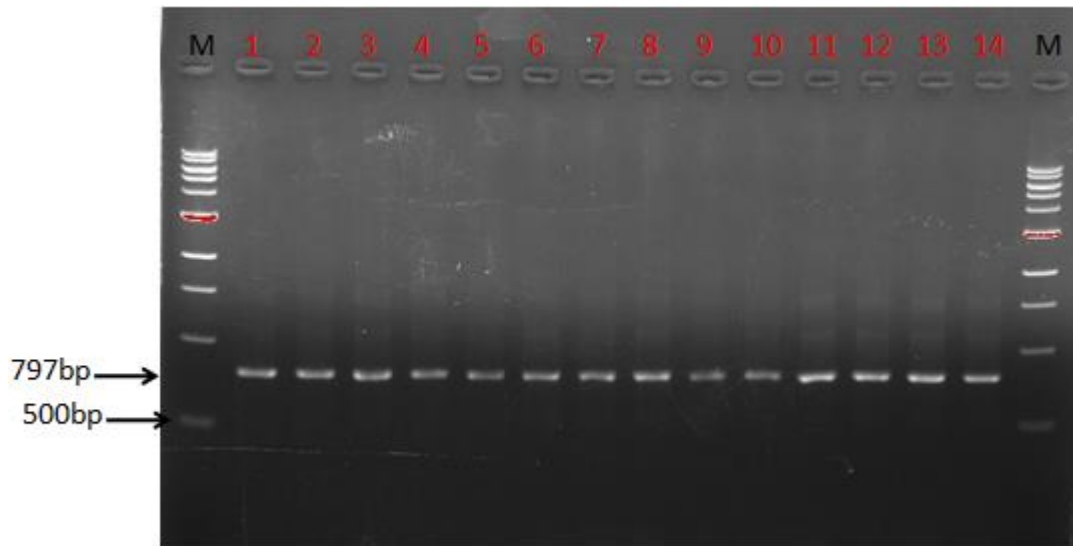
**Table 3.** Prevalence of ESBL-producing uropathogenic *Escherichia coli*.

Sample source	Sample size	Uropathogenic <i>E. coli</i> , No. (%)	ESBL-producing <i>E. coli</i> , No. (%)
Urine	130	73 (56.2)	52 (71.2)

**Plate 1.** Picture of Double Disc Synergy test for ESBL-producing Uropathogenic *Escherichia coli*.

hospitals. In the study done by other researchers, it was found that *E. coli* is usually implicated in urinary tract infections (UTIs) and frequently identified as ESBL-

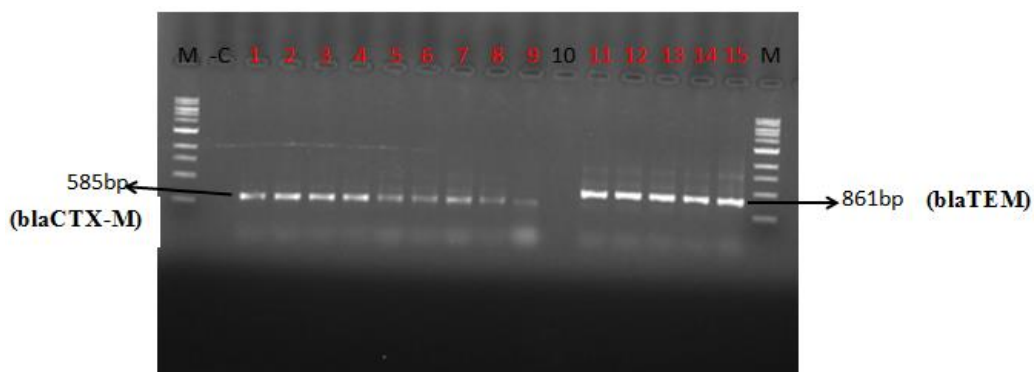
producers (Abhilash et al., 2010; Shanthi and Sekar, 2010; Umadevi et al., 2011). Phenotypic screening tests for ESBL detection only confirm whether ESBL is



**Figure 1.** Gel picture of PCR amplification of 16S rRNA gene of bacterial isolates. Lane M = 1 kb Marker, Lane 1 - 14 = Uropathogenic *E. coli*.

**Table 4.** Distribution of ESBL genes among uropathogenic *Escherichia coli*.

Beta-lactamase (bla) genes	No. of positive isolates [n (%)]
TEM	17 (32.7)
TEM and CTX-M	8 (15.3)
CTX-M	35 (67.3)



**Figure 2.** Gel picture of PCR products of amplified blaCTX-M and blaTEM genes among the bacteria isolates. Lane M= 1 kb Marker, Lane 1- 9 = blaCTX-M, Lane 11-15 = blaTEM, and -C= Negative control.

produced by the isolate but does not detect the presence of ESBL subtype. Currently, many researchers in the world have stated that although molecular methods appear more sensitive, but require specialized equipment and expertise, time consuming, and expensive (Sima et al., 2016; Varun and Parijath, 2014). A definitive identification is achievable only through molecular

techniques. However, molecular methods such as PCR are not readily available in the hospital setting and can only be seen in research facilities. A study by Dia et al. (2015) identified 32 *E. coli* isolates to be ESBLs producers and blaCTX-M gene was reported to be the most frequent ESBL gene with 90.63% frequency. Similarly, Ugbo et al. (2020) and Sharma et al. (2010)

reported 55 and 56% blaTEM frequency, respectively among *E. coli* of clinical origin; and this is in agreement with this present study where we reported 32.7 and 67.3% frequencies for TEM and CTX-M genes, respectively. Roshan et al. (2020) reported 40.3% multidrug-resistant ESBL-producing isolates of *E. coli*; of which blaTEM (83.8%) and blaCTX (66.1%) were the common ESBL genotypes detected. In a study by Abdulaziz et al. (2018), blaCTX-M gene (93.9%) was the most prevalent ESBL genotype. Multiple ESBL gene carriage was also identified to be 45.5% among the uropathogenic *E. coli* (Abdulaziz et al., 2018). These observations by Roshan et al. (2020) and Abdulaziz et al. (2018) are in agreement with our present study, where blaCTX-M genotype was the most prevalent. A study done by Zongo et al. (2015) in Burkina Faso showed that high frequency (75.5%) of ESBL-producers was observed among *E. coli* isolates. They also reported that among the isolates with ESBL-producing ability, CTX-M (65, 49%) was the most prevalent, followed by TEM (25, 73%), and SHV (18, 71%).

## Conclusion

This study has shown the presence of uropathogenic *E. coli* with ESBL-producing ability in the urine samples of hospital attendees in Abakaliki, Nigeria. Our study has also demonstrated the presence of ESBL genes, CTX-M and TEM, in the identified ESBL-producing uropathogenic *E. coli* isolates. Interestingly, blaCTX-M gene was the most predominant ESBL gene among the isolates in our study area. This research work showed that genotypic methods via PCR technique is more reliable for ESBL detection among bacterial isolates as PCR technique detected the presence of blaCTX-M and blaTEM in uropathogenic *E. coli* isolates. Thus, routine clinical detection of ESBL using phenotypic method should be introduced in the clinical setting since molecular methods are expensive to checkmate drug resistance due to ESBL production by bacteria. It is imperative that future studies should incorporate sequencing of isolates and resistance genes amplicons, together with bioinformatics so as to decipher the clonal relatedness/diversity and epidemiological identities of bacterial pathogens. This will greatly help in tracking disease occurrence, origins and sources of bacterial pathogens, and curtailing the spread of multidrug-resistant bacterial pathogens.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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