Original Research Article

Molecular Characterization Of Plasmid-Mediated Extended Spectrum Beta-Lactamase Resistance In Urinary *Escherichia Coli* From Patients In General Hospital, Maitama, Abuja, Nigeria

ABSTRACT

Aims: This study investigates and reports the production of extended spectrum beta-lactamase in *Escherichia coli* isolates from urine of patients sourced from General Hospital, Maitama, Abuja, Nigeria

Study design: Cross sectional study

Place and Duration of Study: Department of Microbiology, Nasarawa State University, Keffi, between August 2020 and February 2022.

Methodology:*Escherichia coli* was isolated from the samples using standard cultural and microbiological methods. Antibiotic susceptibility testing and minimum inhibitory concentrations were evaluated as described by the Clinical and Laboratory Standards Institute (CLSI). The detection of ESBL production in *E. coli* isolates was carried out using double disc synergy test. In addition, molecular detection of ESBL genes was carried out using Polymerase Chain Reaction (PCR) method.

Results:27.5% of samples isolated (33/120) had *E. coli*. Antibiotic resistance in the isolates in decreasing order were as follows: sulphamethoxazole/trimethoprim (91.7%),amoxicillin/clavulanic acid (72.2%), ceftriaxone (69.7%), cefotaxime (66.7%), ciprofloxacin (42.4%), meropenem (42.4%), ofloxacin (39.4%), imipenem (39.4%), gentamicin (33.3%), and nitrofurantoin (20.3%). The most common antibiotic resistant phenotypes wereCTX-AMC-OFX-CRO-SXT-CIP-NET-IMP-MOR (16.7%) and CTX-AMC-OFX-CRO-CN-SXT-CIP-NET-IMP-MOR(16.7%). Multiple antibiotic resistance (MAR) was observed in 96.6% (29/30) of the isolates with the common MAR indices being 0.2 (23.3%), 0.9 (16.7%), and 1.0 (16.7%). Six of the twenty six cefotaxime/ceftriaxone jointly resistant isolates (23.1%) were confirmed ESBL producers. All six of the ESBL positive isolates (100.0%) carried *bla*genes as follows: bla_{TEM} (6/6, 100.0%), bla_{SHV} (2/6, 33.3%), andbla_{CTX-M} (6/6, 100.0%).

Conclusion: The *E.coli*isolates were less resistant to nitrofurantoin, gentamicin, imipenem, ofloxacin and meropenem. In addition, ESBLgenes were detected in confirmed *E. coli* isolates.

Keywords: Escherichia coli; urine; antibiotics resistance; susceptibility; gene.

1. INTRODUCTION

Escherichia coli is known to be a major pathogen which causes a wide spectrum of clinical manifestations, and diseases including UTIs, pneumonia, bacteremia, meningitis and abdominal infections [1]. Antimicrobial such as beta-lactam namely; extended spectrum cephalosporins and penicllins; fluoroquinoles and aminoglycosides are among the most common agent prescribed for treatment of infection caused by Gram negative Enterobactericeae[2, 3]. Cephalosporins are also widely used for treatment of UTIs due to their potency, broad-spectrum of activity and safety profile [2].

The World Health Organization has recently included ESBL producing *E. coli* and many other Enterobacteria in the WHO global priority pathogens list as “priority 1”, critical [2], - and the Center for Disease Control and prevention has also classified these resistant bacteria in the category of “serious threat” based on the clinical and economic impact, incidence of transmissibility and barrier to prevention [3]. In Nigeria, the health sector is not exempted from the challenge of antimicrobial resistance, especially from organisms such as *E. coli* [4].

