Research Article

Phenotypic and Molecular Characterization of \(\beta\)-Lactamases among Enterobacterial Uropathogens in Southeastern Nigeria

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1. Introduction

Urinary tract infections (UTIs) are among the commonest human bacterial infections occurring both in the community and hospital settings, particularly in developing countries with a high rate of casualty and financial cost [1, 2]. UTI exist when the number of microorganisms (\(\geq 10^5\) cells per milliliter) of urine is detected in properly collected mid-stream clean catch urine [3]. UTIs are caused by a variety of pathogens but mostly by the Enterobacteriaceae [1, 4, 5]. Most of the uropathogenic bacteria are from the host bowel flora which enters the bladder through the urethra/bowel reservoir [6, 7]. There have been increasing cases of antibiotic resistance among urinary tract pathogens. Though UTI is treatable, it is now becoming increasingly difficult to control because of antibiotic resistance, especially in the Enterobacteriaceae family [8]. As a result, these bacterial uropathogens are of public health concern with huge social and economic challenges [1, 8, 9]. The most common mechanism of resistance among the Enterobacteriaceae is the production of hydrolytic enzymes, the \(\beta\)-lactamases” [10]. Complications in UTIs are on the increase because of the increasing prevalence of \(\beta\)-lactamases producing uropathogens [4]. Gram-negative bacteria that produce \(\beta\)-lactamases are a major concern in healthcare due to their ability to spread globally and the consequent limited treatment options due to the multiple resistance genes as well as the enzymes’ associated link with resistance to other non-beta-lactam antibiotics [11–13]. Accurate identification of the antimicrobial resistance of a pathogen is decisive for
improved diagnosis, judicious antibiotic use, infection control, and epidemiological surveillance [13].

Molecular genotyping has been used along with phenotyping techniques to screen and confirm expression of antimicrobial drug resistance within a population [11]. Till date, little is known about the molecular basis of antimicrobial resistance in bacteria isolated from UTI in Southeastern Nigeria as inadequate attention has been given to the understanding of the molecular epidemiology of uropathogens in Nigeria, a high-burden country. In appreciation of the above-outlined issues, this study was designed to investigate the antimicrobial susceptibilities, prevalence of \( \beta \)-lactamase phenotypes and genotypes among the enterobacterial uropathogens in Southeastern Nigeria.

2. Materials Methods

2.1. Isolation and Identification. Clean-catch urine samples were collected from patients who had UTI as their primary diagnosis attending Anambra State University Teaching Hospital, Amaku, Awka. The isolates were collected between June 2016 and Feb 2017. Verbal informed consent was obtained from all patients prior to specimen collection, and the study was conducted after obtaining due ethical approval from the Anambra State Ministry of Health (MH/COMM/523/68) and the ethical committee of the hospital (COOUTH/AA/VOOL.1.002). No duplicate samples were collected. The bacterial isolates were identified with respect to their cultural and biochemical characteristics.

2.2. Antibiotic Susceptibility Study. Antibiotic susceptibility testing was done using Kirby-Bauer’s disk diffusion method. The antibiotic disc (Himedia labs, India) containing the following antibiotics was used: cefotixin (30 \( \mu \)g), ceftazidime (30 \( \mu \)g), cefotaxime (30 \( \mu \)g), cefpodoxime (30 \( \mu \)g), aztreonam (30 \( \mu \)g), meropenem (10 \( \mu \)g), ciprofloxacin (30 \( \mu \)g), ofloxacin (5 \( \mu \)g), norfloxacin (10 \( \mu \)g), levofloxacin (5 \( \mu \)g), cotrimoxazole (25 \( \mu \)g), amoxicillin (10 \( \mu \)g), and gentamicin (10 \( \mu \)g). The inhibition zone diameters (IZDs) produced by the bacteria were measured and interpreted as per CLSI guidelines [14].

