



**Antimicrobial Resistance Pattern and Molecular Characterization of
Escherichia coli producing ESBLs from patients with Urinary Tract Infection
in State Specialist Hospital, Maiduguri, Borno State Nigeria**

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ABSTRACT

The emergence of multidrug resistant Escherichia coli associated with urinary tract infections (UTIs) is a serious public healthcare problem globally, causing frequent morbidity and even death due to limited therapeutic options. This study investigated the antimicrobial resistance pattern and molecular characterization of Escherichia coli producing ESBLs from patients with urinary tract infection in State Specialist Hospital, Maiduguri, Borno State Nigeria. The urine samples of 400 patients were obtained from State Specialist Hospital Maiduguri and were cultured for significant bacteriuria. The E. coli isolates were identified using standard microbiological procedures and confirmed using Polymerase chain reaction (PCR) analysis. Antimicrobial resistance patterns of the isolates were determined by the modified Kirby Bauer's disc diffusion technique and the isolates were screened for phenotypic expression of ESBLs enzyme using a combination disc method while the ESBLs genes were detected using PCR analysis. This study identified 7.5% prevalence of E. coli isolates in UTIs patients with higher proportion among the females. The isolates exhibited 60-100% resistance to Cefotaxime, Co-trimoxazole, Meropenem, Ciprofloxacin, Ceftazidime and Amoxicillin/clavulanic acid and 10-24% resistance to Imepenem and Gentamicin. Multidrug resistance (MDR) was exhibited in all the 30(100%) isolates. A total of 27(90%) of the isolates possessed ESBLs genes and the detected genes were blaCTX-M 5(16.7%), blaTEM 14(46.7%), blaSHV/TEM 1(3.3%) and blaCTX-M/TEM 7(23.3%). The findings in this study further emphasizes the need for antimicrobial stewardship with one health approach which will provide timely data for effective antibiotic treatment policy, necessary to preserve the efficacy of antibiotics that are commonly used for the treatment of patients with UTIs.

INTRODUCTION

Urinary tract infection (UTI) is the colonization of the urinary tract by pathogenic microorganisms. Urinary tract infections (UTIs) are some of the most common bacterial infections, affecting about 150 million people globally, annually (McCann *et al.*, 2020). They are often recurrent, frequently difficult to treat and can cause parenchymal damage to the kidney, leading to renal insufficiency and further complications (Prakash *et al.*, 2009). UTIs impose a substantial burden on society and the healthcare system in relation to diagnosis, management, loss of productivity, morbidity and sometimes death (Kudinha, 2017). UTI has been implicated to occur more frequently in women than in men because of their shorter urethra which has closer proximity to the opening of the intestinal tract (Abbo and Hooton, 2014).

UTIs are mainly caused by bacteria however, fungi and some viruses have also been implicated (Kudinha, 2017). The most common etiologic pathogens associated with UTIs include Gram-negative *Enterobacteriaceae* families such as *Escherichia coli*, *Klebsiella*, *Enterobacter*, and *Proteus* species (Chakraborty *et al.*, 2017). *E. coli* is the dominant causative agent in all patient age groups, causing 80–90% of all UTIs (Mlugu *et al.*, 2023). A better understanding of UTI pathogenesis is necessary especially for the most common etiologic agent of urinary tract infections *E. coli* is crucial for the treatment and prevention of UTIs. *E. coli* is a prominent

member of the normal intestinal microbiota of humans and animals (Zhang and Foxman, 2003). The distinctive *E. coli* strain that cause most UTIs have been designated as uropathogenic *E. coli* (UPEC) which possess diverse virulence-associated factors (VFs) that assist them in attaching to, invading and injuring the host and this include adhesins, toxins, siderophores, protective polysaccharide coatings, invasins and serum resistance-associated proteins, the presence and numbers of such VFs predict *in vivo* virulence (Johnson and Russo, 2005).

The high volume of antibiotic prescriptions witnessed in Africa associated with UTIs treatment has led to the increasing emergence of antibiotic resistance in the uropathogens (Tansarli *et al.*, 2013). Furthermore, the misuse and overuse of the common antibiotics in the treatment of UTIs has also compounded the emergence of antibiotics resistance, the increasing resistance to therapeutically important antimicrobial agents and the recent emergence of the virulent and multidrug-resistant *E. coli* have made UTI management challenging (Ikram *et al.*, 2015). Urinary tract infections affect millions of people each year globally and they often cause relapse and are difficult to treat which leads to kidney damage and further complications.

UTIs cause a substantial burden on society and the health system in relation to diagnosis and management. Furthermore, the increasing resistance to therapeutically important antimicrobial agents and the

emergence of multidrug-resistant *E. coli* strains have made UTI management challenging. Hence, this study investigated the antimicrobial resistance pattern and molecular characterization of *Escherichia coli* producing ESBLs from patients with urinary tract infection in State Specialist Hospital, Maiduguri, Borno State Nigeria.

MATERIALS AND METHODS

Materials

Laboratory and PCR Consumable Materials

Hand gloves, sterile cotton swab sticks, Savlon[®] disinfectant, liquid soap, permanent marker, masking tape, water, Primers (Eurofins, USA), DNA Template (Eurofins, USA), Nucleotides and Taq polymerase.

Glass Wares

Measuring cylinders (ASTM E-1272), Wide mouthed bottles, Beakers (ASTM International), Test tubes (Halomedical Borosilicate), Bijou bottles and McCartney bottles (IndiaMARTS).

Plastic Wares

Pipette tips and sterile Petri dishes (JRZ Plastilab, Lebanon) 2mL, 5 mL, 10 mL and 20 mL syringes, PCR Tube, (Eurofins, USA)

Equipment

Incubator (Mettler[®], Germany), triple beam weighing balance, autoclave, hot air oven (Gentlab, UK), refrigerator and dryer (Gentlab Ltd, UK) gel cassette, electrode chamber, comb, tank and UVitetransilluminator (Avebury,

Cambridge U.K) master mix, thermal cycler, (Eurofins, USA).

