

## 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* from urine of patients sourced from the 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 urine samples were collected from urine of patients. *Escherichia coli* isolated from the samples using standard cultural and microbiological methods. Antibiotic susceptibility testing and minimum inhibitory concentrations were evaluated as described by the Clinical 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 (CF: 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*<sub>TEM</sub> (1/6, 16.7%) and *bla*<sub>CTX-M</sub> (1/6, 16.7%). *bla*<sub>SHV</sub> only was not found in any of the isolates.

**Conclusion:** The *E. coli* isolates from urine of patients in General Hospital, Karshi, Abuja, Nigeria was 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*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> as the most common.

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

### 1. INTRODUCTION

Urinary tract infections (UTIs) are widely recognized prevalent diseases one of the popularly known diseases that are that are encountered in medical and clinical practice (REF). These infections are mainly and are caused mainly by Gram negative organisms such as *Escherichia coli* [1, 2, 3, 4]. Antimicrobial and antibiotic resistance during treatment of in patients with UTIs is an increasing global concern example of the ever increasing challenge of antimicrobial resistance [5]. The recent emergence and global dissemination of *E. coli* resistance to antibiotics due to the production of extended-spectrum b-lactamases (ESBLs) is a has shown that *E. coli* antibiotic resistance is currently a real and genuine public health concern [5, 6, 7]. There is currently lack of From data available, not many epidemiological

Comment [SM1]: What type of study

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Strikethrough

Comment [SM2]: There is no mention of MIC in your methods and materials

Comment [SM3]: No MIC was conducted in this study. If you did, there is no mention of that in the methods

Formatted: Strikethrough

Comment [SM4]: I did not see these results in the results section

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Font: Italic

Formatted: Strikethrough

Formatted: Strikethrough

surveillance studies in Abuja ~~that~~ have investigated the molecular ~~characterization~~ nature of *E. coli* strains circulating in the healthcare settings, particularly the General hospitals.

The objectives of this study were to detect ~~and establish antimicrobial resistance profile of~~ *E. coli* in urine ~~samples~~ 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*.

Formatted: Strikethrough

Formatted: Font: Italic

Formatted: Strikethrough

## 2. MATERIAL AND METHODS

### 2.1 Sample collection

A total of 120 urinary samples were randomly collected using appropriate sterile sample containers and transported to the ~~m~~Microbiology ~~l~~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.

Comment [SM5]: Please insert the study area and design. Also add the study area map

Formatted: Strikethrough

Comment [SM6]: This should be in the study area and design

#### 2.1.1 Isolation of *Escherichia coli*

~~Escherichia coli was isolated by~~ The samples collected were subjected to isolation procedure; presumptive *E. coli* was isolated from the urine as follows: ~~inoculating~~ 1ml of urine ~~sample was inoculated~~ into 9 ml of nutrient broth (NB: Oxoid Ltd., UK) and incubated ~~in an incubator~~ (Quincy Lab Inc. Model 12-140E, USA) at 37°C for 24 h. ~~Following incubation,~~ ~~A~~loopful of the 24- h broth was streaked on MacConkey agar (MCA: Oxoid Ltd., UK) plate and incubated at 37°C 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 37°C for 24 h. Colonies with a greenish-metallic sheen appearance were selected as presumptive *E. coli* [8].

Formatted: Font: Italic

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Strikethrough

#### 2.1.2 Identification of *Escherichia coli*

~~Presumptive colonies were phenotypically characterized based on~~ ~~identified~~ *E. coli* was identified ~~done by colony~~ morphological, ~~cultural and biochemical characteristics using Gram staining, catalase and oxidase tests. Gram-negative, catalase positive and oxidase negative isolates were subjected to Motility Test and~~ biochemical tests including (Indole, Methyl Red-Voges-Proskauer, Citrate, Nitrate Reduction Test, Urease Test, H<sub>2</sub>S production ~~motility~~ ~~T~~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.

Formatted: Font: Not Italic

Formatted: Strikethrough

Formatted: Strikethrough

Comment [SM7]: I dont know if in-text should be like this. Maybe include the years here unless if reference is at the end of sentence

Comment [SM8]: Did you use both systems (biochemical and API)?

