Original Research Article

Antimicrobial Resistance Profile and Extended Spectrum Beta-Lactamase Resistance Genes in Urinary *Escherichia coli* from Patients in General Hospital, Karshi, Abuja, Nigeria

ABSTRACT

Aims: This study investigated the antimicrobial resistance profile and extended spectrum beta-lactamase resistance genes in *Escherichia coli* of from urine of patients sourced from General Hospital, Karshi, Abuja, Nigeria

Study design: Investigative

Place and Duration of Study: Department of Microbiology, Nasarawa State University, Keffi, between August 2022 and February 2023.

Methodology: A total of 120 samples were collected fromurine of patients. *Escherichia coli* was isolated from thesamples 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: The prevalence of *E. coli* was 17.5%. Antibiotic resistances in the isolates in decreasing order were as follows: sulphamethoxazole / trimethoprim (SXT: 81.0%), amoxicillin/clavulanic acid (AMC: 61.9%), ofloxacin (OFX: 66.7%), cefotaxime (CTX: 53.4%), gentamicin (CN: 42.9%), ceftriaxone (CRO: 33.3%),imipenem (IPM: 33.3%),meropenem (MOR: 42.4%), nitrofurantoin (NET: 20.3%) and ciprofloxacin (CIP: 23.8%). The commonest antibiotic resistant resistance phenotype was CIP-OFX-SXT (23.8%). Multiple antibiotic resistance (MAR) was observed in 90.5% (19/21) of the isolates, with the common MAR index being 0.3 (23.8%). Six of the twenty one beta-lactam resistant isolates (28.5%) were confirmed ESBL producers. The 6 ESBL positive isolates carried *bla* genes as follows: bla_{TEM} (1/6, 16.7%) and bla_{CTX-M} (1/6, 16.7%). bla_{SHV}only was not found in any of the isolates.

Conclusion: The *E. coli* isolates from urine of patients in General Hospital, Karshi, Abuja, Nigeriawas less resistant to ciprofloxacin, nitrofurantoin, meropenem and imipenem.This implies that the antibiotics are useful in the treatment of infection caused by *E. coli.* Also, ESBL-positive *E. coli* isolates harbored ESBL genes, with $bla_{CTX-M}$and bla_{TEM}as the most common.

Keywords: Escherichia coli; Urine; antibiotic; resistance; ESBL; genes

1. INTRODUCTION

Urinary tract infections (UTIs) are one of the popularly known diseases that are encountered in medical and clinical practice and are caused mainly by organisms such as *Escherichia coli*[1, 2, 3, 4]. Antimicrobial and antibiotic resistance during treatment in patients with UTIs is an example of the ever increasing challenge of antimicrobial resistance [5]. The recent emergence and global dissemination of E. coli resistant to antibiotics due to the production of extended-spectrum b-lactamases (ESBLs) has shown that *E. coli* antibiotic resistance is currently a real and genuine public health concern [5, 6, 7]. From data available, not many epidemiological surveillance studies in Abuja have investigated the molecular nature of E. coli strains circulating in the healthcare settings, particularly the General hospitals.

UNDER PEER REVIEW

The objectives of this study were to detect *E. coli* in urine from General Hospital in Karshi, Abuja, and observe the trend of E. coli resistance to antibiotics and to molecularly characterize E. coli isolates circulating in the hospital. The results of the present study might be helpful and aid the understanding of the situation in UTIs caused by *E. coli*.

2. MATERIAL AND METHODS

2.1 Sample collection

A total of 120 urinary sampleswere randomly collected using appropriate sterile sample containers and transported to the Microbiology Laboratory at the Nasarawa State University, Keffi, for same-day analysis or stored in a refrigerator (Model PRN 1313 HCA, BEKO, Germany) at 5°C for latter-day analysis.The samples were collected from Karshi General Hospital, a secondary health facility located in Karshi town, a suburb in the Federal Capital Territory, Abuja, Nigeria Samples were collected between August 2022 and February 2023.