Culture Media Nutrient agar (NA), nutrient broth (NB), Mueller Hinton agar (MHA), chrom agar, cysteine lactose electrolyte deficient agar (CLED) and eosin methylene blue agar (EMB), blood agar all from Oxoid Limited, England, were used.

Antibiotic Discs

Ceftaxidime 30 µg (CAZ), Amoxicillin-clavulanic acid 20/10 µg (AMC), Ciprofloxacin 5 µg (CIP), Gentamicin 10 µg (CN), Imipenem 10 µg (IPM), Meropenem 10 µg (MER), Trimethoprim-sulfamethoxazole 1.25/23.75 µg (COT), Cefotaxime 30 µg (CTX), Cefotaxime-clavulanic acid 30/10 µg (CTX-CLV)

Chemical Reagents

The following reagents and chemicals were used for this study: Sodium chloride (Fisher Scientific Company New Jersey, USA), normal saline pH 7 (Sigma Chemicals Company St. Louis M.O. USA), 96 % ethanol (BDH Chemical Ltd., England), wash solution (Fermentas, UK), elution buffer (Fermentas, UK), tris-acetate EDTA, sodium dodecyl sulphate, glycerol from (Sigma chemical Ltd., England), ethidium bromide dye (Sigma chemical Ltd., England), 10mM Tris-HCl (Sigma chemical Ltd., England), re-suspension solution (Fermentas, UK) and lysis solution (Fermentas, UK).

Methods

Sample Collection Site

The Sample collection site was the State Specialist Hospital Maiduguri, the hospital is a secondary health care facility that is license by Nigeria Ministry of health with facility code of 08/21/1/1/0024. The hospital has twenty (20) wards with 500 beds capacity and the hospital is located in the heart of Maiduguri along Shehu Laminu way a few kilometers from the general post office in Zango Hausari Maiduguri. It serves as the main service and referral hospital for Maiduguri Metropolis and for the entire local governments of Borno State.

Study Area

This study was conducted in Maiduguri, the capital of Borno State and it's the largest of the six States in North-Eastern Nigeria. It lies on latitude 115°N and longitude 135°E, and occupies an area of 50,778 square kilometers. Borno State is bordered by the Republic of Niger to the North, Chad to the North-East, Cameroon to the East and Yobe State to the North-West, Adamawa State to the South-East. The estimated population of Borno State according to 2006 population census report is 4,098,391. The major languages are Kanuri, Shuwa, Marghi, Mandara and Hausa. Agriculture is the main pre-occupation of the people of Borno, as over 80% of its population engaged in crop cultivation, animal husbandry and fishing (Wathanafa *et al.*, 2022).

Ethical Approval

Ethical approval with reference number SSH/GEN/641/Vol.1 was obtained from the

Hospital Ethical Committee of the State Specialist Hospital Maiduguri, Borno State on 3rd March, 2021

Sample Size Determination

The sample size was determined based on estimated confidence level ($Z\alpha$) of 95 % and maximum tolerable error (e) of 5 %. Using the prevalence rate of 80 % of all UTIs (Mlugu *et al.*, 2023). Required sample size was calculated using the formula (Jaykaran *et al.*, 2011).

As showed below:

$$n = Z_{1-\alpha/2}^2 * P (1 - p) / d^2$$

$$n = 1.96^2 * 0.8 (1 - 0.8) / 0.05^2$$

$$n = 245.824$$

Where,

$Z_{1-\alpha/2}$ = standard normal variate at 5% type 1 error which is 1.96

P = expected proportion in population based on previous studies or pilot studies.

d = Absolute error or precision. The samples 245.824 were rounded up to 400.

Study Design and Sample Collection

A convenient sample selection method was used to select the samples used in this study and descriptive statistics was used to interpreted the results. A cross sectional study of the patients with suspected UTIs in State Specialist Hospital Maiduguri, Borno State were investigated by the collection of 400 samples of urine from patients suspected to have UTIs, who deposited their urine samples at the medical laboratory of the

hospital according to physician instructions. A liquor quantity of each urine samples deposited at the medical laboratory was collected and transported in an ice pack to the Pharmaceutical Microbiology Laboratory within 2 hours of collection for culture and future investigations. The samples collection commenced after the ethical approval was received.

Isolation of *E. coli*

Each urine sample was aseptically inoculated on sterile nutrient agar plate using a sterile loop of 4.0 mm diameter designed to deliver 10 μ L and streaked for discrete colonies before it was incubated at 37 °C for 24 hours. The sample growth that gave bacterial count of $\geq 10^5$ colonies per mL (after multiplying the plate count by 100) was considered as significant while those that gave $< 10^5$ colonies per mL were considered as insignificant (Cheesbrough, 2006).

Each of the discrete colonies that emanated from the urine sample were placed on CHROM agar, cysteine lactose electron deficient (CLED) agar and eosin methylene blue (EMB) agar for identification, the isolates that gave dark pink on CHROM agar, yellow on CLED, and metallic green on EMB were presumptively identified as *E. coli* and the isolates were then probed for *16S rRNA* *E. coli* gene using polymerase chain reaction (PCR) analysis for confirmation.

Deoxyribonucleic acid (DNA) Extraction of *E. coli* Isolates

A loopful of colonies were picked from each of the overnight culture of *E. coli* isolates and suspended in 50 μ L of sterile distilled water

with 1X Tris-EDTA buffer in an Eppendorf tube to extract the DNA of the isolates. The suspension was boiled at 100 °C for 10 mins, the boilate was transferred immediately to the freezer (-20 °C) for 10 minutes and maintained at room temperature before centrifuging at 10,000 rpm for 10 mins. The resulting supernatant of each isolate was collected, stored at 4 °C and used as DNA template in PCR analyses (Odetoyin *et al.*, 2022).

