

# Original Research Article

## THE PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING UROPATHOGENIC *Escherichia coli* FROM MOUAU FEMALE HOSTEL STUDENTS

### ABSTRACT

**Aim:** The study is aimed at determining the antimicrobial susceptibility pattern and production of **Extended-spectrum Beta-Lactamase (ESBLs)** among *Escherichia coli* isolates from urine samples.

**Method:** A total of 35 samples of early morning mid-stream urine samples of about 10-15 ml were collected from **Michael Okpara University of Agriculture Umudike (MOUAU)** female hostel students; using sterile plastic containers. Each urine sample was inoculated on MacConkey Agar (MCA). Isolates were identified based on morphological features, Gram staining, and biochemical characterization. The antibiotic susceptibility test was done by the Kirby Bauer Disc diffusion method on Muller Hinton agar. ESBL detection was done as per **Clinical and Laboratory Standard Institute (CLSI)** guidelines, using the double disc synergy test method.

**Results:** The study reviewed a total of 12 isolates of *Escherichia coli* of which is (34.29%) of which were obtained from urine samples. The susceptibility test results showed that most isolates were susceptible to Ofloxacin with 83.3% susceptibility, followed by Levofloxacin (66.7%), whereas gentamicin, nalidixic acid, cefuroxime, and cefotaxime exhibited a susceptibility rate of 33.3%. On the other hand, the highest resistance rate was to imipenem (96.7%), followed by Amoxicillin and ampiclox with 83.3%, while the least percentage resistance was observed in Ofloxacin with (8.3%) and Levofloxacin with (16.7%). **Extended-spectrum Beta-Lactamase (ESBLs) production among *E. coli* isolates was detected in 25.0% of the potential ESBL producers. The result of the multiple antibiotic resistance index showed that the isolate (E20) was significantly higher than other isolates with  $p < 0.05$ , whereas  $E_3$ ,  $E_7$ ,  $E_{10}$  and  $E_{12}$  had same resistance pattern.**

**Conclusion:** So drug resistance due to ESBL production is a severe threat in UTIs, narrowing down the choice of antibiotics for treatment. So, there is a need to introduce routine screening for ESBL production for all uropathogenic *Escherichia coli*, causing urinary tract infections.

**Keywords:** Antimicrobial, Resistance, Bacteria, Beta-lactamase

### 1. INTRODUCTION

“Urinary tract infection (UTI) represents one of the most common diseases encountered in medical practice. This disease affects people of all age groups, being most common in women” [1]. “The most common bacteria causing UTIs are *E. coli* and other Enterobacteriaceae, accounting for approximately 75% of isolates” [2]. “Other Gram-negative organisms like *P. aeruginosa* and Gram-positive organisms like *Enterococcus* spp. are the common organisms causing UTIs in hospital settings” [3]. “In most UTIs, empirical antimicrobial therapy is initiated before culture and sensitivity results are available. Poor patient compliance and an incomplete course of antibiotic therapy have increased resistance among urinary isolates” [4]. “For uncomplicated lower UTI, such as acute cystitis in otherwise

healthy women, many guidelines recommend not only a revival of old (oral) antibiotics like fosfomycin trometamol, pivmecillinam, and nitrofurantoin, but also a non-antimicrobial measure that has been tested in a pilot study” [5]. “It will be interesting to see the results of the upcoming phase III studies and whether antibiotic therapy can be partially replaced. For prophylaxis of recurrent episodes of uncomplicated UTI, non-antimicrobial measures are already preferred, and antimicrobial prophylaxis is only recommended as a last resort. However, for complicated, nosocomial, and severe UTIs, including pyelonephritis, antibiotic therapy will still be a cornerstone in combination with the treatment of the underlying complicating conditions. Unfortunately, there aren't many novel antimicrobial medications in development by pharmaceutical firms that could help to solve the issue of multi- and extended drug-resistant uropathogens” [5].

“The distribution of urinary pathogens and their susceptibility to antibiotics varies regionally. Hence, it becomes necessary to know these pathogens' distribution and susceptibility to antibiotics in a particular setting” [6]. “Although a variety of cultures can cause UTIs, most commonly isolated organisms are due to *E. coli*, bacteria that are common in the digestive tract and routinely found in stool; other bacteria that may cause UTIs to include species of *Klebsiella*, *Enterococcus*, and *Staphylococcus*. Bacteria known as *E. coli* cause the majority of lower urinary tract infections. *Escherichia coli* are the most commonly encountered member of the family Enterobacteriaceae in the normal colonic flora and the most common cause of opportunistic infections. *Escherichia coli* are gram-negative, non-sporing bacilli, with most strains being motile and generally possessing both sex pili and adhesive fimbriae” [6].

“Antibiotic resistance in uropathogens is spreading globally, affecting hospitalized and outpatient patients, and it varies geographically and is directly correlated with the inappropriate use of antibiotics. The emergence of antimicrobial resistance is critical as the changing rate of antibiotic resistance significantly impacts the empirical therapy of urinary tract infections” [7]. “Before the laboratory results of the urine culture are known, antibiotics are typically administered empirically. Current knowledge of the organisms that cause UTIs and their antibiotic susceptibility is essential to ensuring optimal therapy” [7].