2.1.1 Isolation of *Escherichia coli*

The samples collected were subjected to isolation procedure; presumptive *E. coli* was isolated from the urine as follows: 1ml of urine was inoculated into 9 ml of nutrient broth (NB: Oxoid Ltd., UK) and incubated in an incubator (Quincy Lab Inc. Model12-140E, USA) at 37ºC for 24 h. A loopful of the 24- h broth was streaked on MacConkey agar (MCA: Oxoid Ltd., UK) plate and incubated at 37ºC for 24 h. Pinkish colonies from the 24-hour MCA plates were further streaked on Eosine Methylene Blue agar (EMB: Oxoid Ltd., UK) plates and incubated at 37ºC for 24 h. Colonies with a greenish-metallic sheen appearance were selected as presumptive *E. coli* [8].

2.1.2 Identification of *Escherichia coli*

E. coli was identified done by morphological, cultural and biochemical characteristics using Gram staining, Motility Test and biochemical tests (Indole, Methyl Red-Voges-Proskauer, Citrate, Nitrate Reduction Test, Urease Test, H_2S production Test, etc.) as described in the Bacteriological Analytical Manual [9] and Cheesbrough [8]. The API20E system (Analytical Profile Index) (BioMerieux™, USA), a commercial kit designed for the identification of Enterobacteriaceae and other non-fastidious Gram negative bacteria, was used to confirm the suspected isolates as described in the manufacturer's manual. Colonies with a characteristic pink color on MCA, which grew with a greenish-metallic sheen on EMB agar, Gram-negative, rods, indole positive, citrate negative, methyl-red positive, Voges-Proskauer negative, urease negative, nitrate reduction positive, and a positive motility test indicated *E. coli.* The bacterium was stored in the refrigerator on nutrient agar (Oxoid Ltd, UK) slants and reactivated by sub-culturing on MCA for use in further research.

2.2 Antimicrobial Susceptibility Testing

The antibiotic susceptibility test for *E. coli* isolates from urine was carried outusing the Kirby-Bauer disc diffusion method as modified by the Clinical and Laboratory.Standards Institute (CLSI) [10]. Briefly, 5 colonies of *E. coli* isolates were inoculated into 5 ml of Mueller-Hinton broth (MHB: Oxoid Ltd, UK) and incubated at 37ºC for 24 h after which the 24-h MHB was standardized to the turbidity equivalent to 0.5 McFarland Standard. The 0.5 McFarland Standard was prepared as follows: 99.5 ml of 1% $(\frac{v}{v})$ H_2SO_4 + 0.5 ml of 1.172% $(\frac{w}{v})$ $BaCl_2.2H_2O$. A sterile cotton swab stick was dipped into the standardized *E. coli* suspension and streaked on Mueller-Hinton Agar (MHA: Oxoid Ltd, UK) plates. Antibiotics discs (Oxoid Ltd, UK) were gently placed 15mm apart on the MHA surface using a pair of sterile forceps and the plates were allowed to incubate at room temperature for 1 h before re-incubating at 37ºC for 17 h. The discs used include: Amoxicillin/Clavulanic acid (AMC): (10/20 µg),

UNDER PEER REVIEW

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 millimeter (mm) using a ruler, and the result of the susceptibility test was interpreted using susceptibility breakpoint earlier described by CLSI [10].

2.3 Extended spectrum β-Lactamase production test

The phenotypic confirmatory test for ESBL production by isolates resistant to cefotaxime and ceftazidime was carried out using Double- Disc Synergy Test (DDST) method earlier described by Giriyapur et al.[11]. Briefly, 10^5cfu/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 15 mm (edge-to-edge) from the disc at the centre. 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.

2.3.1 Determination of Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotic resistance index (MARI) of the *E. coli* isolates were determined as described by [12]. MARI is defined as resistance to at least two (2) antibiotics, hence obtaining a MAR value higher than 0.2 indicated a significant and high risk source of acquiring the multidrug resistant *E. coli* from the tested samples

MAR Index=$\frac{a\ (Number\ \ of\ \ antibiotics\ \ \ isolate\ \ is\ resistant\ \ to)}{b\ (Number\ \ of\ \ antibiotics\ \ \ tested\ )}$

(Where a= number of antibiotics to which an isolate is resistant to, while b= number of antibiotics against which isolate was tested).