2.3. Screening for ESBL, MBL, Carbapenemase, and AmpC Production. The isolates were screened for ESBL production by checking their susceptibility against the 30 \( \mu \)g disk each of ceftazidime, cefotaxime, cefpodoxime, and aztreonam. The screen positives were confirmed phenotypically by the modified combined disc on a Mueller-Hinton agar supplemented with 200 \( \mu \)g/ml clexacillin. An isolate was considered an ESBL producer when the IZD around cefotaxime-clavulanate and/or ceftazidime-clavulanate is \( \geq \)5 compared with the IZD around the cefotaxime/ceftazidime disc [15, 16].

Meropenem-resistant isolates were further confirmed for MBL production by the meropenem (MRP)-EDTA combined disc test as described by Behera et al. [17]. An isolate was recorded to be MBL positive if there was a difference of \( \geq 7 \) mm in IZD between the meropenem + EDTA disc and meropenem disc alone [17]. Similarly, the isolates were equally screened for carbapenemase production by checking their susceptibility to meropenem. An organism was considered to be carbapenamase screen positive if the IZD produced by meropenem is between 16–21 mm. The screen positives were confirmed phenotypically using the modified Hodge test (MHT) according to a previously described method [18]. Briefly, standardized inoculums of \( E. \) coli ATCC 25922 were inoculated on a Mueller-Hinton agar plate. A 10 \( \mu \)g meropenem disk (Himedia, India) was applied aseptically at the center of the inoculated Mueller-Hinton agar plate, and a suspension of the test isolate was streaked from the edge of the meropenem disk (10 \( \mu \)g) to the edge of the Mueller-Hinton agar plate. After incubation at 37°C for 18–24 hrs, the Mueller-Hinton agar plates were observed for cloverleaf effect at the intersection of the test isolate and the \( E. \) coli ATCC 25922, which is considered to be carbapenemase screen positive.

2.4. Molecular Studies

2.4.1. DNA Extraction. DNA extraction was carried out using HiPurATM Bacterial Genomic DNA purification Kit (HIMEDIA, category no MB505-50PR HiPurATM Bacterial Genomic DNA purification Kit) by following the manufacturer’s instructions. The extracted DNA was stored at −20°C and used for various molecular studies.

2.4.2. PCR Reactions. The isolates that were screen positive for ESBLs were subjected to multiplex PCR using specific primers for different families of ESBLs (Table 1):

2.4.3. PCR for ESBL (barTEM, barSHV, and barOXA-1-LIKE). Briefly, multiplex PCR reactions were performed in a final volume of 25 \( \mu \)l of the amplification mixture containing 1.25 U of Taq DNA polymerase, 1X Taq buffer, 0.2 mM each of dNTPs, 0.2 \( \mu \)M of each primer, and 2 \( \mu \)l of DNA template. The PCR was carried out with a Biorad thermal cycler (UK) using the following conditions: 94°C for 10 min; 94°C for 30 sec, 60°C for 40 sec, and 72°C for 1 min for 30 cycles, with a final extension at 72°C for 7 min. PCR products were visualized on a 1.8% agarose gel stained with ethidium bromide.

2.4.4. PCR for ESBL (barCTX-M4, barCTX-M6, and barCTX-M9). Multiplex PCR reactions were performed in a final volume of 25 \( \mu \)l of the amplification mixture containing 1.25 U of Taq DNA polymerase, 1X Taq buffer, 0.2 mM each of dNTPs, 0.2 \( \mu \)M of each primer, and 2 \( \mu \)l of DNA template. PCR was carried out with a Biorad thermal cycler (UK) using the following conditions: 94°C for 10 min; 94°C for 30 sec, 60°C for 40 sec, and 72°C for 1 min for 30 cycles, with a final extension at 72°C for 7 min. PCR products were visualized on a 1.8% agarose gel stained with ethidium bromide.
genes: bla\text{VEB}, bla\text{GES}, and bla\text{PER} using specific primers through multiplex PCR.