“Extended-spectrum beta-lactamase (ESBL) production, AmpC production, efflux mechanisms, and porin deficiency are among the mechanisms responsible for drug resistance in Gram-negative bacilli. Multidrug resistance is conferred by the production of beta-lactamases, making infections difficult to treat. Clinicians frequently struggle with selecting appropriate antibiotic therapy for UTIs caused by multidrug-resistant (MDR) bacteria” [8]. “ESBLs are plasmid-mediated  $\beta$ -lactamases that confer resistance to broad-spectrum  $\beta$ -lactam antibiotics, including third and fourth-generation cephalosporins, aztreonam, and extended-spectrum penicillin. These plasmids often encode mutations that confer resistance to other broad-spectrum agents, including aminoglycosides, cotrimoxazole, and fluoroquinolones, resulting in organisms being resistant to most broad-spectrum antibiotics” [7]. “The Detection of ESBLs is a significant challenge for routine clinical microbiology laboratories in resource-limited settings, resulting in underreporting and missing ESBL isolates, resulting in therapeutic failure, a prolonged hospital stay, increased morbidity, increased motility, and high healthcare costs”. [9].

“Urinary tract infections, if not treated promptly, can cause significant morbidity and mortality. As a result, information on the prevalent levels of antimicrobial resistance among common pathogens in a specific location helps make an appropriate choice of empiric therapy” [7]. Hence, this study was carried out to determine the antimicrobial susceptibility pattern and production of ESBL by strains of *Escherichia coli* isolated from urine samples.

## **2. MATERIALS AND METHODS**

### **2.1 Collection of Samples**

Thirty-five samples of early morning mid-stream urine samples of about 10-15 ml were collected from MOUAU female hostel students; using sterile plastic containers. The urine sample bottles were labeled with a code number ranging from (E<sub>1</sub> to E<sub>35</sub>), and the time of collection. The samples were placed in a cold box for transportation to the Microbiology laboratory, where it was stored until analyses were carried out. All samples were analyzed with the microbial culture method.

### **2.2 Sterilization of Materials**

The various glass wares were adequately washed and rinsed using distilled water and sterilized using the autoclave at 121°C for 15 minutes. These glasswares include; Petri-dishes, Bijou bottles, McCartney bottles, conical flasks, and a measuring cylinder.

### **2.3 Preparation of Culture Media**

The media used are MacConkey agar and Mueller Hinton agar. They were prepared according to the manufacturer's instructions for each medium. The required amount of the powdered medium was weighed following the manufacturer's specification and dissolved in distilled water in a conical flask. The dissolved media were autoclaved at 121°C for 15 minutes, cooled, and then poured into Petri dishes.

### **2.4 Inoculation and Isolation**

All samples collected were transported to the laboratory immediately and cultured using the streak plate method on MacConkey agar which was incubated for 24 hours at 37°C for bacterial growth.

### **2.5 Purification of Isolates**

The resulting colonies from the MacConkey agar plates were purified by sub-culturing on freshly prepared Nutrient agar plates. The plates were incubated at 35°C for 24 hours. After overnight incubation, the resulting discrete colonies were stored in an agar slant for further use.

### **2.6 Identification of the Isolates**

Morphological characteristics, gram staining, and biochemical characterization were carried out with all isolates [10].

#### **2.6.1 Gram Staining**

An inoculum of the test organism was emulsified in a drop of physiological saline on a clean and grease-free slide to obtain a thin film. It was passed over a gentle flame to fix the organism on the slide. Crystal violet was added to the fixed smear, allowed to stand for 60 seconds, and rinsed with distilled water. Iodine (mordant) was added, allowed for 1 minute, and then rinsed with distilled water. It was decolorized by adding alcohol and rinsed after 30 seconds with distilled water. It was counter-stained with safranin red for 60 seconds, rinsed with distilled water, and allowed to air dry. Those that retained the crystal violet dye (primary dye) after decolorization with acetone (decolorizer) are referred to as Gram-positive bacteria (violet

color). In contrast, those that were decolorized by the decolorizer and took up safranin red (secondary dye) are known as Gram-negative bacteria, which appear red [10].

## 2.6.2 Motility test

The test **helps** detect motile and non-motile organisms. A drop of a 20 hours peptone medium culture of the test organism, with the help of a Pasteur pipette, was deposited on a clean, grease-free slide. The slide was covered with a slip and viewed under the microscope using an x40 objective lens. The movement of small motile bacteria is distinguished from the on-the-spot vibratory movement (Brownian movement), which is shown by all microorganisms and particles when suspended in a fluid. True bacterial motility refers to an organism's ability to move in multiple or single directions [10].

## 2.6.3 Biochemical Test

### 2.6.3.1 Catalase Test

Using a small sterile applicator stick, 3-4 colonies of the test organism will be immersed in 2ml of freshly prepared 3% H<sub>2</sub>O<sub>2</sub> solution in a test tube. Immediate bubble production indicates a positive test, and no bubbling indicates a negative test [10].