2.3.2 Molecular detection of ESBL resistance genes

2.3.2.1 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°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

UNDER PEER REVIEW

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).

2.3.2.2 DNA amplification of target genes by polymerase chain reaction

Simplex Polymerase Chain Reaction (PCR) was performed in order to amplify the ESBL genes being assessed in the isolates. The presence of *bla_{CTX-M}, bla_{SHV} and bla_{TEM}*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 (Inqaba Biotech, 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 [13]. The reactiontubes were placed in the holes of the thermocycler (Model TC-312, Techne, England) 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 at72°C for 3 min and a hold at 4°C infinitely.

2.3.2.3 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, 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 (VilberbLourmat TFX-35-M serial no NoV02 8104, France).

3. RESULTS AND DISCUSSION

3.1 Prevalence of *Escherichia coli*

The phenotypic identification of the isolated *E. coli* isolates showed that they were Gram-negative, rod shaped, indole positive, citrate negative, urease negative, nitrate positive, and production of hydrogen sulphide gas was observed. These characteristics were in tandem with the standard results of Genus *Escherichia* [8]. The prevalence of *E. coli* in the samples and in relation to gender is as shown in Table 2 and Table 3. 21 (17.5%) were *E. coli* positive.

3.2 Antibiotic Resistance Profile of the *Escherichia coli* isolates

The antibiotic resistance profile of the *E. coli* isolates is shown in Table 3. The highest resistance was to Sulphamethoxazole/Trimethoprim (81.0%), and the least resistance was to ciprofloxacin (23.8%). Low resistance was also observed to imipenem (33.3%), meropenem (33.3%) and ceftriaxone (33.3%).

3.3 Antibiotic Resistant Phenotypes of the *Escherichia coli* isolates

The antibiotic resistant phenotypes of the *E. coli* isolates is as shown in Table 4. The common phenotypes observed was CIP-OFX-SXT (23.8%).

3.4 Multiple Antibiotic Resistance (MAR) Index

UNDER PEER REVIEW

The MAR indices of the isolates is as shown in Table 5. All but 2 of the isolates were MAR isolates, showing resistance to at least two antibiotics tested. The commonest MAR index was 0.3 (23.8%).

3.5 Phenotypic confirmation of Extended-Spectrum Beta-Lactamase production

All of the 21 beta-lactam resistant isolates tested showed enhanced zones of clearing towards the amoxicillin-clavulanic acid disc when examined by DDST method.

3.6 Molecular Detection of Extended-Spectrum Beta-Lactamase Genes

The distribution of the ESBL resistance genes screened for in the ESBL-positive *E. coli* isolates is as follows: of the 21 ESBL-positive isolates screened, 6 (28.5%) carried the *bla*genes. 1 (16.7%) harbored $bla_{CTX-M}$and 1 (16.7%) carried bla_{TEM}. None harbored bla_{SHV} only.Some isolates carried two *bla*genes or more (either of the combinations: $bla_{\text{CTX-M}}$, and bla_{TEM}; bla_{TEM} and $bla_{\text{SHV;}}$ and $bla_{\text{TEM}}bla_{\text{CTX-M}}$ and bla_{SHV}).

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]

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

Gender	No. (%) *E. coli* (n=21)
Male	2(9.5)
Female	19 (90.4)
Total	21 (100.0)

Table 3. Antimicrobial resistance phenotypes of *Escherichia coli* isolated from Urine from General Hospital, Karshi, Nigeria

Antimicrobial Resistance Phenotypes	Frequency (n=21)
CRO	1(4.8)
AMC	1(4.8)
AMC, SXT	1(4.8)
CTX, CN	1(4.8)
CIP, OFX, SXT	5(23.8)
CTX, AMC, CRO, SXT, OFX	3(14.3)
AMC, CN, OFX, CIP	1(4.8)