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Strikethrough

### 2.2 Antimicrobial Susceptibility Testing

The antibiotic susceptibility test for *E. coli* isolates from urine was carried out using the Kirby-Bauer disc diffusion method as ~~described~~ ~~modified~~ by the Clinical and Laboratory Standards Institute (CLSI) [10]. Briefly, 5 colonies of *E. coli* isolates were inoculated into 5 ml of

Formatted: Strikethrough

Mueller-Hinton broth (MHB: Oxoid Ltd, UK) and incubated at 37°C 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% ( $\frac{w}{v}$ ) H<sub>2</sub>SO<sub>4</sub> + 0.5 ml of 1.172% ( $\frac{w}{v}$ ) BaCl<sub>2</sub>.2H<sub>2</sub>O. 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 37°C for 17 h. The discs used included: 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). Following 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 Giriapur et al.[11]. Briefly, 10<sup>5</sup>cfu/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.

**Comment [SM9]:** The referenced author used 20 and 25 mm. What made you decide on the 15mm?

#### 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

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

(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. After discarding the supernatant, the pellet was resuspended in 100 µl of sterile distilled water and vortexed. The tube was centrifuged again at 14000 g for 10 min, and the supernatant was discarded carefully. The cells were re-

**Comment [SM10]:** Which method.

**Comment [SM11]:** I think you should name the reference  
This author did not do any PCR work

**Comment [SM12]:** Please rephrase

**Formatted:** Strikethrough

**Formatted:** Strikethrough

**Formatted:** Strikethrough

suspended in 500 µl of normal saline and heated at 95°C for 20 min. The heated bacterial suspension was cooled on ice for 10 mins and spun for 3 min at 14000 rpm for 3 min. The supernatant containing the DNA was transferred to a 1.5-ml microcentrifuge tube and stored at -20°C for other downstream reactions.

**Comment [SM13]:** Consistency. Min or mins?  
Correct all others

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).

### 2.3.2.2 DNA amplification of target genes by polymerase chain reaction

**Comment [SM14]:** Any controls used for your study?

To detect the presence of *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub> and *bla*<sub>TEM</sub> genes, a Simplex Polymerase Chain Reaction (PCR) was performed as described in order to amplify the ESBL genes being assessed in the isolates. The presence of *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub> and *bla*<sub>TEM</sub> 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 reaction tubes 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 follows: 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.

**Comment [SM15]:** Insert reference

**Formatted:** Strikethrough

**Formatted:** Strikethrough

**Formatted:** Strikethrough

**Formatted:** Strikethrough

**Comment [SM16]:** Manufacture and country

**Comment [SM17]:** How much of the bromide was used.

**Comment [SM18]:** How much

**Formatted:** Strikethrough

**Formatted:** Strikethrough

**Formatted:** Strikethrough

**Formatted:** Strikethrough

**Comment [SM19]:** I think you should remove the headings and combine the results in one or two paragraphs since they are short.

**Comment [SM20]:** I think you should separate the discussion from the results. Also please revisit your results and discussion

**Formatted:** Strikethrough

### 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 containing ethidium bromide 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) was used 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 (Vilber Lourmat TFX-35-M serial no NoV02 8104, France).

**Comment [SM21]:** There is nowhere in the introduction or materials where you talk about gender. Maybe you should write a sentence on UTIs and gender in the introduction. Also specify in the sample collection that this much sample were from this gender etc.

**Comment [SM22]:** You need to write your results here and when you are done, refer the reader to the table. Not the other way around. Also introduce your results e.g. A total of ...urine samples were tested of which ....were positive for E.coli.

**Comment [SM23]:** See above comment

**Comment [SM24]:** Table 3 is multidrug resistance not individual antibiotics. You need to include a table showing individual antibiotics tested. Then you can add the multidrug table

## 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%).

**Comment [SM25]:** See above comment. Report your results to your readers

### 3.4 Multiple Antibiotic Resistance (MAR) Index

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*<sub>CTX-M</sub> and 1 (16.7%) carried *bla*<sub>TEM</sub>. None harbored *bla*<sub>SHV</sub> only. Some isolates carried two *bla* genes or more (either of the combinations: *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub>; *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>; and *bla*<sub>TEM</sub>*bla*<sub>CTX-M</sub> and *bla*<sub>SHV</sub>).

**Table 1. Primers and their sequences**

S/N	Target genes	Sequence	Amplicon size (bp)	References
1	<i>bla</i> <sub>TEM</sub>	5'-TCGGGGAAATGTGCGCG-3' 5'-TGCTTAATCAGTGAGGCACC-3'	972	[13]
2	<i>bla</i> <sub>SHV</sub>	5'-GGGTTATTCTTATTGTGCGC-3' 5'-TTAGCGTTGCCAGTGCTC-3'	615	[14]
3	<i>bla</i> <sub>CTX-M</sub>	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. (%) <i>E. coli</i> (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**