### 2.6.3.2 Oxidase Test

This is used to identify bacteria that produce an enzyme known as cytochrome oxidase, which is an enzyme of the bacterial electron transport chain. Two drops of freshly prepared oxidase reagent were placed in filter paper on a Petri dish. A colony of test organisms was picked with a sterile glass rod and smeared on the filter paper. The deep purple color was observed for a positive result, while negative result shows no color [10].

### 2.6.3.3 Indole Test

“This was performed on the bacterial isolates to determine the ability of the organisms to convert tryptophan into indole. The test organisms were inoculated into a bijoux bottle containing 3ml of sterile peptone water and incubated at 35-37°C for 48 hours. Indole production was tested by adding 5-7 drops of Kovac's reagent and examined for a ring of red color in the surface layer within 5 minutes. A ring of red layer indicated a positive test. The absence of a ring of red layer indicates a negative test” [10].

### 2.6.3.4 Hydrogen Sulphide (H<sub>2</sub>S) Production Test

“A hydrogen sulphide (H<sub>2</sub>S) production test is used to detect hydrogen sulphide (H<sub>2</sub>S) gas produced by an organism. An inoculum from each isolate will be transferred aseptically to a sterile triple sugar iron agar (TSIA) slant. The inoculated slants were incubated at 35°C for 24 hours, and the results were observed. Hydrogen sulfide combines with the iron in the media to produce iron sulfide (FeS). The presence of an H<sub>2</sub>S-producing organism is detected by turning the agar slants into black color” [10].

#### **2.6.3.5 Citrate Utilization Test**

“This is used to detect the ability of an organism to use citrate as its sole source of carbon and energy. Slopes of Simmons citrate agar were prepared in bijou bottles. Using a sterile wire loop, the slope was first streaked, and then the butt stabbed, and they were incubated at 37°C for 48 hours. A bright blue color is a positive test, whereas green color indicates a negative result” [10].

### **2.7 Antibiotic Susceptibility Testing**

The antibiotic susceptibility test was done by the Kirby Bauer Disc diffusion method on Muller Hinton agar. Discrete colonies from 24-hour nutrient agar plates were suspended into sterile normal saline in a tube to achieve a bacteria suspension equivalent to 0.5 McFarland turbidity standards. To drain surplus fluid, a cotton swab was dipped in the bacterial mixture and rubbed against the tube's side. The entire surface of the agar plates was then inoculated with the swab of inoculums, ensuring the confluent growth of bacteria. Antibiotics disc containing these antibiotics (Ceftriaxone, Cefotaxime, Cefuroxime, Cefixime, Imipenem, Gentamicin, Levofloxacin, Ofloxacin, Nalidixic acid, Amoxicillin, Nitrofurantoin, and Ampilox), were placed onto the inoculated plates with a sterile flame forceps and the plates incubated at 37°C for 18-24hrs. After incubation, the diameter zone of inhibition produced by each antibiotic against the isolates was measured with a meter rule. The drugs were interpreted as sensitive, intermediates, or resistant, following the direction of the Clinical and Laboratory Standard Institute, CLSI [11]. The zone of inhibition ≤27mm for cefotaxime was considered a potential ESBL producer [12].

### **2.8 ESBL Detection**

Screening and a phenotypic confirmatory test were carried out to assess the detection of ESBL, as recommended by the CLSI.

### 2.8.1 Initial Screening Test

Initial screening tests recommended by the CLSI were done by the Disc diffusion method by putting a disc of cefotaxime on Muller- Hinton agar for antibiotic sensitivity testing. Zone sizes of the above antibiotics were measured as per CLSI guidelines.

- Cefotaxime zone  $\leq 27$  mm

The zones above may indicate ESBL production so confirmed by the following test.

### 2.8.2 Phenotypic confirmatory test

The isolated colonies were inoculated in nutrient broth at 37°C for five hours. The turbidity was adjusted to 0.5 McFarland standards, and lawn culture was made on Mueller-Hinton agar using a sterile swab. An Amoxicillin disc (20/10ug) was placed in the center of the plate. Ceftriaxone (30ug), ceftazidime (30ug), and aztreonam (30ug) were positioned on both sides of the Amoxicillin with a 15 mm center-to-center distance to the centrally located disc. Overnight at 37°C, the plate was then incubated. Extended-spectrum B-Lactamase production was interpreted as positive if the 3<sup>rd</sup>-generation cephalosporin disc inhibition was increased towards the Amoxicillin ( $\geq 5$ mm) disc or if none of the discs were inhibitory alone, but bacterial growth was inhibited where the two antibiotics were diffused together [12].

## 2.9 STATISTICAL ANALYSIS

The data for this research work was analyzed using a statistical package for social sciences (SPSS) version 23 for ANOVA, descriptive statistics, and multiple comparisons at  $P < 0.05$  for two duplicates.