PCR Based Genotypic Detection of *E. coli*

All organisms suspected to be *E. coli* by their phenotypic characteristics were confirmed genotypically as *E. coli* by amplifying their *16S rRNA* gene using Polymerase chain reaction (PCR), the primers used were ECO-1 GACCTCGGTTTAGTTCACAGA and ECO-2 CACACGCTGACGCTGACCA at 585 base pair (Mamun *et al.*, 2016). *E. coli* strain 25922 was used as the positive control while water was used as the negative control. A 25 μ L reaction mixture contained 12.5 μ L of 2X master mix, 10 pmol each of the primers (Eurofins, USA), 2.4 μ L of the DNA template and made up with nuclease free water. Amplification conditions were as follows: Initial denaturation at 95 °C for 5 mins; 35 cycles of denaturation at 94 °C for 45 s, annealing at 45 °C for 45 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 5 mins. Each PCR product (10 μ L) was electrophoresed on a 1.5 % (w/v) agarose gel in 1X TAE. Gels containing 5 μ L of 10 μ g/ml of ethidium bromide were visualized under ultraviolet (UV) light using an UVitec transilluminator (Avebury, Cambridge UK) (Odetoyin *et al.*, 2022).

Antibiotics Susceptibility Testing

Each of the *E. coli* isolates was standardized by suspending three to five colonies of the overnight culture in to a 5 mL sterilized Normal Saline solution in a test tube and the turbidity was matched with that of 0.5 McFarland's turbidity standard (Bello *et al.*, 2021).

The resulting suspension was then inoculated on a sterilized Mueller Hinton agar (MHA) plate by swabbing the surface of the agar after which each of the following eight antibiotic discs Cefotaxime (CTX, 30 µg), Ceftazidime (CAZ, 30 µg), Amoxicillin/clavulanic acid (AMP, 30), Ciprofloxacin (CIP, 5 µg), Gentamicin (CN, 10 µg), Imipenem (IMP, 10 µg), Meropenem (MER, 10 µg), and Cotrimoxazole (COT, 25 µg) were placed on the inoculated plates. The plates were then allowed on the bench for 15 to 30 minutes before incubating at 37°C for 24 hours. Diameters of clear zones of inhibition were then measured to the nearest millimeter and interpreted as susceptible or resistant in accordance with the antimicrobial susceptibility breakpoint (CLSI, 2020). Multi-drug resistance (MDR) in this study was defined as the isolates that were resistant to at least one agent in three or more classes of antimicrobial agents used (Magiorakos *et al.*, 2012) and all the antibiotics used in this study belonged to one of the following six classes; Penicillins, Cephalosporins, Aminoglycosides, Fluroquinolones, Carbapenems and Sulfonamides.

Phenotypic Detection of ESBL

The screening for ESBLs producing *E. coli* isolates were investigated using the

combination disc diffusion method. All the isolates that were found to be resistant to either of the two 3rd generation Cephalosporin antibiotics that were used in this study were subjected to phenotypic ESBLs screening. Each of these *E. coli* isolates was standardized to 0.5 McFarland's turbidity standard and the resulting suspension was then swabbed on the surface of the sterilized MHA plates. Discs of Cefotaxime (CTX, 30 µg) and Cefotaxime/clavulanic acid (CTX-CLV, 30/10 µg) were placed on MHA plates at equal distance from each other and then allowed on the bench for 15 to 30 minutes before incubating at 37°C for 24 hours for the detection of ESBL producing isolates. All of the isolates that showed diameter clear zone of inhibition around Cefotaxime/Clavulanic acid ≥ 5 mm compared to diameter clear zone of inhibition around the single disc of Cefotaxime were labeled positive to ESBL producing *E. coli* phenotypically (Silago *et al.*, 2020). The *E. coli* isolates were then further probed for ESBLs genes using PCR analysis.

Genotypic Detection of ESBLs Encoding Genes

All the *E. coli* isolates resistant to Ceftazidime were further probed for ESBLs producing genes using PCR analysis. PCR amplification reactions were carried out under the following conditions: initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 50 °C for TEM, SHV and CTX-M for 30 s and extension at 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes. A 25 µL reaction mixture

contained 12.5 µL of 2X master mix, 10 pmol each of the primers (Eurofins, USA), 2.4 µL of the DNA template and made up with nuclease free water. Amplification conditions were as follows: Initial denaturation at 95 °C for 5 mins; 35 cycles of denaturation at 94 °C for 45 s, annealing at 45 °C for 45 s, and extension at 72 °C for 1 mins; followed by a

final extension at 72 °C for 5 mins. Each PCR product (10 µL) was electrophoresed on a 1.5% (w/v) agarose gel in 1X TAE. Gels containing 5 uL of 10 µg/ml of ethidium bromide were visualized under ultraviolet (UV) light using an UVitec transilluminator (Avebury, Cambridge UK) (Odetoyin *et al.*, 2022).

