MOLECULAR CHARACTERIZATION AND MULTI-DRUG-RESISTANT PROFILES OF UROPATHOGENIC *ESCHERICHIA COLI* FROM ADULTS ATTENDING NATIONAL ORTHOPEDIC HOSPITAL ENUGU, NIGERIA

Authors:
Unaeze, B.C[1*].; Abueme, O[1].;Enweani-Nwokelo, I.B[1].; Nwankpa, S.N[2].; Amuche, E.D[3].

Author Affiliations:
[1]Department of Medical Microbiology and Public Health, faculty of Medical Laboratory Sciences, Nnamdi Azikiwe University, Awka, Nnewi campus, Anambra State, Nigeria.
[2]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Paul University Awka, Anambra State, Nigeria.
[3]Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Chukwuemeka Odumegwu Ojukwu University, Igbariam campus, Anambra State, Nigeria.

[*]**Corresponding Author:**
Dr. Bright Chukwuebuka Unaeze
bc.unaeze@unizik.edu.ng
Orcid Number 0000000162119371

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ABSTRACT

Background: Antimicrobial resistance (AMR) in *Escherichia coli* poses a significant challenge in both community and hospital settings.

Aim: The study aimed to ascertain the prevalence, molecular types and susceptibility pattern of multidrug resistance patterns of *E.coli* isolated from urine of adults with urinary tract infections attending National Orthopaedic Hospital, Enugu Nigeria.

Materials and Methods: Two hundred and thirty (230) urine samples were collected from adults aged 18-65 years attending the hospital using sterile screw capped containers. Structured questionnaire was used to obtain the demographic data of the participants. Urine samples were inoculated directly onto MacConkey agar, CLED and Chrome agar using streak plate method and incubated at 37^0C for 24 hrs. Colonies were identified based on their cultural, morphological and biochemical characteristics. Antimicrobial susceptibility and resistance of the isolates were determined by Kirby Bauer standard disc diffusion method on Mueller Hinton agar. Molecular analysis was done using multiplex and Linear PCR on multidrug resistant *E. coli* isolates.

Results: Out of the 230 samples analysed, 60(26.08%) were positive for *E. coli,* of which 42(24.9%) were obtained from the female participants, suggesting a gender-based predisposition to UTIs($p>0.05$). Age group 35-45 years had the highest prevalence (31.14%) of infection. Out of the 60 samples positive for *E.coli*, 25(41.66%) were multidrug resistant *E. coli*. The result shows that there was no statistically significant ($p>0.05$) association between gender, age in multidrug resistant *E. coli.* The *E.coli* isolates were highly resistant (86.6%) to Streptomycin and Penicillin, and had least resistance to Nitrofurantoin (1.66%). Molecular analysis identified the presence of blaCTX-M and blaTEM resistance genes, while blaSHV was not detected. All the isolates resistant to Streptomycin and Penicillin were positive for blaTEM genes, while the isolates resistant to Nalidixic acid, Amoxicillin-clavulanic acid and Trimethoprim-Sulfamethoxazole were positive for blaCTX-M genes.

Conclusion: The high level of resistance to commonly used antibiotics and the detection of resistance genes such as blaCTX-M and blaTEM highlights the growing threats of antimicrobial resistance in the area.

Keywords: *Multidrug resistant, molecular characterization, E.coli, UTIs*

INTRODUCTION

Escherichia coli (E. coli) is an important pathogen causing infections ranging from mild cases to life threatening conditions, it is linked to increased rate of morbidity and mortality due to multidrug resistance *E. coli*, it is also known as one of the major threats to global health in the recent times.[1,2] *Escherichia coli* is a versatile commensal and pathogenic member of the human microflora. As the primary causative pathogen in urosepsis, *E. coli* places an immense burden on healthcare systems worldwide.[3] Also, the major resistance mechanism of the Enterobacteriaceae resistance is primarily due to the production of beta-lactamase hydrolyzing enzymes like extended spectrum beta-lactamases (ESBL), AmpC beta-lactamases, and carbapenemases (Carbapenemase-Producing Enterobacteriaceae (CPE). These resistance genes are found on plasmids that can spread between Enterobacteriaceae, causing infections. These plasmids often contain additional resistance genes, making them difficult to treat. Asymptomatic carriage in healthy children and community-acquired infections, especially with ESBL, are increasingly reported.[4]