## 3. RESULTS

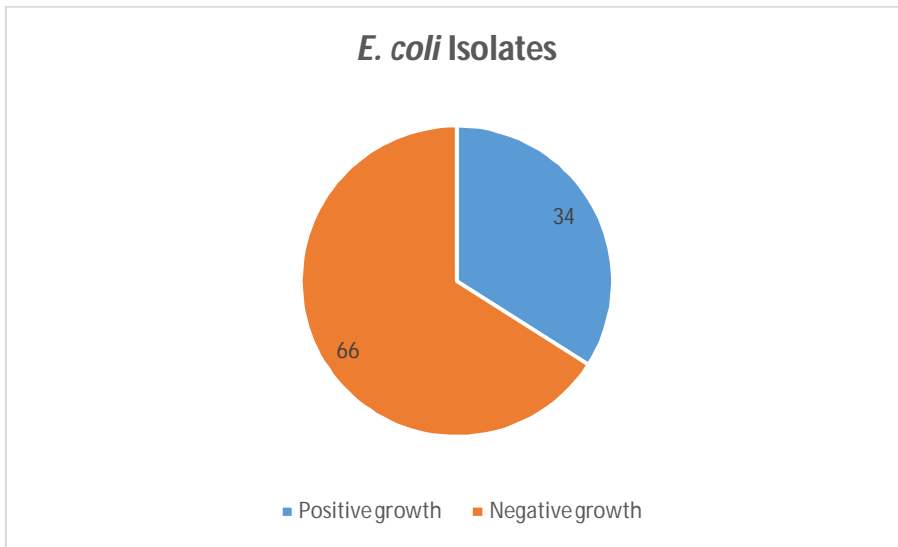
The colony characteristics, morphology, and biochemical reaction of the probable *E. coli* isolates are shown in table 1. Out of the 35 urine samples analyzed, significant growth of suspected *E. coli* colony-forming units was observed in 12 samples.

The biochemical interpretation revealed that *E. coli* isolates and cultures exhibited a higher level of indole, catalase, motility, methyl red, glucose, lactose, and sucrose as a result of different enzymatic reactions. The indole test results show that the organism was able to produce indole from tryptophan using the enzyme typtophanase [13], which was detected by adding kovac's reagent. Catalase testing reveals that the *E. coli* isolates contain catalase enzyme, which mediates the breakdown of hydrogen peroxide into oxygen and water, as evidenced by the formation of bubbles. The methyl red test demonstrates that *E. coli* could produce and maintain stable acid end products from glucose fermentation in the glucose broth, causing the methyl red indicator to remain red because the acid pH level was not higher than 4.4.

The citrate utilization test also demonstrates that *E. coli* could not use citrate as the sole carbon source because it lacked a citrate transporter, resulting in the medium's inability to change color from green to blue.

Table 1: Colonial description and biochemical characteristics of the isolates from the urine samples

S/N	CULTURAL MORPHOLOGY	MICROSCOPIC FEATURES	BIOCHEMICAL TEST								SUGAR FERMENTATION					Gram Reaction	PROBABLE ISOLATES
			Catalase	Oxidase	Coagulase	Indole	Citrate	Motility	Methyl Red	Voges-P	Urease	Glucose	Lactose	Mannitol	Sucrose		
1	Pink Pigment	Short Rod	+	-	-	+	-	+	+	-	-	AG	AG	NAG	A	-	<i>Escherichia coli</i>
2	Cream mucoid	Short rod	+	-	-	-	-	-	+	+	-	AG	AG	AG	AG	-	<i>Klebsiella sp</i>
3	Brown colonies	Short rods	+	+	-	-	+	+	-	+	-	A	A	NA	A	-	<i>Proteus sp</i>



**Figure 1: Values of percentage occurrence of *Escherichia coli* from the urine samples are at  $p < 0.05$  for duplicates.**

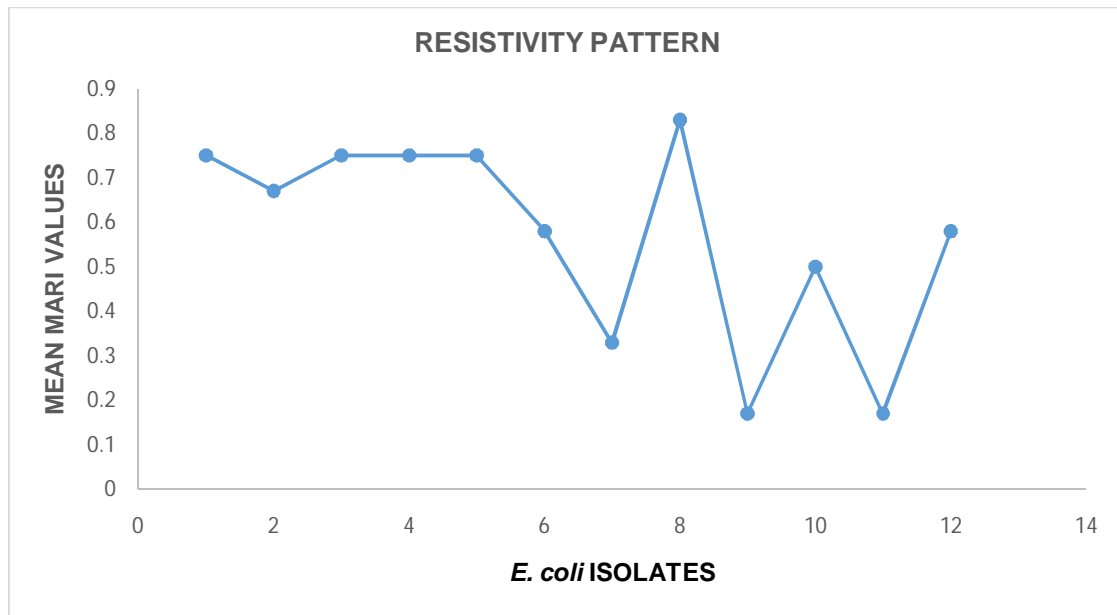
The percentage occurrence of *E. coli* isolates from the urine samples analyzed shows that *E. coli* was isolated from 34.0% of the total samples investigated (Figure 1).