Table 1: Selected Primers used for Molecular Identification of ESBL Genes

Target Gene	Primer Name	Primer Sequence (5'- 3')	Amplicon size (bp)	Reference
SHV	SHV-F	CGCCTGTGTATTATCTCCCT	293 bp	(Ungo-kore <i>et al.</i> , 2019)
	SHV-R	CGAGTAGTCCACCAGATCCT		
TEM	TEM-F	CTCACCGGCTCCAGATTTATC	440 bp	(Wang <i>et al.</i> , 2017)
	TEM-R	CCGCATACACTATTCTCAGAATG		
CTX-M	CTX-M-F	CGCTGTTGTTAGGAAGTGTG	874 bp	(Timothy <i>et al.</i> , 2020)
	CTX-M-R	GGCTGGGTGAAGTAAGTGAC		

RESULTS

Study Population

A total of 400 patients suspected to have UTIs comprising of 153 (38.3%) males and 247 (61.8%) females of ages ranging between 1 and 84 years were involved in this study. The age and gender distribution of this

population is shown in the Table 2.1 revealing age group 20-29 as the group with the highest samples and group 80-89 as the group with the lowest samples. A total of 207 (51.8%) of the 400 samples collected yielded growth but only 50 (24.2%) samples showed significant growth $\geq 10^5$ CFU/mL among which 18 (36%) were males and 32 (64%) were females.

Table 2.1: Age and Gender Distribution of Significant Bacteriuria

Age Group (Years)	Number of Samples	Number of Significant Bacteriuria (%)	Gender	
			Male	Female
0-9	20	2 (4)	2	0
10-19	66	9 (18)	3	6
20-29	112	15 (30)	4	11

30-39	92	8 (16)	4	4
40-49	52	9 (18)	1	8
50-59	35	5 (5)	2	3
60-69	16	1 (2)	1	0
70-79	5	1 (2)	1	0
80-89	2	0	0	0
Total	400	50 (100)	18 (36)	32 (64)

Phenotypic and PCR Based Genotypic Detection of *E. coli* Isolates

A total of 50 isolates yielded significant bacteriuria, out of which 30 (60%) isolates were identified as *E. coli* phenotypically and the 30 isolates were also confirmed to possess the *E. coli* 16S rRNA gene using PCR analysis as shown in Table 2.2.

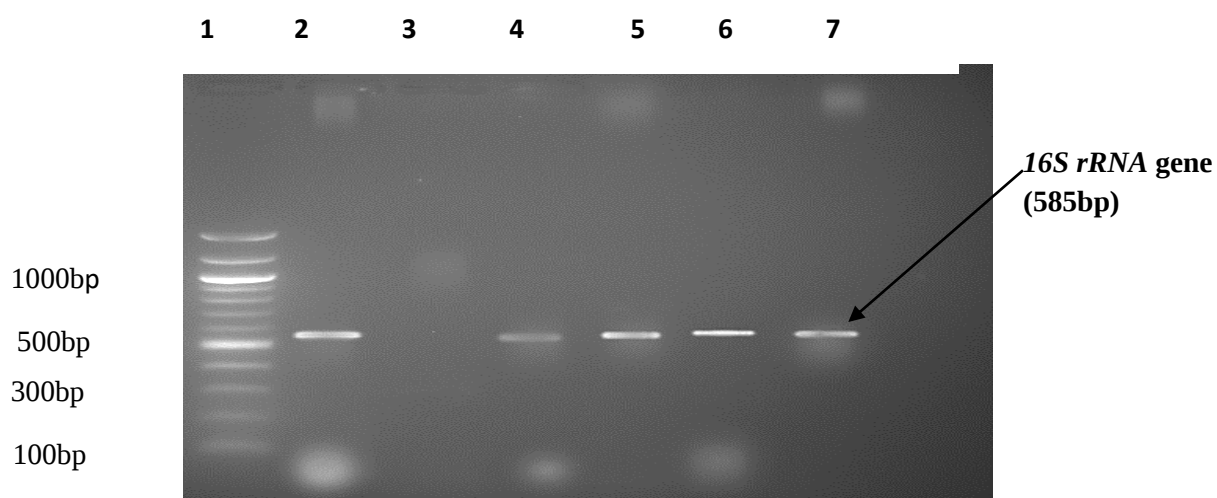
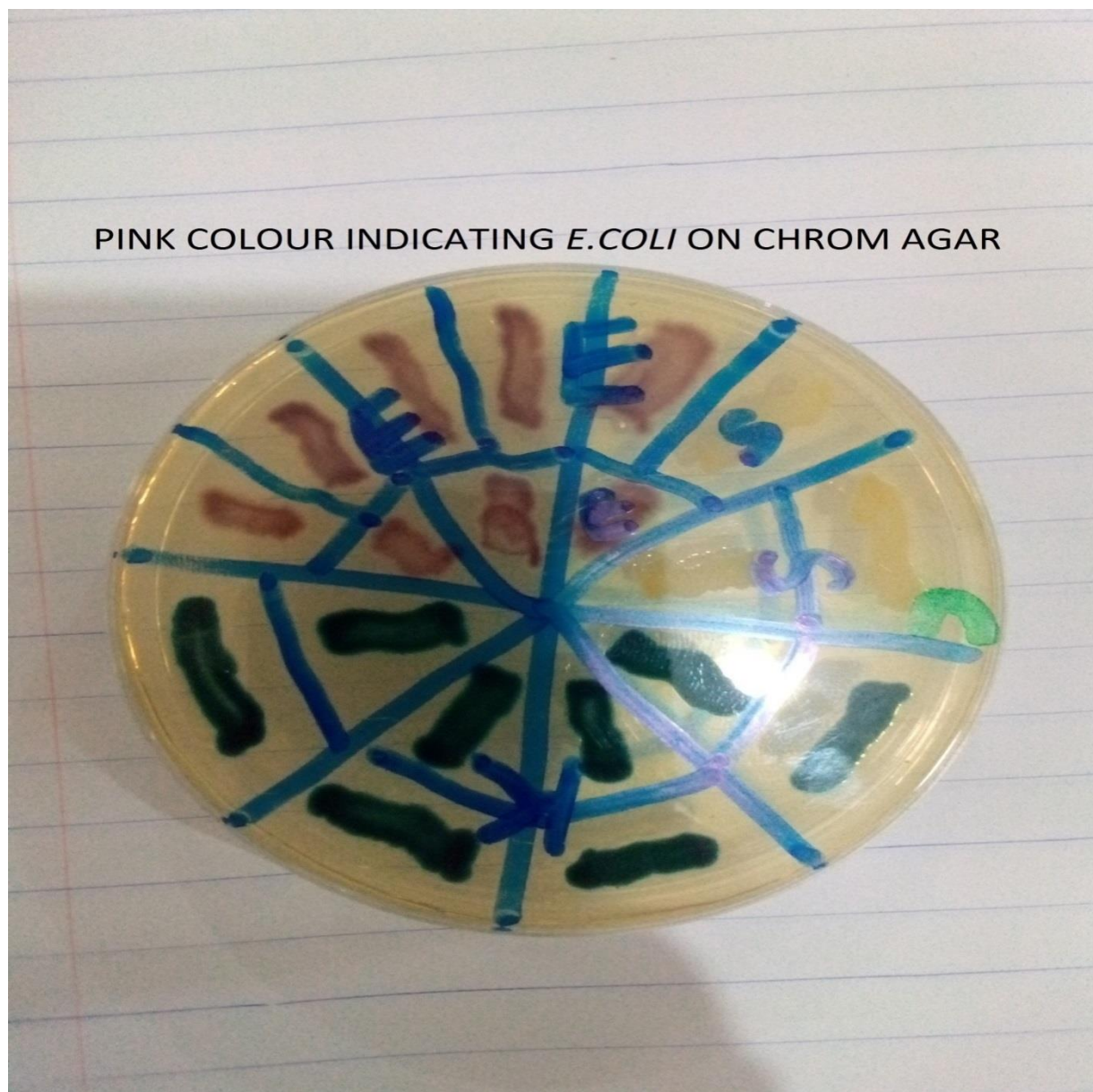