Escherichia coli is a commensal bacterium of the family Enterobacteriaceae. It is a common cause of urinary tract, blood stream, food borne infections, cholecystitis, respiratory illnesses and meningitis, and is linked with community associated as well as nosocromial infections.[5,6] It can also serve as a genetic reservoir for plasmid-mediated antibiotic resistance that can be transferred to other pathogens and thus, represent on important index pathogen for understanding the epidemiology of antibiotic resistance.[7]

Urinary tract infections(UTI) are the major common bacterial infection in humans after respiratory tract infection because of a wide used of broad-spectrum antibiotics and indiscriminate use of antibiotics.[8] Thus, it accounts for approximately 200 million cases across the globe yearly, with females having higher (40%) prevalence rates than males (12%) that experiencing one symptomatic episode of the urinary tract infection in their lifetime.[9] Infections with *E. coli* is associated with increased length of hospital stay, higher cost of care, drain on limited resources, and high rates of morbidity and mortality.[2] UTIs are mostly caused by Gram-negative bacteria; thus, the foremost pathogen responsible for uncomplicated cystitis and pyelonephritis is *Escherichia coli* followed by other species of *Enterobacteriaceae*, like *Proteus mirabilis* and mostly *Klebsiella pneumoniae*, and Gram-positive pathogens includes *Enterococcus faecalis* and *Staphylococcus saprophyticus*.[10] Further, high recurrence rates and increasing antimicrobial resistance among uropathogens threaten to greatly increase the economic burden of these infections.[10] Also, UTIs are the most frequent infectious diseases affecting humans and represent an immense public health threat with a substantial economic onus. However, UTIs are responsible for approximately 7 million physician visits annually with 15% of all community-prescribed antibiotics.[11]

Multidrug-resistant organisms (MDROs) pose a global public health threat, increasing in community-acquired and hospital-acquired infections, with prevalence varying by region.[12] Multidrug-resistant bacteria are the principal cause of failure in the treatment of infectious diseases, resulting in increases in the term and magnitude of mobility, higher rates of mortality, and a greater health cost burde.[13]

Multidrug resistance (MDR) is the ability of a microorganism (like bacteria, viruses, fungi, or parasites) or a cell (like a cancer cell) to resist the effects of multiple different drugs.

MATERIALS AND METHODS

This study was conducted at the National Orthopaedic Hospital, Enugu State, Nigeria. The National Orthopaedic Hospital, Enugu (NOHE), established on January 17, 1975, is a federal government specialty hospital located in Enugu State, Nigeria. A descriptive and cross-sectional study design was used in this study which involved adults with urinary tract infections. A consecutive convenience sampling technique was used to recruit participants for urine samples from adults with urinary infections.

Method of Sample Collection

Every adult with urinary infections who came to the hospital was recruited. The clean-catch midstream technique was used to obtain a sterile, uncontaminated sample in a sterile universal bottle was employed. A structured questionnaire was used in the study to collect demographic data and medical history of the participants involved in the study. For individuals with an indwelling urinary catheter, the tube is clamped before the sample is extracted using a sterile procedure.

Microbial Analysis

Isolation and Identification of *E. coli* was done using MacConkey agar, CLED and Chrome agar culture media. The selective media show unique peculiarities for *E. coli*, while MacConkey agar media is a lactose fermenter, which has the potency of fermenting lactose and gives a pink to red coloration colonies.

Isolation of *Escherichia coli*

Urine samples were collected from the participants and inoculated directly onto MacConkey, CLED, and Chrome agar using the streak plate method. The plates were thereafter incubated at 37^0C for 24 hours in an inverted position. Also, identification of the *E. coli* was done using biochemical test procedures

Purification of the isolates

The culture plates that showed discrete colonies were selected after 24 hours, and aseptically, the colonies were streaked on a sterile culture plates that were prepared according to the manufacturer's description. The streaked plates were placed in a bacteriological incubator in inverted positions at 37^0C for 24 hours as described in.[14]

Characterization of the pure isolates.

The pure isolates were characterized using morphological and biochemical characteristics:

Gram staining, motility test, and biochemical reactions.