**Table 2:** Drug Sensitivity and Resistance Pattern of *E. coli* Isolates from the urine Samples

Isolates	GN	OFX	AUG	LBC	ACX	ZEM	CRO	NF	CTX*	CXM	IMP	NA	S%	I%	R%
E <sub>3</sub>	5 <sup>R</sup>	0 <sup>R</sup>	5 <sup>R</sup>	28 <sup>S</sup>	5 <sup>R</sup>	0 <sup>R</sup>	8 <sup>R</sup>	26 <sup>S</sup>	14 <sup>R</sup>	28 <sup>S</sup>	8 <sup>R</sup>	0 <sup>R</sup>	25.0	0.0	75.0
E <sub>6</sub>	12 <sup>R</sup>	25 <sup>S</sup>	5 <sup>R</sup>	20 <sup>S</sup>	5 <sup>R</sup>	0 <sup>R</sup>	7 <sup>R</sup>	30 <sup>S</sup>	2 <sup>R</sup>	25 <sup>S</sup>	5 <sup>R</sup>	5 <sup>R</sup>	33.0	0.0	67.0
E <sub>7</sub>	15 <sup>I</sup>	28 <sup>S</sup>	2 <sup>R</sup>	0 <sup>R</sup>	0 <sup>R</sup>	5 <sup>R</sup>	5 <sup>R</sup>	12 <sup>R</sup>	5 <sup>R</sup>	8 <sup>R</sup>	0 <sup>R</sup>	14 <sup>I</sup>	8.3	16.6	75.0
E <sub>10</sub>	10 <sup>R</sup>	25 <sup>S</sup>	5 <sup>R</sup>	27 <sup>S</sup>	0 <sup>R</sup>	5 <sup>R</sup>	0 <sup>R</sup>	5 <sup>R</sup>	5 <sup>R</sup>	5 <sup>R</sup>	10 <sup>R</sup>	28 <sup>S</sup>	25.0	0.0	75.0
E <sub>12</sub>	10 <sup>R</sup>	28 <sup>S</sup>	5 <sup>R</sup>	14 <sup>I</sup>	8 <sup>R</sup>	5 <sup>R</sup>	2 <sup>R</sup>	10 <sup>R</sup>	28 <sup>S</sup>	0 <sup>R</sup>	0 <sup>R</sup>	10 <sup>R</sup>	16.7	8.3	75.0
E <sub>15</sub>	5 <sup>R</sup>	30 <sup>S</sup>	5 <sup>R</sup>	5 <sup>R</sup>	0 <sup>R</sup>	28 <sup>S</sup>	28 <sup>S</sup>	10 <sup>R</sup>	28 <sup>S</sup>	5 <sup>R</sup>	5 <sup>R</sup>	25 <sup>S</sup>	41.7	0.0	58.4
E <sub>18</sub>	29 <sup>S</sup>	15 <sup>I</sup>	5 <sup>R</sup>	28 <sup>S</sup>	26 <sup>S</sup>	14 <sup>I</sup>	26 <sup>S</sup>	20 <sup>S</sup>	8 <sup>R</sup>	28 <sup>S</sup>	0 <sup>R</sup>	5 <sup>R</sup>	41.7	16.7	33.4
E <sub>20</sub>	5 <sup>R</sup>	26 <sup>S</sup>	5 <sup>R</sup>	27 <sup>S</sup>	0 <sup>R</sup>	5 <sup>R</sup>	0 <sup>R</sup>	5 <sup>R</sup>	5 <sup>R</sup>	8 <sup>R</sup>	0 <sup>R</sup>	13 <sup>R</sup>	16.7	0.0	83.4
E <sub>21</sub>	28 <sup>S</sup>	28 <sup>S</sup>	26 <sup>S</sup>	30 <sup>S</sup>	25 <sup>S</sup>	10 <sup>R</sup>	26 <sup>S</sup>	15 <sup>I</sup>	28 <sup>S</sup>	28 <sup>S</sup>	0 <sup>R</sup>	27 <sup>S</sup>	75.0	8.4	16.7
E <sub>22</sub>	26 <sup>S</sup>	25 <sup>S</sup>	5 <sup>R</sup>	25 <sup>S</sup>	5 <sup>R</sup>	15 <sup>R</sup>	28 <sup>S</sup>	16 <sup>I</sup>	12 <sup>R</sup>	0 <sup>R</sup>	0 <sup>R</sup>	28 <sup>S</sup>	41.7	8.4	50.0
E <sub>26</sub>	25 <sup>S</sup>	17 <sup>S</sup>	21 <sup>S</sup>	15 <sup>I</sup>	0 <sup>R</sup>	24 <sup>S</sup>	26 <sup>S</sup>	15 <sup>I</sup>	28 <sup>S</sup>	16 <sup>I</sup>	5 <sup>R</sup>	14 <sup>I</sup>	50.0	33.3	16.7
E <sub>29</sub>	5 <sup>R</sup>	19 <sup>S</sup>	5 <sup>R</sup>	20 <sup>S</sup>	15 <sup>R</sup>	14 <sup>R</sup>	18 <sup>I</sup>	16 <sup>I</sup>	15 <sup>R</sup>	11 <sup>R</sup>	13 <sup>I</sup>	5 <sup>R</sup>	16.7	25.0	58.3
S%	4(33.3)	10(83.3)	2(16.7)	8(66.7)	2(16.7)	2(16.7)	5(41.7)	3(25.0)	4(33.3)	4(33.3)	0(0.0)	4(33.3)			
I%	1(8.3)	1(8.3)	0(0.0)	2(16.7)	0(0.0)	1(8.3)	1(8.3)	4(33.3)	0(0.0)	1(8.3)	1(8.3)	2(16.7)			
R%	7(58.3)	1(8.3)	10(83.3)	2(16.7)	10(83.3)	9(75.0)	6(50.0)	5(41.7)	8(66.7)	7(58.3)	11(96.7)	6(50.0)			