Plate 1a: A gel picture showing the bands of the amplified *Eco* gene of *Escherichia coli* isolates. Lane 1: 100bp ladder; Lane 2 (Positive control - *E. coli* 25922); Lane 3 (Negative control); Lanes 4-7 (Positive isolates-PE1-PE4)



Picture of CHROM Agar with *E. coli* Isolates

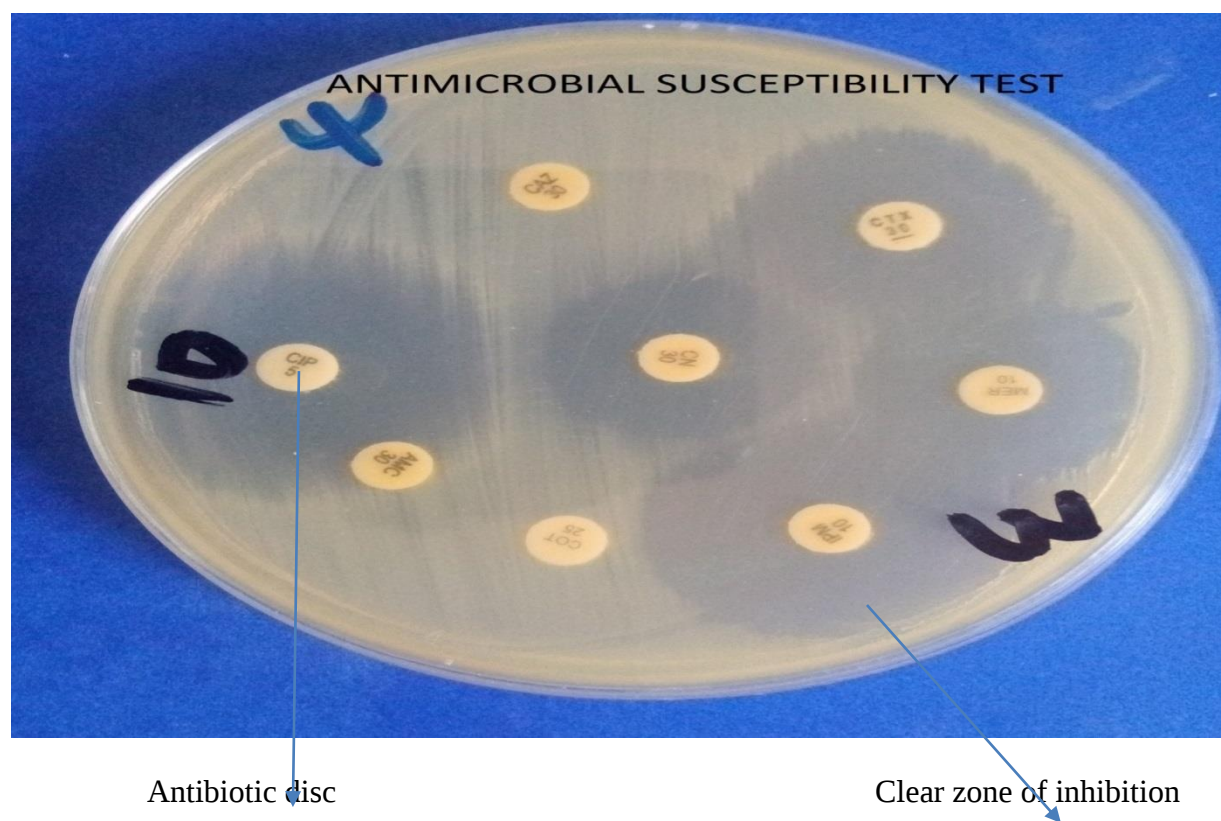
Table 2.2: Age and Gender Distribution of *E. coli* Isolates