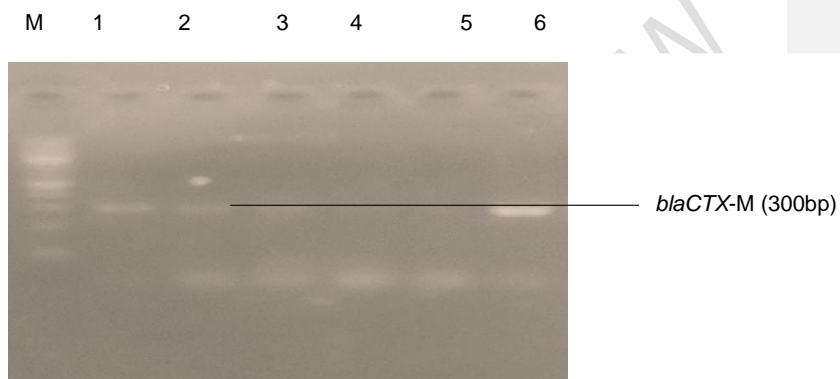
Antimicrobial Resistance Phenotypes	Frequency (n=21)
CRO	1(4.8)
AMC	1(4.8)
AMC, SXT	1(4.8)
CTX, CN	1(4.8)
CIP, OFX, SXT	5(23.8)
CTX, AMC, CRO, SXT, OFX	3(14.3)
AMC, CN, OFX, CIP	1(4.8)
CTX, OFX, CRO, CN, SXT, NET	1(4.8)
CTX, AMC, CN, SXT, CIP, IMP, MOR	1(4.8)
CTX, AMC, CRO, CN, SXT, IMP, MOR	2(9.5)
AMC, 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]



**Figure 1:** Agarose gel electrophoresis of the amplified *blaCTX-M* genes from the *E. coli* isolates. Lanes 1-3 and 6 represent the *blaCTX-M* bands 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 (REF). 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 studies conducted – reported by Ekenget *et al.* [16], Farooq *et al.* [17] and Amadu *et al.* [18], respectively. 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 patients in our study is not in agreement with the findings of Shitu *et al.* [19] which reported high prevalence in males (37.5%) than the females (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].

Formatted: Highlight

Comment [SM26]: I think you are doing it wrong. This is an in-text referencing

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 Meduguet *et al.* [21] which reported 82.9% and with 88.4%, respectively. 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  $\beta$ -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].

Comment [SM27]: What are you referring to?

Comment [SM28]: It is not clear as to how many isolates were resistant in the results, making it difficult to follow

Formatted: Strikethrough

Comment [SM29]: If its only 1 isolate, it cant be high

Comment [SM30]: How many isolates? What is the percentage?

Formatted: Strikethrough

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 (100%) [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 (REF). 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].

Formatted: Strikethrough

It has been noted that *E. coli* which produce extended-spectrum  $\beta$ -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 of ESBL-producing *E. coli* was similar when compared with studies 30% in both Qatar (30%) [33] and Iran [34], but lower than other studies about 41% in Turkey (41%) [35], and higher than reports in other countries, about 16.8% in Sweden (16.8%) [36] and around 2% in Spain (2%) [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*<sub>TEM</sub> and

Formatted: Strikethrough

Comment [SM31]: How much reported?

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Font: Italic

Comment [SM32]: A number at the start of sentence

Formatted: Highlight

*bla*<sub>CTX-M</sub> 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 significant number of *E. coli* with ESBL production. The ESBL-producing *E. coli* was less 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  $\beta$ -lactamase genes, *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub>. 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.

Comment [SM33]: Which committee?

## REFERENCES

1. Murugan K, Savitha T, Vasanthi S. Retrospective study of antibiotic resistance among uropathogens from rural teaching hospital, Tamilnadu, India. *Asian pacific Journal of tropical Disease*. 2012 Oct 1;2(5):375-80.
2. Chakupurakal R, Ahmed M, Sobithadevi DN, Chinnappan S, Reynolds T. Urinary tract pathogens and resistance pattern. *Journal of clinical pathology*. 2010 Jul 1;63(7):652-4.
3. Karlowsky JA, Lagacé-Wiens PR, Simner PJ, DeCorby MR, Adam HJ, Walkty A, Hoban DJ, Zhanel GG. Antimicrobial resistance in urinary tract pathogens in Canada from 2007 to 2009: CANWARD surveillance study. *Antimicrobial agents and chemotherapy*. 2011 Jul;55(7):3169-75.
4. Baral P, Neupane S, Marasini BP, Ghimire KR, Lekhak B, Shrestha B. High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. *BMC Research notes*. 2012 Dec;5(1):1-9.
5. Mukherjee K, Tribedi P, Mukhopadhyay B, Sil AK. Antibacterial activity of long-chain fatty alcohols against mycobacteria. *FEMS microbiology letters*. 2013 Jan 1;338(2):177-83.
6. Khanna N, Boyes J, Lansdell PM, Hamouda A, Amyes SG. Molecular epidemiology and antimicrobial resistance pattern of extended-spectrum- $\beta$ -lactamase-producing Enterobacteriaceae in Glasgow, Scotland. *Journal of antimicrobial chemotherapy*. 2012 Mar 1;67(3):573-7.
7. Vaidya VK. Horizontal transfer of antimicrobial resistance by extended-spectrum  $\beta$  lactamase-producing enterobacteriaceae. *Journal of laboratory physicians*. 2011 Jan;3(01):037-42.
8. Cheesbrough M. District laboratory practice in tropical countries. 2006. Cambridge University Press.