2.4.5. \textit{PCR for MBL, AmpC, and KPC}. The 25 isolates that were screen positive for MBLs by the phenotypic test were subjected to multiplex PCR using specific primers for different families of MBLs like bla\text{VIM}, bla\text{IMP}, bla\text{SPM}, bla\text{SIM}, and bla\text{GIM} [19]. The multiplex reaction conditions were 94°C for 5 min; 94°C for 30 sec, 52°C for 40 sec, and 72°C for 50 secs for 36 cycles, with a final extension at 72°C for 5 min. PCR products were visualized on a 1.8% agarose gel stained with ethidium bromide. PCR was equally carried out for AmpC (multiplex PCR) and KPC and NDM (uniplex PCR) using the primers and reaction conditions as in Table 2.

3. Results

A total of one hundred (100) enterobacterial uropathogens, \textit{E. coli} (58), \textit{Salmonella} (15), \textit{K. pneumoniae} (14), \textit{Citrobacter freundii} (10), and \textit{Enterobacter aerogenes} (3), were isolated and identified from 300 urine specimens collected from patients that present with clinical symptoms of UTI and positive urine culture ($\geq10^5$ CFU/mL).

The antibiotic susceptibility of the isolates shows that most of the \textit{E. coli} isolates (Table 3) were resistant to cefpodoxime, cotrimoxazole, and meropenem, immediately susceptible to aztreonam, cefotaxime, and ceftazidime but susceptible to the fluoroquinolones. \textit{Salmonella} isolates, on the other hand (Table 4), had a very good susceptibility profile to the 3\textsuperscript{rd} generation cephalosporins (cefepoxide, ceftriaxone, cefotaxime, and ceftazidime), immediately susceptible to cefotaxin but were resistant to ofloxacin and cotrimoxazole. \textit{K. pneumoniae} isolates were resistant to cefpodoxime, cefotaxime, and cotrimoxazole but susceptible to the fluoroquinolones (Table 5). Table 6 shows the summary of multiple antibiotic resistant indices (MARIs) of uropathogens. Only \textit{Salmonella} spp and \textit{E. aerogenes} had a MARI $<0.2$.

### Table 1: Primer sequence/PCR conditions for the ESBL resistance genotyping [12, 40].

| Genes | Primer sequences (5’-3’) | Annealing temp. (°C) | No. of cycles (2–4) | Amplicon size (bp) |
|-------|--------------------------|----------------------|----------------------|-------------------|
| TEM | F: CATTTCCCGTGCGCCCTTATTC  
R: CGTTCACTCATGATGTGCCTGAC | 60 | 30 | 800 |
| SHV | F: AGCGCGTTGAGGCAAATTAAC  
R: ATCCGGCAGATAAAAATCACCAC | 60 | 30 | 713 |
| OXA-1-like | F: GGCCACCACTTTCACTTTCAAG  
R: GACCACAAGTTTCTGTAAGTG | 60 | 30 | 564 |
| CTX-M-1 | F: TTAGAAAAATGGCGCCTGTA  
R: CGATATCGTTGTTGTATCAT | 60 | 30 | 688 |
| CTX-M-2 | F: CGTAAAGCGCCAGATGAGAC  
R: CGATATCGTTGTTGTATCAT | 60 | 30 | 404 |
| CTX-M-9 | F: TCAAGCCGTCGGATCTGGT  
R: TGATTCTCGGCCTGAAG | 60 | 30 | 561 |
| GES 1–9, 11 | F: AGTCGGCTAGACCGGAAAG  
R: TGTTTCGTCGCCGCTGA | 60 | 30 | 399 |
| PER 1,3 | F: GCAGGATAGAAAGCGT  
R: TCTGGCTTTGACTCGGCTGA | 60 | 30 | 520 |
| VEB 1–6 | F: CATTTCCCGATGAAATTTGACTCTG  
R: CAGAATGTTCCTTTGGACTCTG | 60 | 30 | 648 |