The use of antibiotics is considered as a great factor in the emergence, selection and dissemination antibiotic-resistant organisms in medicine [5]. The trend of antimicrobial resistance among *E. coli* in hospitalsand health care centres is a cause of concern especially due to the possibility and potential for the transfer of these pathogens to the human population [5].Recently, 60.0% ESBL producing *E. coli* were associated with suspected cases of UTIs in Abuja and other parts of Nigeria [6, 7, 8].[9] also identified ESBL and ciprofloxacin co-resistance strain A and B as the most common strains associated with suspected cases of UTIs in tertiary hospitals, Nasarawa State, Nigeria. This study however investigates the antimicrobial resistance profiles, and ESBL resistance genes of the ESBL producing isolates from urine of patients with suspected UTIs in Maitama, Abuja.Nigeria.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Media

Bacteriological media that were used in this study included: MacConkey Agar (MCA), Mueller-Hinton Agar (MHA), Nutrient agar (NA), Luria-Bertani (LB) broth, Eosine Methylene Blue (EMB) Agar, Nutrient Broth (NB), Simmons Citrate Agar (SCA), Methyl red/Voges-Proskauer (MR/VP) medium and Peptone water (PW). All the media were sourced from Oxoid Ltd. (U.K.).

2.1.2 Equipment

The equipment used in this study include: Autoclave (Certoclav, Model SM280E, Surgifriend Medicals, England), Oven (HotboxSize One, Galengkamp, U.K.), Incubator (Model12-140E, Quincy Lab Inc), Refrigerator/Freezer (Model PRN 1313 HCA, BEKO, Germany), Thermocycler (Model TC-312, Techne, England), Gel electrophoresis machine (Max Fill Scie-plas Model HU10 serial no5237), Laminar air flow cabinet (PCR-8 re-circulating laminar flow prestation, Labcaire product 220/240v), Microscope (Model CME 1349522X, Leica, USA), Spectrophotometer (Eppendorf Biophotometer 8.5 mm, Lichtstrahihohe), UV illuminator (VilberbLourmat TFX-35-M serial no NoV02 8104), Centrifuge (Model 5417R: Touch plate Super Mixer, CAT No 1291, Lab-line Instrument Inc USA), Microwave oven (HINARI Life Style 800watts model MX310TCSL), Electronic weighing balance (Model QT 600: Touch plate Super Mixer, CAT No 1291, Lab-line Instrument Inc USA),Vortex machine (Touch plate Super Mixer, CAT No 1291, Lab-line Instrument Inc USA), and Gel Doc system (Biorad, U.K.)

2.1.3 Chemicals and reagents

The chemicals and reagents used in this study included: Carbolfuschin, Crystal violet, Ethanol, Creatinine, Potassium hydroxide and Kovac's reagents, obtained from BDH chemical Ltd, England; Ethidium bromide, Iodine solution, EDTA and Glycerol obtained from Sigma Chemical Ltd, England; and Agarose gel from Schwarz/ Mann Biotech.

2.1.4 Bacteria Isolates

Confirmed *E. coli*isolates from the urine of patients were obtained and used for this study. The antibiotic resistance profiles of the isolates are as shown in Table 4.

2.2 Methods

2.2.1 Antibiotic Susceptibility Testing

The antibiotic susceptibility test for *E. coli*isolates was carried outusing the Kirby-Bauer disc diffusion method as modified by the Clinical and LaboratoryStandards Institute - CLSI [10]. Briefly, 5 colonies of *E. coli*isolates were inoculated into 5 ml of Mueller-Hinton broth (MHB) and incubated at 37ºC for 24 hours after which the 24-hour

MHB was standardized to the turbidity equivalent to 0.5 McFarland standards. The 0.5 McFarland standard was prepared as follows: 99.5 ml of 1% ($\frac{v}{v}$) H_2SO_4 + 0.5ml of 1.172% ($\frac{w}{v}$) $BaCl_2.2H_20$. A sterile cotton swab stick was dipped into the standardized *E. coli*suspension and streaked on MHA plates. Antibiotics discs were gently placed on the MHA plates using a pair of sterile forceps and the plates were allowed to incubate at room temperature for 1 hour before re-incubating at 37ºC for 17 hours. The discs used include:Amoxicillin/Clavulanic acid (AMC): (10/20 µg), Sulphamethoxazole/ Trimethoprim (SXT :) (25 µg), Ceftriaxone (CRO): (30 µg), Cefotaxime (CTX :) (30 µg), Nitrofurantoin (NET :) (30 µg), Ofloxacin (OFX :) (5 µg), Gentamicin (CN :) (10 µg), Meropenem (MOR :) (30 µg), Ciprofloxacin (CIP :) (5 µg) and Imipenem (IPM :) (30 µg).After incubation, the diameters of the zones of inhibition were measured to the nearest millimetre (mm) using a ruler and the result of the susceptibility test was interpreted using susceptibility breakpoint earlier described by CLSI [10].