9. Bergey's Manual of Determinative Bacteriology. Identification Flow Charts. Available at: <http://mysite.science.uottawa.ca/jbasso/microlab/IDFlowcharts.pdf>. Accessed on November 2019.
10. CLSI C. Performance standards for antimicrobial susceptibility testing: 25th informational supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute. 2015.
11. Giriapur RS, Nandihal NW, Krishna BVS, Patil AB, Chandrasekhar MR. Comparison of disc diffusion methods for the detection of extended-spectrum Beta lactamase-producing enterobacteriaceae. *Journal of Laboratory Physicians*. 2011;3(1):33.
12. Krumperman PH. Multiple antibiotic indexing *Escherichia coli* to identifying risk sources of faecal contamination of foods. *Applied Environmental Microbiology*. 1983;6(1):165-170.
13. Porteous LA, Armstrong JL, Seidler RJ, Watrud LS. An effective method to extract DNA from environmental samples for polymerase chain reaction amplification and DNA fingerprint analysis. *Current microbiology*. 1994;29(5):301-307.
14. Nkene IH, Ngwai YB, Omede MU, Samuel J, Envuladu EY, Abimiku RH. Extended spectrum beta-lactamase producing *Escherichia coli* from urine of symptomatic and asymptomatic subjects in Keffi, Nigeria. *International Journal of Research Studies in Biosciences*. 2015;3(12):1-5.
15. Bassey A, Ngwai YB, Bassey BE, Nkene IH, Abimiku RH, Parom SK. Phenotypic and molecular detection of extended spectrum  $\beta$ -lactamase in *Escherichia coli* from patients in Nigerian National Petroleum Corporation Medical Services, Abuja, Nigeria. *Annual Research & Review in Biology*. 2018 Sep 7:1-7.
16. Ekeng BE, Ochang EA, Elem DE, Owai PA, Monjol BE, Ukwah IH, Nwagboso CI, Abraka BA, Ereh SE. Antibiotic Resistance Pattern of Uropathogens in a Tertiary Care Hospital in Calabar, Nigeria. *Annual Research & Review in Biology*. 2021 Jun 15;36(6):10-8.
17. Farooq M, Gul A, Mahmood A, Anwar M, Mir TS, Altaf A. Extended-Spectrum Beta-Lactamase (ESBL) Producing, Multidrug-Resistant (MDR) Uropathogens and Their Antimicrobial Susceptibility Profile: A Tertiary Care Hospital Experience. *Pakistan Armed Forces Medical Journal*. 2022 Feb 28;72(1):20-4.
18. Amadu DO, Nwabuisi C, Yunusa T, Nasir IA, Oladejo JM, Seibu E, Fadeyi A, Usman Y, Aliyu D. Prevalence and associated factors associated with Uropathogenic *Escherichia coli* isolates from catheterized persons at Ilorin Tertiary Hospital, Nigeria. *Afro-Egyptian Journal of Infectious and Endemic Diseases*. 2019 Jun 1;9(2):119-28.
19. Shitu S, Gambo BA, Musa MO, Abubakar AA, Attahiru M. Prevalence of Multidrug Resistant *Escherichia Coli* in Suspected Cases of Urinary Tract Infection Among Patients Attending Ahmadu Bello University Medical Center, Zaria. *UMYU Journal of Microbiology Research (UJMR)*. 2020 Dec 30;5(2):123-30.
20. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infectious Disease Clinics*. 2014 Mar 1;28(1):1-3.
21. Medugu N, Aworh MK, Iregbu K, Nwajobi-Princewill P, Abdulraheem K, Hull DM, Harden L, Singh P, Obaro S, Egwuenu A, Thakur S. Molecular characterization of multi drug resistant *Escherichia coli* isolates at a tertiary hospital in Abuja, Nigeria. *Scientific reports*. 2022 Sep 1;12(1):14822.
22. Murray BE. Resistance of Shigella, Salmonella, and other selected enteric pathogens to antimicrobial agents. *Reviews of Infectious Diseases*. 1986 May 1;8(Supplement\_2):S172-81.
23. Demir M, Kazanas H. Uropathogens and antibiotic resistance in the community and hospital-induced urinary tract infected children. *Journal of Global Antimicrobial Resistance*. 2020 Mar 1;20:68-73.