3.1. Phenotypic Screening of the Uropathogens for Beta-Lactamase Production. The screening tests showed 96% of the uropathogens (58 \textit{E. coli}, 15 \textit{Salmonella}, 10 \textit{K. pneumoniae}, 10 \textit{C. freundii}, and 3 \textit{E. aerogenes}) were screen positive for ESBL production while 58% (21 \textit{E. coli}, 15 \textit{Salmonella}, 13 \textit{K. pneumoniae}, 6 \textit{C. freundii}, and 3 \textit{E. aerogenes}) were screen positive for AmpC.

3.2. Results of Molecular Studies. Out of the 58 ESBL screen positive \textit{E. coli}, 35 (60.3%) were confirmed positive with PCR (Table 7). The predominant gene was bla\text{TEM}. Forty-two of the \textit{E. coli} isolates were positive for various MBL genes by PCR, bla\text{SPM} was the most predominant MBL gene. Ten (10) of the 42 \textit{E. coli} had coexpression of more than one MBL gene: $[3(bla\text{IMP} + bla\text{SPM}), 1(bla\text{SPM} + bla\text{GIM}), 3(bla\text{SPM} + bla\text{SIM}), 1(bla\text{SPM} + bla\text{VIM} + bla\text{SIM}), 2(bla\text{IMP} + bla\text{SPM} + bla\text{GIM} + bla\text{SIM})]$. Two out of the 21 AmpC screen positives were phenotypically positive for AmpC and only one of these was confirmed positive by PCR. Only 2 \textit{E. coli} isolates were KPC positive by PCR while none of the \textit{E. coli} isolates were positive for the NDM gene. Seven out of the 15 ESBL screen positive \textit{Salmonella} isolates were confirmed by PCR to harbor bla\text{TEM} + bla\text{SHV} genes, 3 isolates harboring bla\text{CTX-M-2} ($n = 1$), bla\text{GES} ($n = 1$) and bla\text{PER} gene ($n = 1$). Of the 7 MBL screen positive \textit{Salmonella}, 2 were PCR confirmed positive: 1 (bla\text{IMP} + bla\text{SPM} + bla\text{VIM}) and 1 (bla\text{IMP} + bla\text{VIM} + bla\text{GIM}). Nine of the 10 ESBL screen positive \textit{K. pneumoniae} were phenotypically and PCR positive, 5 of which had coexpression of bla\text{TEM}, bla\text{SHV}, and bla\text{OXA-1-LIKE}. Of the 13 AmpC screen positive \textit{K. pneumoniae}, none was confirmed to be a AmpC producer. Three isolates of \textit{K. pneumoniae} were positive for MBL genes: bla\text{IMP} ($n = 1$), bla\text{IMP} + bla\text{VIM} + bla\text{GIM} ($n = 1$), and bla\text{IMP} + bla\text{GIM} + bla\text{VIM} + bla\text{SIM} ($n = 1$). All the 10 \textit{C. freundii} were positive for ESBL genes. Bla\text{TEM} was the predominant ESBL gene. It existed in combination with bla\text{GES} in 5 isolates and with bla\text{VEB} in 1 isolate. Two out of the 21 AmpC screen positives were phenotypically positive.
For AmpC, and only one of these was confirmed positive by PCR. Only 2 E. coli isolates were KPC positive by PCR.

4. Discussion

Enterobacteriaceae are the highest reported causes of UTI and are usually resistant to several antibiotics resulting in recurrent UTIs, especially in the high-risk population [16, 20, 21].