2.2.2 Extended spectrum β-Lactamase production test

The phenotypic confirmatory test for ESBL production by isolates resistant to cefotaxime and ceftriaxome was carried out using Double- Disc Synergy Test (DDST) method earlier described by Fetahagic et al. [11]. Briefly,105cfu/ml bacterial suspension was streaked on sterile Mueller-Hinton agar plates and amoxicillin/clavulanic acid (30 µg) disc was placed at the centre of the plate. Cefotaxime (30 µg) and ceftriaxone (30 µg) discs were then placed 15mm (edge-to-edge) from the centre disc. Enhancement of zone of inhibition in the area between the amoxicillin-clavulanic acid disc and any one of the β-lactam discs compared with the zone of inhibition on the far side of the drug disc was interpreted as indicative of the presence of an ESBL in the tested strain.

2.2.3 Molecular detection of extended spectrum β-Lactamase genes

Isolates that were confirmed ESBL producers were screened to detect the presence of some ESBL resistance genes namely: bla_{SHV}, $bla_{TEM}$and bla_{CTX-M}.

2.2.4 DNA extraction

The bacterial DNA was extracted by a method as earlier described by [12] with minor modification. Ten (10) milliliters of an overnight broth culture of the bacterial isolate in 1 ml Luria-Bertani (LB) were spun at 14000 rpm for 3 min. The supernatant was discarded, and the harvested cell pellet was resuspended in 1 ml sterile distilled water and transferred into 1.5 ml centrifuge tube and centrifuged at 14000 rpm for 10 min. The supernatant was discarded carefully. The pellet was resuspended in 100 µl of sterile distilled water by vortexing. The tube was centrifuged again at 14000 g for 10 min, and the supernatant was discarded carefully. The cells were re-suspended in 500 µl of normal saline and heated at

95°C for 20 min. The heated bacterial suspension was cooled on ice for 10mins and spun for 3 min at 14000 rpm. The supernatant containing the DNA was transferred to a 1.5-ml microcentrifuge tube and stored at -20^{o}C for other downstream reactions.

Estimation of the concentration, purity and yield of the DNA sample was accessed using absorbance method (measurement of absorbance) with the spectrophotometer (Nanodrop 1000). For DNA concentration, absorbance readings were performed at 260 nm (A_{260}) and the readings were observed to be within the instrument's linear range (0.1 – 1.0). DNA purity was estimated by calculating the A_{260}/A_{280} ratio and this was done by the spectrophotometer's computer software (where A_{260}/A_{280} ratio ranges from 1.7 – 1.9).

2.2.5 DNA amplification of extended spectrum β-Lactamase genes

Simplex Polymerase Chain Reaction (PCR) was performed in order to amplify the ESBL genes being assessed in the isolates. The presence of *blaCTX-M, blaSHV and blaTEM*genes were tested for using previously published primer sets and conditions. The primer sequences and expected amplicon sizes for each gene are listed in Table 1.

The reactions were carried out in 20 µl reaction volume made up of 10 µl of Mastermix (InqabaBiotectm, South Africa), 0.32 µl of primers (0.16 µl each of forward and reverse primers), 3 µl of DNA and 6.68 µl of nuclease-free water. The primer concentration stood at 0.2 M. The reaction tubes were placed in the holes of the thermal cycler and the door of the machine was closed.

Conditions for amplification of all the genes during the reactions were set as 3 min of initial denaturation at 95°C, followed by 35 amplification cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 40 sec, initial extension at 72°C for 50 sec, final extension at 72°C for 3 min and a hold at 4°C infinitely.

2.2.6 Agarose gel electrophoresis

Exactly 7 µl of the amplified DNA was transferred into the wells of a 1.5% Agarose gel by stabbing the wells using a micropipette and this was done carefully to ensure that each well had only one sample. Each gel had one well which contained a DNA ladder (1500 bp, Thermo Scientific, Inqaba Biotech, South Africa) in order to estimate the size of the DNA amplicons. Electrophoresis was run at 125 volts for 20 min, after which the gels were viewed using ultra-violet trans-illuminator.