Age Group (Years)	Number of Samples	Number of <i>E. coli</i> Isolate (%)	Gender (%)	
			Male	Female
0 – 9	20	1 (3.3)	1 (3.3)	0
10-19	66	5 (16.6)	2 (6.7)	3 (10)
20-29	112	9 (30)	3 (10)	6 (20)
30-39	92	7 (23.3)	3 (10)	4 (13.3)
40-49	52	3 (10)	0	3 (10)
50-59	35	3 (10)	1 (3.3)	2 (6.7)

60-69	16	1 (3.3)	1 (3.3)	0
70-79	5	1 (3.3)	1 (3.3)	0
80-89	2	0	0	0
Total	400	30 (100)	12 (40)	18 (60)

Antibiotic Resistance Pattern of *E. coli* Isolates

The results of antimicrobial resistance pattern of the 30 *E. coli* isolates were interpreted using CLSI (2020) interpretative chart (Appendix II). The *E. coli* isolates in this study showed high resistance of 86.7-100% to Co-trimoxazole, Meropenem, Ciprofloxacin, Ceftazidime and Amoxicillin/clavulanic acid, a moderate resistance was recorded to Cefotaxime at 60%. However, this study revealed a high susceptibility rate of 76.7-90% to both Imipenem and Gentamicin, as shown in Table 2.3.



Picture of Antimicrobial Susceptibility Test of *E. coli* Isolates

Table 2.3: Antibiotic Resistance Pattern of *E. coli* Isolates

Antibiotics	Number of Resistant Isolates (%) (n = 30)	Gender	
		Male	Female
Cefotaxime	18 (60)	8	10
Ceftazidime	30 (100)	12	18
Amoxicillin/Clavulanic acid	30 (100)	12	18
Ciprofloxacin	27 (90)	10	17
Gentamicin	7 (23.3)	3	4
Imipenem	3 (10)	2	1
Meropenem	26 (86.7)	12	14
Co-trimoxazole	26 (86.7)	11	15

Multidrug Resistance of *E. coli* Isolates

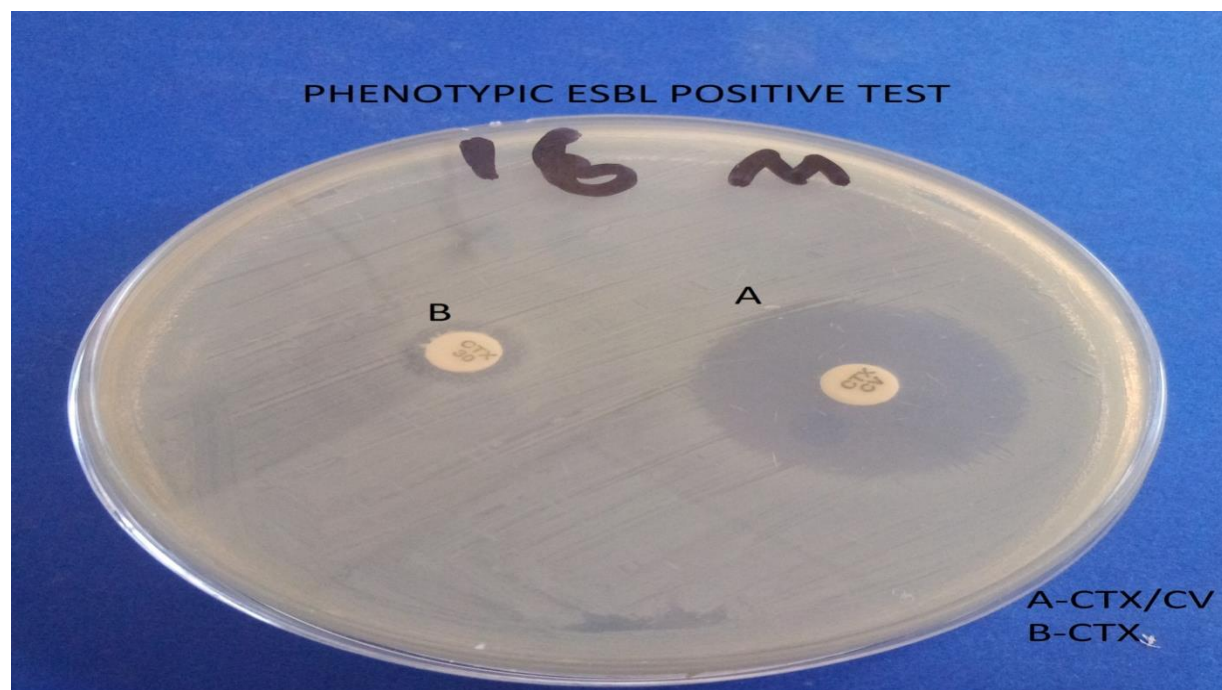
Multidrug resistance in this study was defined as the resistance of an isolate to at least one agent in three or more classes of antimicrobial agents used (Magiorakos *et al.*, 2012). All the *E. coli* isolates in this study exhibited multidrug resistance as shown in Table 2.4.