They present a public health challenge and thus deserve adequate attention. For an in-depth understanding of the underlying resistance genotypes and mechanisms, this study characterized the enterobacterial uropathogens with respect to drug resistance and their $\beta$-lactamase production capacities. Antibiotic resistance is a key clinical and public health challenge in treating UTI. Emergence of $\beta$-lactamase producers among the Enterobacteriaceae reduces therapeutic options because the isolates often coexpress resistance to other classes of antibiotics. Our predominant isolates (E. coli, Salmonella spp., and K. pneumoniae) showed variable resistance to most antibiotics tested. This is similar to the findings of Ekwealor et al. [1]. The fluoroquinolones and gentamicin were highly active against E. coli isolates and thus can be prescribed for the empiric treatment of UTI caused by

| Table 2: Primer sequence/PCR conditions for the MBL, AmpC, KPC, and NDM resistance genotyping [41–42]. |
|---------------------------------------------------------------|
| **Genes** | **Primer sequences (5'-3')** | **Annealing temperature (°C)** | **No. of cycles (2–4)** | **Amplicon size (bp)** |
|-----------|-----------------------------|-------------------------------|-------------------------|------------------------|
| VIM       | F: GAT GGT TGT TGG TCG CAT  | 52                            | 36                      | 390                    |
|           | R: CGA ATG CGC AGC ACC AGA  |                               |                         |                        |
| IMP       | F: GGA ATA GAG TGG CTT AAT  | 52                            | 36                      | 180                    |
|           | CTC R: CCA AAC YAC TAS GTT  |                               |                         |                        |
|           | ATC T                               |                               |                         |                        |
| GIM       | F: TCG ACA CAC CTT GTG CTG AA  | 52                            | 36                      | 477                    |
|           | R: AAC TCC AAA TTT TGC CAT GC     |                               |                         |                        |
| SPM       | F: AAA ATC TGG GTA CGC AAA CG    | 52                            | 36                      | 271                    |
|           | R: ACA TTA TCC GCT GGA ACA AGG   |                               |                         |                        |
| SIM       | F: TAC AAG GGA TTC GGC ATC G     | 52                            | 36                      | 570                    |
|           | R: TAA TGG CCT GTT CCC ATG TG    |                               |                         |                        |
| MOXM      | F: GCT GCT CAA GGA GCA CAG GAT  | 64                            | 25                      | 520                    |
|           | R: CAC ATT GAC ATA GGT GTG TGC C |                               |                         |                        |
| CITM      | F: TGG CCA GAA CTG ACA GGC AAA  | 64                            | 25                      | 462                    |
|           | R: TTT CTC CTG AAC GTG GCC       |                               |                         |                        |
| DHAM      | F: AAC TTT CAC AGG TGT GCT GGG T | 64                            | 25                      | 405                    |
|           | R: CCG TAC GCA TAC TGG CTT TGC  |                               |                         |                        |
| ACCM      | F: AAC AGC CTC AGC AGC CGG TTA  | 64                            | 25                      | 346                    |
|           | R: TTC GCC GCA ATC ATC CCT AGC   |                               |                         |                        |
| EBCM      | F: TCG GTA AAG CGG ATG TTG CGG  | 64                            | 25                      | 302                    |
|           | R: CTT CCA CTG CGG CTG CCA GTT   |                               |                         |                        |
| FOXM      | F: AAC ATG GGG TAT CAG GGA GAT G| 64                            | 25                      | 190                    |
|           | R: CAA AGC GGG TAA CCG GAT TGG  |                               |                         |                        |
| NDM-1     | F: ACC GGC TGG ACC GAT GAC CA   | 58                            | 35                      | 264                    |
|           | R: GCC AAA GGT GGG CGC GGT TG    |                               |                         |                        |
| KPC       | F: CATTCAAGGGCTTCTGTCGTG         | 55                            | 30                      | 538                    |
|           | R: ACGACGGCGATATGCTATTG          |                               |                         |                        |