Table 1. Primers and their sequences

S/N	Target genes	Sequence	Amplicon size (bp)	References
1	bla_{TEM}	5´-TCGGGGAAATGTGCGCG-3´ 5´-TGCTTAATCAGTGAGGCACC-3´	972	[13]
2	bla_{SHV}	5´-GGGTTATTCTTATTTGTCGC-3´ 5´-TTAGCGTTGCCAGTGCTC-3	615	[14]
3	bla_{CTX-M}	5´-ACGCTGTTGTTAGGAAGTG-3´ 5´-TTGAGGCTGGGTGAAGT-3´	857	[14]

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Escherichia coli*

The cultural, morphological and biochemical finger print of *E. coli* isolated from urine of patients in General Hospital, Maitama, Abuja, Nigeria was observed. Pinkish colony on MCA

which grew with greenish metallic sheen on EMB agar was Gram negative rod and had biochemical reactions namely: indole-positive, methyl red-positive, Voges- Proskauer-negative, citrate-negative, ONPGpositive, among others indicated *E. coli*.

3.2 Occurrence of *Escherichia coli*

A total of 120 samples were isolatedfor this study.33 isolates were confirmed *E. coli,* out of which 6 (13.3%) were male and 27 (36.0%) female as shown on Table 2. The age distribution of the study population was also observed as shown in Table 3.

Table 2. **Occurrence of *Escherichia coli* in the urine of patients in relation to Gender**

Gender	No. (%) *E. coli*(n=33)
Male	6(13.3)
Female	27 (36.0)
Total	33 (27.5)

Table 3. **Age Distribution of the Study Population**

Age (Years)	No. (%) *E. coli*
≤ 10	1(3.33)
11-20	3 (9.1)
21-30	5 (15.1)
31-40	15 (45.4)
41-50	6 (18.1)
>50	3 (33.3)
Total	30 (25.0)

3.2 Antimicrobial Resistance Profile

The antimicrobial resistance in the *E. coli*isolates from urine of patients attending General Hospital Maitama, Abuja Municipal Area, Nigeria is as shown in Table 4. The resistance in the isolates were as followssulphamethoxazole/trimethoprim (91.7%), amoxicillin/clavulanic acid (72.2%), ceftriaxone (69.7%), cefotaxime (66.7%), ciprofloxacin (42.4%), meropenem (42.4%), ofloxacin (39.4%), imipenem (39.4%), gentamicin (33.3%), and nitrofurantoin (20.3%).

Table 4. Antimicrobial Resistance and Sensitivity pattern of *Escherichia coli* isolates (n=33)

Antibiotic	Resistance (%)
sulphamethoxazole/trimethoprim	30 (91.7%)
amoxicillin/clavulanic acid	24 (72.2%)
Ceftriaxone	23 (69.7%)
cefotaxime	22 (66.7%),
ciprofloxacin	14 (42.4%),

meropenem	14 (42.4%),
Ofloxacin	13 (39.4%)
imipenem	13 (39.4%)
gentamicin	10 (33.3%)
nitrofurantoin	7 (20.3%)

3.3Antimicrobial Resistance Phenotypes

Resistance to the antibiotics tested was observed in the 30 confirmed *E. coli* isolates. The distribution of the resistant isolates into phenotypes is shown in Table 5. The commonest phenotype was the CTX-AMC-OFX-CRO-SXT-CIP-NET-IMP-MOR, and CTX-AMC-OFX-CRO-CN-SXT-CIP-NET-IMP-MORcombinations all having 5 isolates each(16.7%)

3.4 Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR), which is the resistance of microorganisms to at least two (2) antibiotics was observed in all, bar one of the isolates. One isolate (3.3%) had a MAR index of < 0.20. The commonest indices were 0.2 (23.3%), 0.9 (16.7%), 1.0 (16.7%) and 0.3 (13.3%) as shown in Table 6.