UNDER PEER REVIEW

CTX, OFX, CRO, CN, SXT, NET	1(4.8)
CTX, AMC, CN, SXT, CIP, IMP, MOR	1(4.8)
CTX, AMC, CRO, CN, SXT, IMP, MOR	2(9.5)
AMC, OFX, CN, SXT, CIP, NET, IMP, MOR	1(4.8)
CTX, AMC, OFX, CRO, CN, SXT, CIP, NET, IMP, MOR	3(14.3)

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

Table 4. Multiple Antibiotic Resistance (MAR) Index of *Escherichia coli* isolated from Urine from General Hospital, Karshi, Nigeria

No of antibiotics isolate resistant to (a)	No. of antibiotics tested (b)	MAR Index $(\frac{a}{b})$	No. (%) MAR isolates (n=21)
10	10	1.0	3(14.3)
9	10	0.9	0(0.0)
8	10	0.8	3(13.3)
7	10	0.7	1(4.8)
6	10	0.6	1(4.8)
5	10	0.5	0(0.0)
4	10	0.4	4(19.0)
3	10	0.3	5(23.8)
2	10	0.2	2(9.5)
1	10	0.1	2(9.5)

**MAR isolates are those with resistance to at least two antibiotics [24]*

UNDER PEER REVIEW

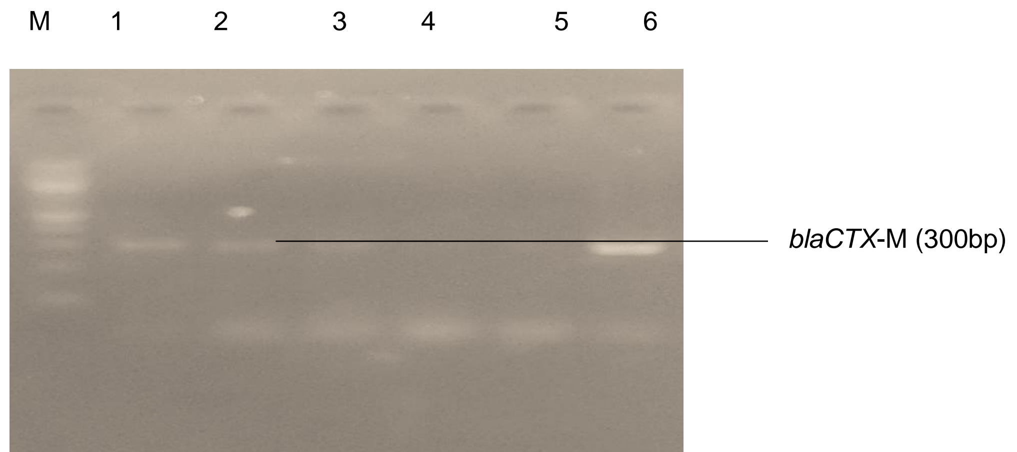

Figure 1: Agarose gel electrophoresis of the amplified *blaCTX*-Mgenes from the *E. coli* isolates. Lanes 1-3 and 6 represent the *blaCTX*-Mbands while Lanes 4 and 5 were negative for *blaCTX*-M. Lane M represents the 1500bp molecular ladder.

Figure.2: Agarose gel electrophoresis of the amplified *blaTEM* genes from the *E. coli* isolates. Lanes 1-3, 5 and 6 represent the *blaTEM* bands while Lanes was negative for *blaTEM.* Lane M represents the 1500bp

There has been an increase in the incidence of UTIs in recent years globally, which is caused by a variety of microorganisms, particularly Gram-negative bacteria. This study revealed that *E. coli* was likely the uropathogen responsible for infection, with an occurrence rate of 17.5%, which is similar to studies carried out by [14] and Bassey *et al.* [15] but less than 31.19%, 59.85% and 55.0% in the study reported by Ekeng*et al*. [16], Farooq *et al*. [17] and Amadu *et al*. [18]. The prevalence of the infected women (19/21; 90.4%) was higher than in men (2/21; 9.5%).). The high percentage occurrence of the urinary isolates in female

than the male patient in our study is not in agreement with the findings of Shitu *et al*. [19] which reported high prevalence in male (37.5%) than the female (36.5%) but agreed with the study conducted by Bassey *et al*. [15], Amadu *et al*. [18] and Nkene*et al*. [14]. There are many factors that can affect the interpretation of UTIs in women, particularly *E. coli*. Among these factors are the anatomical structure of the urogenital system, and women have a shorter distance between the urethral and anal opening [20].