| Table 3: Antibiotic susceptibility pattern of E. coli ($n = 58$). |
|---------------------------------------------------------------|
| **S/no** | **Antibiotics** | **Resistant** | **Intermediate** | **Susceptible $n$** |
|----------|----------------|--------------|-----------------|---------------------|
| 1        | Cefpodoxime (CPD) | 35 (60.34)  | 19 (32.76) | 4 (6.90) |
| 2        | Ceftriaxone (CTR) | 0 (0)       | 10 (17.24) | 48 (82.76) |
| 3        | Aztreonam (AT) | 1 (1.72)    | 36 (62.07) | 21 (36.21) |
| 4        | Cefotaxime (CTX) | 8 (13.79)   | 33 (56.90) | 17 (29.31) |
| 5        | Ceftazidime (CAZ) | 1 (1.72)    | 34 (58.62) | 23 (39.66) |
| 6        | Meropenem (MRP) | 9 (15.52)   | 7 (12.06)  | 42 (72.41) |
| 7        | Cefoxitin (CX) | 3 (5.17)    | 11 (18.97) | 44 (75.86) |
| 8        | Ofloxacin (OF) | 4 (6.90)    | 4 (6.90)   | 50 (86.21) |
| 9        | Ciprofloxacin (CIP) | 4 (6.90)   | 10 (17.24) | 44 (75.86) |
| 10       | Norfloxacin (NX) | 5 (8.62)    | 1 (1.72)   | 51 (87.93) |
| 11       | Levofloxacin (LE) | 4 (6.90)    | 0 (0)      | 54 (93.10) |
| 12       | Cotrimoxazole (COT) | 29 (50)     | 1 (1.72)   | 26 (44.83) |
| 13       | Gentamicin (GEN) | 6 (10.34)   | 6 (10.34)  | 45 (77.59) |
| 14       | Amoxicillin (AMX) | 16 (27.59) | 3 (5.17)   | 20 (34.38) |
Similarly, in Libya, Abubaker et al. [5] reported a very good susceptibility of uropathogenic *E. coli* to ciprofloxacin, and a very low resistance to gentamicin was equally reported by Elsayed et al. [4] in Egypt.

Unlike the *E. coli* isolates, the *salmonella* spp. was resistant to the fluoroquinolones. The susceptibility test for *K. pneumoniae* showed that amoxicillin, cefpodoxime, ceftazidime, aztreonam, and cefoxitin exhibited very poor antipneumococcal activity while the fluoroquinolones showed very good activity and is in agreement with the reports of Sikarwar & Batra [22] that a fluoroquinolone, ciprofloxacin, had a 90% antibacterial activity against uropathogens. It was observed that *K. pneumoniae* isolates (Table 5) were more resistant to most of the antimicrobial agents tested than *E. coli* and *Salmonella* isolates. A similar scenario of multidrug resistance (MDR) of uropathogenic *Klebsiella* spp. has been reported in Libya [5]. It should be noted that all the isolates had poor susceptibility to cotrimoxazole and amoxicillin. This is in agreement with what was reported in Ethiopia where a high level of resistance (>70%) was recorded for cotrimoxazole and ampicillin by uropathogens [23]. Several researches have reported increasing prevalence of trimethoprim-sulfamethoxazole-resistant uropathogenic strains and suggested fluoroquinolones as an alternative treatment choice for UTI [24]. *E. coli* and *Salmonella* were very sensitive to aztreonam and ceftazidime. This observed low resistance rates may be due to
less use of these drugs in treating bacterial infections in Nigeria. A significant sensitivity to gentamicin was noted with *E. coli* and *C. freundii* ([Tables 3 & 8]). Two related studies in Abakilikii and Enugu both in Southeastern Nigeria equally reported a remarkable susceptibility of *K. pneumoniae* in the neighboring southeastern state, Enugu, by Ejikeugwu et al. [4]. Commonly, in our hospitals predisposing factors for the emergence of antibiotic-resistant urinary tract pathogens to gentamicin [18, 25]. This might be because gentamicin being a parenteral preparation might be used with much restriction. Improper antibiotic use, dose, and duration of administration have been reported as predisposing factors for the emergence of antibiotic-resistant strains in a locality [4]. Commonly, in our hospitals ceftriaxone is used empirically for inpatients and amoxicillin-clavulanate for outpatients by the physicians. The choice of drug treatments will further be determined by the sensitivity tests.