3.5 Molecular Detection of Extended Spectrum Beta-lactamase Genes

Six of the thirty ESBL positive *E. coli* isolates (20.0%) carried the *bla*genes as follows: bla_{SHV} (2/6; 33.3%), bla_{CTX-M} (6/6; 100.0) and bla_{TEM} (6/6; 100.0). Some isolates carried two *bla*genes (either of the combinations: bla_{CTX-M}, and bla_{TEM}; bla_{TEM} and $bla_{SHV;}$ and $bla_{TEM}bla_{CTX-M}$and bla_{SHV}).

Table 5. Antimicrobial Resistance Phenotypes of *Escherichia coli* isolated from urine of patients attending General Hospital in Abuja Municipal Area, Nigeria

Antimicrobial Resistance Phenotypes	GHM (n=30)
SXT	1(3.3)
CTX, AMC	2(6.7)
AMC, CRO	3(10.0)
SXT, CN	1(3.3)
OFX, CIP	1(3.3)
CTX, AMC, SXT	1(3.3)
AMC, SXT, CRO	1(3.3)
CRO, CTX, AMC	1(3.3)
CTX, AMC, CRO, SXT	3(10.0)
CTX, AMC, CN, SXT, CIP, MOR	1(3.3)
CTX, AMC, CRO, CN, SXT, MOR	1(3.3)
CTX, OFX, CRO, CN, SXT, CIP, IMP, MOR	3(10.0)
CTX, AMC, OFX, CRO, SXT, CIP, NET, IMP, MOR	5(16.7)
CTX, AMC, OFX, CRO, CN, SXT, CIP, NET, IMP, MOR	5(16.7)

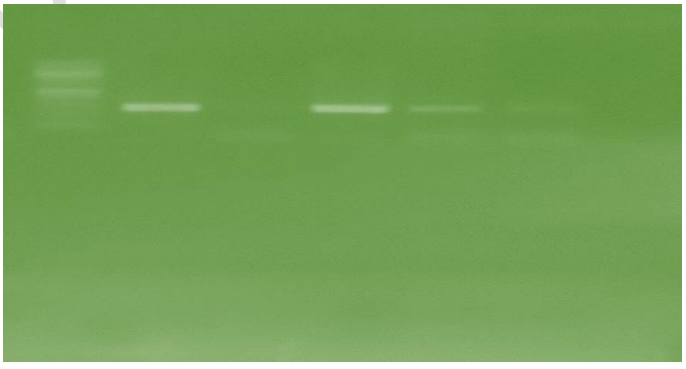
AMC=Amoxicillin/Clavulanic acid; CTX=Cefotaxime; CRO=Ceftriaxone; CIP=Ciprofloxacin; CN=Gentamicin; IMP=Imipenem; OFX=Ofloxacin; MOR=Meropenem; NET=Nitrofurantoin; SXT=Sulfamethoxazole/Trimethoprim

Table 6. Multiple Antibiotic Resistance (MAR) index of resistant *Escherichia coli* isolated from urine of patients attending General Hospital Maitama, Abuja, Nigeria

No of antibiotics isolate resistant to (a)	No. of antibiotics tested (b)	MAR Index $(\frac{a}{b})$	No. (%) MAR isolates (n=30)
10	10	1.0	5(16.7)
9	10	0.9	5(16.7)
8	10	0.8	3(10.0)
7	10	0.7	0(0.0)
6	10	0.6	2(6.7)
5	10	0.5	0(0.0)
4	10	0.4	3(10.0)
3	10	0.3	4(13.3)
2	10	0.2	7(23.3)
1	10	0.1	1(3.3)

**MAR isolates are those with resistance to at least two antibiotics [15]*

1 M 2 3



blaSHV(147bp)

SHV

3.6 Discussion

There has been a significant increase in the number of infections due to ESBL E. coli, and African countries have their fair share. [16]. Many strain of *E. coli* have also been shown to be multi-resistant [17, 18, and 19]. Interestingly, reports have shown that the heavy usage of antibiotics is a risk factor for the acquisition of ESBL-producing organisms and this has resulted in the increase in resistance of many common antibiotics, such as,ampicillin, tetracycline, gentamicin and cephalosporins(third generation) [19].