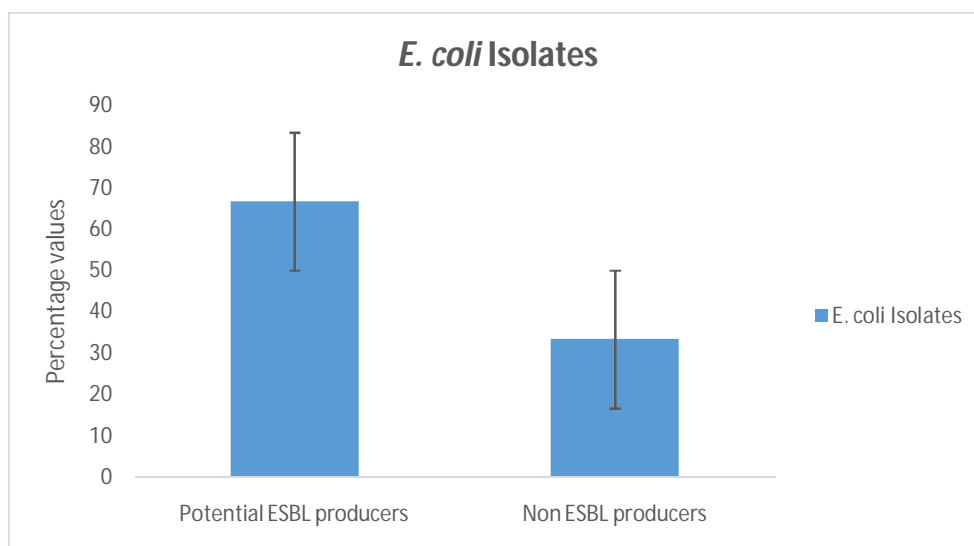
Key: CXM = cefuroxime, CTX = cefotaxime, ZEM = Cefaxime, GN = gentamicin, OFX = ofloxacin, AUG = amoxicillin, ACX = ampiclox, NF = nitrofurantoin CRO = ceftriazone, IMP = imipenem, LBC = levofloxacin, and NA = Nalidixic Acid.

The antibiotics sensitivity and resistance pattern of *E. coli* Isolates from the urine samples are shown in Table 2. Screening of the isolates for antimicrobial susceptibility showed that most isolates were susceptible to Ofloxacin with 83.3% susceptibility, while 96.7% of the isolates were resistant to imipenem.



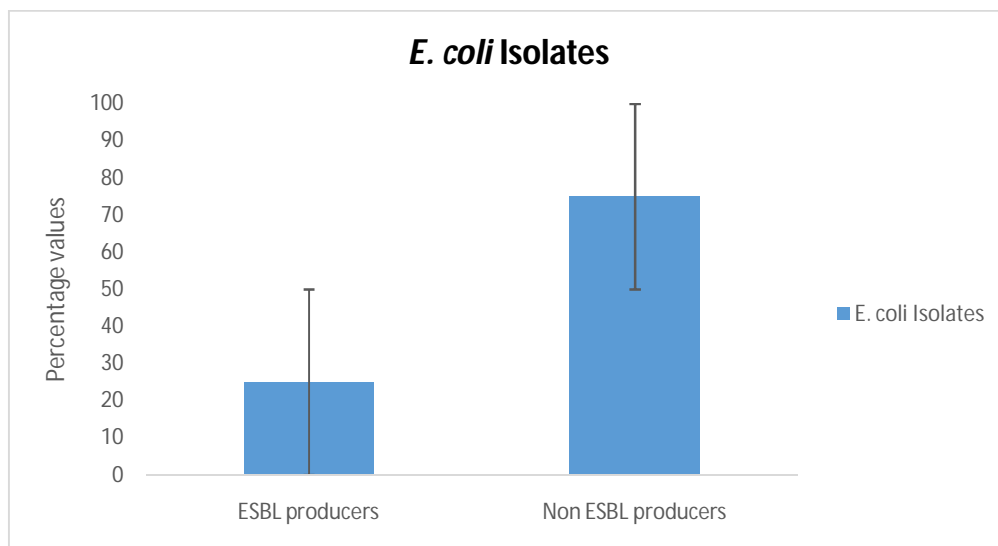
**Figure 2: Scattered plot of the Resistivity Pattern and Multiple Antibiotic Resistance (MAR) Index of the *E. coli* isolates**