Sixteen (27.6%) of the screen positive *E. coli* were phenotypically confirmed to be ESBL producers (Table 9). Similar rates (27.7%) of ESBLs have been reported from a neighboring southeastern state, Enugu, by Ejikeugwu et al. [18] and 26.1% in southwestern Nigeria [26]. Lower prevalence (6.7%) of ESBLs was detected phenotypically among uropathogenic *E. coli* in northern Nigeria [5]. However, higher prevalence of ESBL-producing uropathogenic *E. coli* (38.9%) was reported in Nepal [11], 40% in Potohar region of Pakistan by Ali et al. [24], and 83% in Doha, Qatar [20]. The rates of resistance of ESBL-producing bacteria to antibiotics have previously been reported to be geographically dependent. This is due to the differences in antimicrobial usages and infection control measures in these locations [27].

On the molecular level, the prevalence of ESBL production was *E. coli* (60.34%), *C. freundii* (100%), *K. pneumoniae* (64.28%), and *Salmonella* spp. (46.66%). These high rates are of serious issue as the spread of these enzymes is normally driven by mobile genetic elements which facilitate the horizontal transmission of the resistance genes among bacteria of other species [28]. In addition, they often carry genes that encode high levels of resistance to many other antibiotics and cause high therapeutic failures among infected patients [16, 29]. The increasing prevalence of infections caused by antibiotic-resistant bacteria makes the empirical treatment of UTI difficult and the outcome unpredictable. It is thus associated with higher cost of therapy, increased risk of complications, morbidity, and mortality [4, 16]. Many studies reported that urine of UTI patients harbors ESBL-producing *E. coli* [5, 30]. A similar observation was noted by Iroha et al. [31] in the neighboring Enugu state where 81.8% of ESBL-producing strains of *E. coli* was isolated from urine of outpatients in a tertiary care hospital. ESBLs have been reported among 51–90% of *Enterobacteriaceae* in Asia. Similar to our findings, Padmavathy et al. [32] reported that the percentage of ESBL-producing *E. coli* was 66.9% in Chennai, India.

The high levels of ESBL producers are a major threat to infection management as this may have contributed to the antibiotic resistance reported in this study. ESBL-producing organisms are known to contain plasmids with genes that encode resistance to quinolones, aminoglycosides, and cotrimoxazole. This is exemplified in the resistance profile of *K. pneumoniae* (Table 5). The high prevalence of bla*TEM* among the *C. freundii* isolates (Table 8) might be responsible for their high resistance to the ß-lactams (amoxicillin (80%), cefotaxime (80%), and ceftazidime (60%)) as observed in Table 6. It has been reported previously that resistance to oxyimino-cephalosporins (e.g., cefpodoxime and ceftazidime), is caused mostly by TEM-type of ESBL [14]. However, ESBL-producing *E. coli* and *C. freundii* isolates were susceptible to fluoroquinolones. This finding is in line with a similar study done in Southeastern Nigeria by Iroha et al. [33]. They advised limited use of any cephalosporin on an ESBL-positive *E. coli* infection. Since *E. coli* isolates showed high prevalence of resistance to various antibiotics, strategies to control the increase in resistant uropathogens would be important. The observed low resistance of *E. coli* (13.8%) and *Salmonella* spp (13.3%) to cefotaxime and high susceptibility to ceftriaxone (>80%) might be due to the low prevalence of bla*CTX-M* gene in this study. This analogy can also explain the high resistance profile of *K. pneumoniae* (64.9%) to cefotaxime as 5 of the 14 *K. pneumoniae* isolates harboured the bla*CTX-M* gene. Among the Gram-negative pathogens,
bla<sub>CTX-M</sub> genes have been reported as a vital mechanism of resistance to cefotaxime and ceftriaxone [8]. Our findings are in line with the reports of Eskandari-Nasab et al. [34] in which the bla<sub>CTX-M</sub> genes were predominant in Klebsiella spp. Similarly Kuldeep and Nitika [21] stated that majority of ESBLs in E. coli are derived from the common plasmid mediated broad-spectrum bla<sub>TEM</sub>. Majority of ESBLs are derived from plasmid mediated penicillinases of the TEM and SHV families [35]. Low levels of bla<sub>GES</sub>, bla<sub>VEB</sub>, and bla<sub>PER</sub> were reported in this study. It has been stated that the most frequently detected clinically important ESBLs belong to the TEM, SHV, and CTX-M families while GES, VEB, and PER are of less prevalence [28, 36]. Although, the frequency of ESBL-producing isolates is increasing, the rate of infection can be minimized by regular surveillance and monitoring in order to institute effective and credible treatment of UTI.