In this present study, 27.5% of the total samples were found to be carriers of *E. coli.* Also, 18.1% of the *E. coli* isolates were discovered to carry ESBL genes. This observation is similar to the findings in other studies that ESBL producing organisms can be found or detected in both community and hospitalized patients with varying prevalence levels; Odongo *et al*,in Uganda [20] observed a 10% prevalence, and a similar study by Odoki*et al* in Uganda [21] showed 41.9%. The varying prevalence in the different studies could be due to population variation and differences in sample sizes. The prevalence was observed to be higher in females (36.0%) than males (13.3%). Many studies have also documented the prevalence of *E. coli* in UTI patients to be higher in females compared to the males [22, 23]. This could be due to the proximity of the anus to the urethra of females the urethral tube of the female is short, hence, the distance travelled by the organism to the bladder is shortened.

The prevalence was high in the age group 31-40 (45.4%), compared to other age groups. The findings are similar to the ones reported by Lin *et al*, [24] in Taiwan (30%), Asare *et al*, [25] in Ghana (44%)., but differs from studies by, Aiyegoro*et al* [26], Collingwood *et al*[27] where juveniles <17 were recorded as prevalent carriers.

In our study, all but one of the isolates (96.7%) were observed to be multiple resistant, that is, resistant to two or more antibiotics (including βlactam antibiotics, trimethoprim-sulfamethoxazole, ciprofloxacin amongst others). Resistance to the antibiotics used was as follows: sulphamethoxazole/trimethoprim (91.7%), amoxicillin/clavulanic acid (72.2%), ceftriaxone (69.7%), cefotaxime (66.7%), ciprofloxacin (42.4%), meropenem (42.4%), ofloxacin (39.4%), imipenem (39.4%), gentamicin (33.3%), and nitrofurantoin (20.3%). The high level of resistance observed to augmentin, sulphamethoxazole/trimethoprim, and ceftriaxone (all >50%) is very likely due to selective pressure as a result of uncontrolled and indiscriminate or inappropriate use of these antibiotics in hospitals and in other places around the country. This could be said to be encouraged by the lack of sufficient knowledge and awareness of the antibiotic policy recently released. The availability of antibiotics hawked and sold over the counter in Nigeria could be said to be a contributing factor.

Very few publications have been made and documented on the molecular characterization of ESBL genes from *E. coli* isolated from urine of patients in hospitals in Abuja. This characterization plays an important role in epidemiological studies as well as management of outbreaks, or even controlling and preventive measures. In this study, 6 of the 30 *E. coli* isolated and confirmed positive for ESBL phenotype (20%) carried ESBL genes. This finding is similar to a study by Wang *et al*, in China (39%), Sadeghi *et al* in Iran (40%), and Pandit *et al*, in Nepal (40%), [28, 29, 30], but is different from findings by Kuta in Minna (60%) [31], Ugwu *et al* (60.3%) [32] in Anambra and 93% by Abujnah in Libya [33].The high levels of ESBL producers are a major threat to infection and disease management ESBL-producing organisms are known to contain plasmids with genes that encode resistance to quinolones, aminoglycosides, and cotrimoxazole [34].

In conclusion, this study has described the emergence of ESBLs among E. coli isolates obtained from hospitalizedpatients in Abuja. Also, similar situationseems to be occurring in other countries; thespread of E. coli-producing ESBLs comes as a great challenge to health systems especially in developing countries, and this emphasizes the need for implementationof strict hospital infection control policies, includingthe review of current therapeutic modalities, control ofthe use of non-prescribed antibiotics and continuousmonitoring of antibiotic sensitivity profiles of E. coliisolates.

4. CONCLUSION

Escherichia coli, the organism of interest, observed in the UTI patients had a low prevalence. Also observed was a significantly high resistance to trimethoprim/sulfamethoxazole, ciprofloxacin and augmentin. Continuous use of these drugs may be as a result of antimicrobial resistance and treatment failures. Urine culture should be done when UTI is suspected to serve as a guide. There is also a need to continuously monitor antibiotic resistance so as to curb or reduce resistance emergence.

CONSENT

Not applicable

ETHICAL APPROVAL

Appropriate ethical approval was obtained before the start of the study.

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