The resistivity pattern and multiple antibiotic resistance (MAR) index of the *E. coli* isolates shows that most of the isolates exhibited multi-antibiotic resistance, with 80% having a MAR of over 0.2 (Figure 2). The result of the multiple antibiotic resistance index showed that the isolate (E20) was significantly higher than other isolates with  $p < 0.05$ , whereas E<sub>3</sub>, E<sub>7</sub>, E<sub>10</sub> and E<sub>12</sub> had same resistance pattern.



**Figure 3: Bar chat for phenotypic screening of *Escherichia coli* isolates based on CLSI (2021) breakpoint**

The phenotypic screening of *Escherichia coli* isolates based on CLSI (2021) breakpoint shows that out of the twelve (12) *Escherichia coli* isolates screened for ESBL production, only 8(66.7%) of the isolates revealed diameters of zones of inhibition ( $\leq 27$  mm) when tested against cefotaxime (25ug) antibiotics which confirmed them to be suspected ESBL producers (Figure 3).



**Figure 4: Bar chart for the phenotypic Confirmatory Test for ESBL-Producing isolates based on Double Disc Synergy Test (DDST)**

The phenotypic confirmatory test for ESBL-Producing isolates based on the Double Disc Synergy Test (DDST) shows that 2 (25.0%) of the suspected ESBL-producing *Escherichia coli* isolates were confirmed to be ESBL producers.

## 4. DISCUSSION, CONCLUSION, AND RECOMMENDATION

### 4.1 Discussion

Urinary Tract Infections are caused by the microbial invasion and subsequent multiplication in the urinary tract. The study investigated the antimicrobial susceptibility profile and detection of extended-spectrum beta-lactamase production by *Escherichia coli* isolated from urine samples obtained from MOUAU female students in the Babylon hostel. The study was of 6 months duration from May 2021 to October 2021. "The urine samples were cultured in duplicate on MacConkey agar, and twelve isolates were found to be lactose fermenters, as evidenced by the presence of pink colonies. These characteristics are consistent with the corresponding cultural feature of *E. coli* mentioned by Al-Baer and Hussein" [14]. In addition, Van Belkum et al. [15] stated that "for rapid identification of *E. coli* and processing of Enterobacteriaceae, colonies were first examined for lactose fermentation on MacConkey agar by direct examination".

Pure colonies were identified using Gram staining and other microscopically distinct characteristics. The bacterial isolates were gram-negative rods with peritrichous flagella and were non-spore-forming. The necessary biochemical tests were performed on bacterial isolates that were presumed to be *E. coli* based on microscopic features. The results presented in Table (1) showed that all twelve *E. coli* isolates were given a positive tests for the catalase, indole, and methyl red tests but negative tests for the oxidase, Vogas-Proskauer, and citrate tests. Kithar et al. [16] also stated that such characteristics are usually consistent with those of *E. coli*. In this study, a total of 12 isolates of *Escherichia coli* of which is (34.29%) were obtained from the 35 urine samples collected. The rate of *Escherichia coli* isolates from the urine samples was found to be moderately high. A higher rate of *Escherichia coli* isolation was indicated in the study by Nas et al. [6], who in his research reported on the prevalence and antibiotic susceptibility pattern of *Escherichia coli* isolated from urine samples of urinary tract infection patients. Rahman et al. [17] also reported that *E. coli* was the commonest organism accounting for 62.90 percent of the uropathogens.

The susceptibility test results showed that most isolates were susceptible to Ofloxacin, with 83.3% susceptibility. There was equally high susceptibility to Levofloxacin (66.7%), whereas gentamicin, nalidixic acid, cefuroxime, and cefotaxime exhibited a susceptibility rate of 33.3%.

This is not in agreement with the study by Mustafa et al. [7], who reported that *Escherichia coli* showed alarming reduced susceptibilities to fluoroquinolones like norfloxacin and Ofloxacin. This result is contrary to the findings from a previous study by Nas et al. [6], whose result showed high sensitivity of isolates to gentamicin (82%), ciprofloxacin (75%), streptomycin (70%) and chloramphenicol (65%). On the other hand, high degree of resistivity rates to ampicillin (85%), Amoxicillin (85%), spectrin (90%), erythromycin (75%), and tetracycline (75%) were detected.