Enterobacteriaceae resistance determinants in southeastern Nigeria. Ejikeugwu et al. [38] had reported increasing reports of MBL-producing Gram-negative bacteria in Anambra State University Teaching Hospital, Awka. The genotypic method has a higher specificity/sensitivity than the phenotypic method as thus should be a method of choice for detection of ESBL-producing strains. Limitations of the study are that we didn’t record the patient’s demographics and history of their antibiotic consumption. We also could not screen specifically for OXA-48 genes.

### Table 9: Differences between bla-phenotypic and bla-PCR positives.

| Organisms          | ESBL Phenotypic positive | PCR-positive | MBL Phenotypic positive | PCR-positive | AmpC Phenotypic positive | PCR-positive |
|--------------------|--------------------------|--------------|-------------------------|--------------|-------------------------|--------------|
| E. coli (58)       | 16                       | 35           | 3                       | 10           | 2                       | 1            |
| C. freundii (10)   | 5                        | 10           | 0                       | 0            | 0                       | 1            |
| K. pneumoniae (14) | 9                        | 9            | 0                       | 3            | 2                       | 0            |
| Salmonella spp (15)| 1                        | 7            | 0                       | 2            | 2                       | 0            |
| Total              | 31                       | 61           | 3                       | 15           | 4                       | 2            |

bla<sub>CTX-M</sub> genes in our study is likely to be responsible for the observed high susceptibility of E. coli (75%) and intermediate susceptible of Salmonella to cefoxitin. Conversely, a study in Chennai, India, reported that 61.9% of the uropathogenic E. coli isolates expressed an AmpC phenotype [32].

### 5. Conclusion

The uropathogens were found to be resistant to various antimicrobial classes studied. The study showed high prevalence of drug-resistant genes among the enterobacterial uropathogens. Majority of the enterobacterial uropathogens harbored more than one antibiotic-resistant gene. Our study has notably shown that all the ESBL genes, the most predominant gene in E. coli and C. freundii was bla<sub>TEM</sub>-in Salmonella spp was a combination of bla<sub>TEM</sub>-SHV, and in K. pneumoniae, bla<sub>CTX-M1</sub> was predominant among the enterobacterial uropathogens isolated from patients of Anambra State University Teaching Hospital, Awka. The genotypic method has a higher specificity/sensitivity than the phenotypic method as thus should be a method of choice for detection of ESBL-producing strains. Limitations of the study are that we didn’t record the patient’s demographics and history of their antibiotic consumption. We also could not screen specifically for OXA-48 genes.

### Data Availability

The data used to support the findings of this study are included within the article.

### Ethical Approval

The study was ethically approved by the Anambra State Ministry of Health (MH/COMM/523/68) and the ethical committee of the hospital (COOUTH/AA/VOOL.1.002) while informed consent was taken from the patients.

### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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