Table 2.4: Prevalence of Multi-drug Resistant

Number of Antibiotic Classes with Resistance	Number of Resistant Isolates (%)
1	0
2	0
3	2 (6.7)
4	7 (23.3)
5	15 (50)
6	6 (20)
≥3	30 (100)

Phenotypic Detection of ESBLs

A total of 11 (36.7%) *E. coli* isolates were found to express ESBLs enzymes phenotypically and were distributed among 5 males and 6 females



A-CTX/CV= Cefotaxime/Clavulanic acid

B-CTX= Cefotaxime

Picture of ESBLs Positive Result with Combination Disc

Table 2.5: Gender Distribution of *E. coli* Isolates with ESBLs Phenotypically

Age group (Years)	Phenotypic ESBLs Test		Total (%)
	Positive	Negative	
0-9	0	1	1 (3.3)
10-19	1	4	5 (16.7)
20-29	3	6	9 (30)
30-39	4	3	7 (23.3)
40-49	0	3	3 (10)
50-59	2	1	3 (10)
60-69	0	1	1 (3.3)
70-79	1	0	1 (3.3)
Total	11 (36.7)	19 (63.3)	30 (100)

Genotypic Detection of ESBLs

A total of 27 (90%) *E. coli* isolates were found to possess the screened ESBLs (CTX-M, TEM, SHV) genes as shown in Plate 4.2. A total of 5 (16.7%) *E. coli* isolates were observed to possess *bla*CTX-M while 14 (46.7%) possessed *bla*TEM genes. However, 1 (3.3%) isolate was observed to have *bla*TEM and *bla*SHV genes while 7 (23.3%) isolates were observed to have *bla*CTX-M and *bla*TEM genes. All the 27 (90%) isolates were found to exhibit multidrug resistance and the distribution of the ESBLs genes among the gender is shown in Table 2.6.

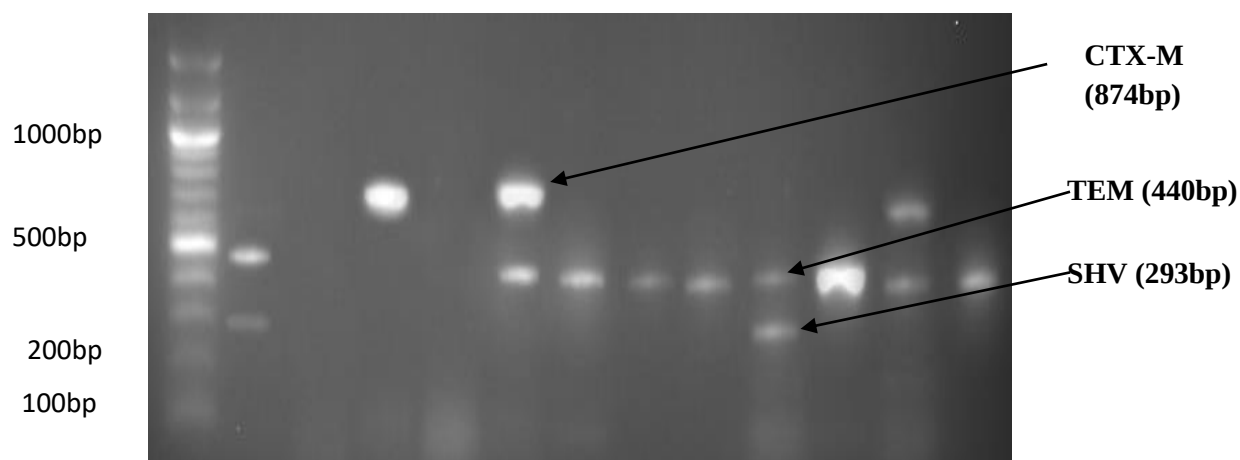


Plate 2a: A representative gel picture showing the amplified ESBL genes of *Escherichia coli* isolates. Lane 1: 100bp ladder; Lane 2: *Escherichia coli* strain 48(Positive control); Lane 3: *Klebsiella Pneumoniae* NCTC 13810 (Negative control); Lane 4: PE1 (CTX-M); Lane 5: PE4(Negative); Lane 6: PE2 (CTX-M, TEM); Lane 7: PE5 (TEM); Lane 8: PE6 (TEM); Lane 9: PE7(TEM); Lane 10: PE8 (TEM, SHV); Lane 11: PE9 (TEM); Lane 12: PE11 (CTX-M, TEM); Lane 13: PE12 (TEM)

Table 2.6: Gender

ESBLs Gene	Number of Isolates (%) (n = 30)	Male	Female
TEM	14 (46.7)	6	8
CTX-M	5 (16.7)	3	2
SHV	0	0	0
SHV/TEM	1 (3.7)	0	1

CTX-M/TEM	7 (3.3)	2	5
Total	27 (90)	11	16

DISCUSSION

Urine samples of 400 patients were obtained from the State Specialist Hospital, in this study with 153 (38.3%) males and 247 (61.8%) females with ages ranging between 1 and 84 years. This distribution showed that there are high number of female patients who reported to the hospital with complaints of UTIs compared to their male counterpart, and agrees with reports that females are more liable to UTIs possibly because of their shorter urethra that is at closer proximity to the opening of their intestinal tract (anus) (Abbo and Hooton, 2014). Also, the age group with the highest samples was 20 – 29 years implying that the sexually active ages are more vulnerable to UTIs and this tallies with the findings of Aiyegoro *et al.* (2007) and Abdu *et al.* (2018).

The studied urine samples indicated that 50 (24.2%) yielded significant bacteriuria which was distributed among 18 (36%) males and 32 (64%) females, this may be because of their shorter urethra that is at closer proximity to the opening of their intestinal tract (anus) and the female gender tend to visit the hospital more often for medical services compare to their male counterpart and this agrees with the findings of Abdu *et al.* (2018) in Maiduguri, Nigeria and Odoki *et al.* (2019) in Uganda.

The prevalence of *E. coli* isolates in UTIs patients was 30/400 (7.5%) which is lower than the 39.2% reported by Mohammed *et al.* (2016) in Maiduguri, Nigeria and the 49 %

obtained by Orrett (2001) in Trinidad, which may be due to the difference in time, health facilities and vehicular sources of *E. coli*. This study also showed that, females had a higher prevalence of *E. coli* isolates in 18 (60%) than their male counterpart with 12 (40%) and this is in agreement with an earlier study carried out by Abdu *et al.* (2018) in Maiduguri, Nigeria, Odongo *et al.* (2020) in Uganda and Oladeinde *et al.* (2011) in Okada, Edo State Nigeria. The highest prevalence of *E. coli* isolates (30%) was recorded in the age group between 20 and 29 and this finding conforms with Abdu *et al.* (2018) in Maiduguri and Okafor and Nweze (2020) in Nsukka, Nigeria.