“Antibiotic resistance has been reported since the beginning of using these agents for treating infections, and it is a growing problem around the world” [18]. Here, the results of the study showed a very high resistance rate among *E. coli* isolated from urinary tract-infected students. The highest resistance rate was to imipenem (96.7%), followed by Amoxicillin and ampiclox with 83.3%, while the least percentage resistance was observed in Ofloxacin with (8.3%) and Levofloxacin with (16.7%). Almost all the isolates were resistant to imipenem (96.7%). The high resistance to imipenem was contrary to findings by Rahman et al. [13], who reported a 64.0% resistance rate. 83.3% of the isolates were also resistant to imipenem, Amoxicillin, and ampiclox, which was similar to findings by Nas et al. [6], who reported a high degree of resistivity rates to ampicillin (85%), Amoxicillin (85%). Also, the high resistance rate of *E. coli* to ampiclox and Amoxicillin in this study justifies the study conducted by Sabir et al. [19], who reported 100% resistant of *E. coli* to beta-lactam drugs. This high resistance to Amoxicillin and ampiclox is evidence of antibiotic abuse. “Antimicrobial resistance in *E. coli* has been reported worldwide, and increasing rates of resistance among *E. coli* are a growing concern in both developed and developing countries. The higher resistance against the above antimicrobials could be a result of repeated or prolonged use or exposure of uropathogens to antibiotics. Repeated use of antibiotics can damage urethral flora, allowing uropathogens to colonize and subsequently infect the urinary tract, leaving clinicians with very few choices of drugs for the treatment of UTI” [20].

Eighty percent (80%) of the isolates had multiple antibiotic resistance indexes (MAR) above 0.2. **The result of the multiple antibiotic resistance index showed that the isolate (E20) was significantly higher than other isolates with  $p < 0.05$ , whereas E<sub>3</sub>, E<sub>7</sub>, E<sub>10</sub> and E<sub>12</sub> had same**

**resistance pattern.** This means that the strains had been previously exposed to antimicrobial agents. It also indicated that the isolates originated from a source where antibiotics are often used, such as hospital wards. A previous study by Rahman et al. [13] has demonstrated a higher multidrug-resistant index exhibited by *E. coli* isolates. This high prevalence of multidrug-resistant isolates may be ascribed to the misuse of antibiotics, which ultimately induces resistance. This can be attributed to various factors poor personal hygiene of the MOUUAU female hostel

“Additionally, Gram-negative bacteria can have several resistance mechanisms to antimicrobial agents. One of these mechanisms is the production of Extended-Spectrum Beta Lactamases (ESBL) that makes the bacteria resistant to beta-lactam antibiotics and prone to developing resistance to other antibiotic classes, including quinolones, aminoglycosides, and cotrimoxazole” [21]. The present study also demonstrated the prevalence of ESBL-producing *E. coli* strains was 16.7%. This result is deficient compared to the findings from a previous study by Rahman et al. [13], which showed that ESBL production by *E. coli* strains from urine was found in 31% of isolates. “The prevalence is dynamically rising due to multiple factors; lack of early treatment of the case, fast transmission of ESBL strains to the community and healthcare institutions, and environmental stress. Inappropriate use of carbapenems, increased use of third-generation cephalosporin and quinolones in the community, stool-mediated infections, and the existence of the ESBL gene in the plasmid are other possible factors” [22]. “Having plasmids and other mobile genetic materials can help ESBL-producing bacterial strains to be resistant to other multiple antimicrobial agents. This may give physicians limited treatment options and increase the mortality and morbidity of patients infected with ESBL-producing bacteria” [23].

## 4.2 Conclusion

*Escherichia coli* is one of the leading causes of urinary tract infections in humans. The finding of this study revealed that *E. coli* was observed as the most common etiologic agent of UTI, and its prevalence accounted for 34.29% of the urine samples examined. The result showed high sensitivity of the isolates to Ofloxacin (83.3%) and Levofloxacin (66.7%). On the other hand, high degree of resistivity rates to imipenem (96.7%), followed by Amoxicillin and

ampiclox with 83.3%, while the least percentage resistance was observed in Ofloxacin (8.3%) and Levofloxacin with (16.7%). The judicious use of antibiotics is recommended to help limit the increasing rate of drug resistance in the pathogens.

#### 4.3 Recommendations

*Escherichia coli* causes Urinary tract infections (UTIs) throughout the world, so to improve infection control measurement knowledge about the distribution and frequency of Urinary tract infection (UTI) is very important. Other centers should also publish their data regarding urinary pathogens which will help clinicians in recommending empirical treatment for urinary tract infections. So, drug resistance due to ESBL production is a serious threat to UTIs narrowing down the choice of antibiotics for treatment. So, there is a need to introduce routine screening for ESBL production for all uropathogenic *Escherichia coli* causing urinary tract infections.

#### CONSENT AND ETHICAL APPROVAL

The authors declare that all experiments have been examined and approved by the appropriate ethics committee. Informed consent was obtained from all relevant authorities.

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