In this study, the antimicrobial susceptibility testing of *E. coli* showed that 81.0% of them were highly resistant to sulfamethoxazole/trimethoprim and ofloxacin (66.7%). This finding is similar to studies conducted with by Shitu *et al*. [19] with 82.9% and Medugu*et al*. [21] with 88.4%. High resistance may primarily be attributed to the diversity of genes in various bacteria that regulate the efflux of antibiotics from the cell [21]. Furthermore, the high resistance of the isolates to amoxicillin/clavulanic acid, sulfamethoxazole/trimethoprim and ofloxacin may be due to misused, ineffective therapy, lack of compliance to dosage schedules and prolonged use of single agent for treatment of bacterial diseases which is common in the developing countries like Nigeria [15]. Although it has been suggested that certain Penicillins, when taken with β-lactamase inhibitors (clavulanic acid), are useful in treating some infections caused by bacteria that produce ESBLs [22], in this study, 66.7% of *E. coli* were resistant to Amoxicillin-clavulanic acid. This finding is in agreement with a similar laboratory-based recent study conducted in Turkey [23], while less of it was found in a study conducted in Poland [24].

In the current study, approximately 33.3% of *E. coli* were resistant to Imipenem. This finding was slightly lower when compared with other results from studies in Iraq, roughly 95.2% [25,26], and 100% in Baghdad [27]. It is disturbing that from recent data, *E. coli* have been resistant to Imipenem in UTIs such as observed in Duhok city [28] and Erbil city [29], as well as in Saudi Arabia [30]. In other words, *E. coli* resistance to Imipenem is steadily on the rise, and this could be due to the use of over-the-counter antibiotics and the ease of buying antibiotics without a prescription. The public health sector has not verified the guidelines for how to control the selling of antibiotics. In addition, ciprofloxacin (23.8) and nitrofurantoin (33.3%) had the least resistance against these isolates in this study. A similar result was found for Nitrofurantoin in a study conducted in Ethiopia [31].

It has been noted that *E. coli* which produce extended-spectrum β-lactamase (ESBL) are becoming more common in the community globally [32]. In this study, all isolates of *E. coli* were subjected to phenotypic and molecular detection of ESBL production/genes and 6 (28.5%) of *E. coli* were positive for ESBL production. The prevalence rate ESBL-producing *E. coli* was similar when compared with studies 30% in both Qatar [33] and Iran [34], but lower than other studies about 41% in Turkey [35], and higher than other countries, about 16.8% in Sweden [36] and around 2% in Spain [37]. The isolates of *E. coli* were subjected to PCR using TEM, CTX-M, and SHV-specific primers for the detection of ESBL genes. 28.5% of them were identified as possessing ESBL genes, TEM and CTX-M, respectively. The detection of both bla_{TEM} and $bla_{CTX\text{-}M}$ resistance genes in the ESBL-producing *E. coli* suggests that these genes may be responsible for the observed resistance to beta-lactam antibiotics.

.CONCLUSION

This study shows that *E. coli* is one of the most predominant bacteria among urinary patients in the hospital, and especially among women. It also shows significantnumberof *E. coli* with ESBL production The ESBL-producing *E. coli* wasless resistant to ciprofloxacin, nitrofurantoin, meropenem and imipenem, hence these are considered to be the effective antibiotic choices for the treatment of UTI infections. In addition, ESBL infection are increased in the urinary tract because of two β-lactamase genes, $bla_{CTX\text{-}M}$ and bla_{TEM}.

UNDER PEER REVIEW

Effective monitoring of antibiotic prescription and use will be helpful to reduce and manage the cases of antibiotic resistance

CONSENT

Not applicable

ETHICAL APPROVAL

Ethical approval was sought and gotten from appropriate committee before the commencement of the study.

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UNDER PEER REVIEW

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