24. Kot B, Gruzewska A, Szweda P, Wicha J, Parulska U. Antibiotic resistance of uropathogens isolated from patients hospitalized in district hospital in central Poland in 2020. *Antibiotics*. 2021 Apr 16;10(4):447.
25. Saeed CH, AL-Otraqchi KI, Mansoor IY. Prevalence of urinary tract infections and antibiotics susceptibility pattern among infants and young children in Erbil city. *Zanco Journal of Medical Sciences (Zanco J Med Sci)*. 2015 Apr 1;19(1):915\_922-.
26. Alkhateeb NE. Antibiotic resistance of urinary tract pathogens and rationale for empirical antibiotic therapy in children with urinary tract infection. *Zanco Journal of Medical Sciences (Zanco J Med Sci)*. 2016 Dec 15;20(3):1458\_1466-.
27. Hussein NH. Prevalence and antimicrobial susceptibility patterns of bacteria isolated from Urinary Tract Infections (UTIs) in children at children hospital in Baghdad. *Al-Kindy College Medical Journal*. 2017;13(1):102-7.
28. Assafi MS, Ibrahim NM, Hussein NR, Taha AA, Balatay AA. Urinary bacterial profile and antibiotic susceptibility pattern among patients with urinary tract infection in duhok city, kurdistan region, Iraq. *International Journal of Pure and Applied Sciences and Technology*. 2015 Oct 1;30(2):54.
29. Aka ST, Haji SH. Evaluation of multi drug resistance among extended spectrum  $\beta$ -lactamase-producing *Escherichia coli* causing urinary tract infection in Erbil City. *Zanco Journal of Medical Sciences (Zanco J Med Sci)*. 2015 Aug 16;19(2):998-1004.
30. Alqasim A, Abu Jaffal A, Alyousef AA. Prevalence of multidrug resistance and extended-spectrum  $\beta$ -lactamase carriage of clinical uropathogenic *Escherichia coli* isolates in Riyadh, Saudi Arabia. *International journal of microbiology*. 2018 Sep 16;2018.
31. Agegnehu A, Worku M, Nigussie D, Lulu B, Tadesse BT. Pediatric febrile urinary tract infection caused by ESBL producing Enterobacteriaceae species. *BioMed research international*. 2020 Dec 2;2020:1-8.
32. Calbo E, Román V, Xercavins M, Gómez L, Vidal CG, Quintana S, Vila J, Garau J. Risk factors for community-onset urinary tract infections due to *Escherichia coli* harbouring extended-spectrum  $\beta$ -lactamases. *Journal of Antimicrobial Chemotherapy*. 2006 Apr 1;57(4):780-3.
33. Awean GZ, Salameh K, Elmohamed H, Alshmayt H, Omer MR. Prevalence of ESBL urinary tract infection in children. *J Adv Pediatr Child Health*. 2019;2(1):004-7.
34. Rezai MS, Salehifar E, Rafiei A, Langae T, Rafati M, Shafahi K, Eslami G. Characterization of multidrug resistant extended-spectrum beta-lactamase-producing *Escherichia coli* among uropathogens of pediatrics in North of Iran. *BioMed research international*. 2015 May 3;2015.
35. Kizilca O, Siraneci R, Yilmaz A, Hatipoglu N, Ozturk E, Kiyak A, Ozkok D. Risk factors for community-acquired urinary tract infection caused by ESBL-producing bacteria in children. *Pediatrics International*. 2012 Dec;54(6):858-62.
36. Kaarme J, Riedel H, Schaal W, Yin H, Nevéus T, Melhus Å. Rapid increase in carriage rates of Enterobacteriaceae producing extended-spectrum  $\beta$ -lactamases in healthy preschool children, Sweden. *Emerging Infectious Diseases*. 2018 Oct;24(10):1874.
37. Rodríguez-Bano J, Navarro MD, Romero L, Martínez-Martínez L, Muniain MA, Perea EJ, Pérez-Cano R, Pascual A. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. *Journal of clinical microbiology*. 2004 Mar;42(3):1089-94.