In this study *E. coli* isolates were found to have 100% resistance to ceftazidime and amoxicillin-clavulanic acid which agrees with Okafor and Nweze (2020) in Nsukka, Nigeria and 90% of resistance was exhibited to ciprofloxacin which contrasts with that by Abdu *et al.* (2018) who reported 54% in Maiduguri, Nigeria but agrees with Odongo *et al.* (2020) in Uganda. This study also revealed that 87% of the *E. coli* isolates were resistant to co-trimoxazole which was far lower than the 48% reported by Abdu *et al.* (2018) in Maiduguri, Nigeria. However, the 60% and 87% cefotaxime and co-trimoxazole resistance recorded respectively were observed to be similar with the findings of Onanuga *et al.* (2020) in Bayelsa, Nigeria. This may be attributed to the cheap cost and availability of these drugs, self-medications as well as the extensive use of these agents in

the treatment of uncomplicated UTIs in both hospital and community settings, since extensive use of these antimicrobial agents favours the development of antibiotic-resistant bacteria and hence, a major risk factor for antibiotic resistance in a community (Odetoyin *et al.*, 2018). This therefore, implies that these agents should no longer be considered for the empiric therapy of UTIs.

Meropenem which is one of the last resort agents that are used in treating life threatening multi-drug resistant ESBLs producing *E. coli* infections experienced 87% resistance in this study which is uncommon and alarming. This level of resistance is higher than the 4.1% reported by Suwaiba *et al.* (2020) in Kaduna, Nigeria, and indicates the need for regular antimicrobial surveillance programmes aimed at monitoring antimicrobial resistance, and use of antibiotics, especially the carbapenems.

This study revealed that the genotypic ESBLs prevalence of 27 (90%) was higher than the phenotypic ESBLs prevalence of 11 (37%), thus implying that some of the ESBLs genes that cannot be picked phenotypically due to their dormancy were identified genotypically, emphasizing the importance of molecular investigations.

The *E. coli* isolates in this study were multidrug resistant by hydrolyzing beta-lactams antibiotics, thereby making them as non-susceptible to most commonly used antibiotics (Adamus *et al.*, 2018). In this study *E. coli* isolates that were probed for ESBLs genes using PCR analysis showed prevalence of 90% which is almost similar with the 88.8 % reported by Ibrahim *et al.*

(2023) in the United Kingdom, but higher than the 64.7% reported by Sheriff *et al.* (2021) in Damaturu, Nigeria and the 23.8% recorded by Mohammed *et al.* (2016) in Maiduguri, Nigeria. This observed higher prevalence could probably be attributed to an increase in the misuse of third generation cephalosporins, especially in the treatment of UTIs.

The encoding ESBLs genes identified were *blaTEM* (46.7%), *blaCTX-M* (16.7%), *blaTEM/blaSHV* (3.3%) and *blaTEM/blaCTX-M* (23.3%). The ESBL encoding genes were present in 90% of the *E. coli* isolates, which was a higher rate than the 62.3% reported by Ugwu *et al.* (2020) in Anambra State southeastern Nigeria and the 40.3% reported by Pandit *et al.* (2020) in Nepal. The increasing rate at which *E. coli* are harbouring ESBL genes is disturbing thereby hindering successful UTIs treatment and increasing human risk burden (Bello *et al.* 2021).

The high prevalence of *blaTEM* (51.9%) ESBL genes observed in this study concurs with Bello *et al.* (2021) who reported 50% among ESBL producing uropathogenic *E. coli* in Ilorin - Kwara State, Nigeria. Higher rate of *blaTEM* (93.5%) was documented among ESBL producing uropathogenic *E. coli* by Hassuna *et al.* (2020) in Egypt and lower rate (26.3%) was reported by Abba *et al.* (2019) in Benue, Nigeria. The *blaTEM* genes are plasmid mediated resistant genes that have been reported among *Enterobacteriaceae* including *E. coli* conferring resistance to the penicillin and cephalosporins antibiotics. (Bush and Bradford, 2016; Rahman *et al.*, 2018). Higher

prevalence of *bla*CTX-M (18.5%) recorded in this study differs from the lower prevalence of *bla*CTX-M (10.7%) reported by Onanuga *et al.* (2020) in Bayelsa, Nigeria. This may be due to different health facilities were vehicular sources of *E. coli* collected.

The *E. coli* isolates that co-harboured more than one ESBL genes had a prevalence of 26.7% in this study, which is lower compare with the 64% reported by Bello *et al.* (2021) in Ilorin - Kwara State, Nigeria. The genotypes complexity among these uropathogenic *E. coli* circulating within Maiduguri metropolis could be the compounding factor to multidrug resistance and the ineffective empirical treatment, management and control of *E. coli* associated UTIs (Bello *et al.* 2021).

Lastly this study has shown that 90% of the MDR *E. coli* isolates possess ESBLs gene which is similar to the 88.8% reported by Ibrahim *et al.* (2023) in the United Kingdom, and this implies that the MDR in the isolates

was probably due to ESBLs genes acquisition.

CONCLUSIONS

A high prevalence of multidrug resistant ESBLs producing *E. coli* isolates characterized with low haemolysin production and resistance to carbapenems were revealed in this study. This observed situation is alarming and could cause a serious public health problem in the treatment of UTIs in this area. Thus, calls for prudent use of antibiotics and continuous antibiotics resistance surveillance cannot be overemphasized in the control of global antibiotics resistance.

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