The following biochemical tests were carried out: indole test, catalase test, urease test, oxidase test, citrate test, coagulase test, and the following sugar fermentations were done: H_2S production, glucose, maltose, sorbitol, methyl red, voges-proskauer etc

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing (AST) is a laboratory procedure that determines the effectiveness of antimicrobial drugs, such as antibiotics, against specific bacteria causing an infection. The antibiotics used include: ofloxacin (OFX), pefloxacin (PEF), ceftriaxone (CEF), nalidixic acid (NA) and trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CPX), penicillin (PN), cefuroxime (CEP), amoxicillin-clavulanate (AU), gentamicin (CN), streptomycin (S), nitrofurantoin (N).

Modified Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility of isolates already identified and confirmed by biochemical tests.

Discrete colonies of isolates on solid agar media was emulsified in 3ml of sterile physiological saline and the turbidity adjusted to 0.5 McFarland standards. The standardized suspensions were inoculated on Muller Hillton agar using a sterile swab to ensure even distribution and confluent growth. The antibiotic disc was aseptically placed using antibiotic dispenser. A pre-diffusion time of 30 minutes was allowed and the plate incubated at 37^0 C for 24 hours. After incubation, the inhibition zone diameter produced by each antibiotic disc was measured to the nearest millimeter and recorded.

The results were interpreted according to the Clinical and Laboratory Standards and Institute

guideline (CLSI 2024). This diameter was then compared to pre-defined clinical breakpoints.

Ethical Approval

An ethical approval was obtained from the research ethics committee of Orthopedic hospital Enugu, Enugu State, Nigeria.

Sample Size Calculation

A prevalence rate of 18.8% was adopted based on the findings of Okafor and Nweze[16], who investigated the occurrence and antibiotic susceptibility of *Escherichia coli* isolated from patients diagnosed with urinary tract infections (UTIs) in Nsukka, southeastern Nigeria. This figure reflects the burden of UTI within the studied population and provides a reliable estimate to be used in calculating the sample size for similar epidemiological research. Utilizing this prevalence in the sample size formula ensures that the study is adequately powered to detect meaningful associations or differences while minimizing the risk of Type II error. Thus, the sample size was calculated using the[41] formula using a true prevalence of the said population.

N= $(Z^2 \times P \times (1 - P)) / d^2$

Z: confidence level, P: expected prevalence, d: margin of error, and N= sample size

Sample size determination for patients with urinary tract infection using the prevalence as shown above:

N= $(1.96)^2$ X 0.188 X (1-0.188) / 0.05^2

N= 0.586 / 0.0025

N= 234.4

So the Sample size calculated was **234**.

Genotypic identification of resistance genes and sequencing of the isolates

The multidrug isolates that were positive for ESBLs were selected and screened for blaTEM, blaSHV, and blaCTX-M for amplification of resistance genes using the polymerase chain reaction technique. The protocol for the molecular analysis was done according to the method described by.[42] The polymerase chain reaction (PCR) was carried out using the One Taq Quick Load 2X Master Mix with Standard Buffer (New England Biolabs, MA, U.S.A.), which is composed of 20 mM Tris-HCI, 1.8 mM MgCl2, 22 mM NH4Cl, 22 mM KCI, 0.2 mM dNTPs, 5%glycerol, 0.06% IGEPAL CA-630,0.05% Tween 20,Xylene Cyanol FF,Tartrazine, and 25 units/ml of Taq DNA polymerase. This was vortexed at low speed and placed in a thermal cycler machine, with cycling parameters and primers used.

Multiplex PCR was used to detect the genes for SHV and CTX-M, while conventional linear PCR was used for the BlaTEM type ESBL gene.

The PCR products were analyzed on a 1.5% agarose gel stained with ethidium bromide (1µg/mL), with a 100 bp DNA ladder (New England Biolabs, USA) used as the DNA molecular weight marker. Electrophoresis was carried out at 90 volts until the dye front reached the other end of the gel (about 1 hour 20 mins). The gel was visualized under an ultraviolet trans-illuminator.

Data Analysis

Data obtained was summarised using descriptive statistics of mean and standard error of the mean. Risk factors, prevalence and susceptibility, and their associations

with multi-drug resistance were analysed using Chi-square, Pearson's correlation, and multiple logistic regression analysis. Values were considered significant at $p \leq 0.05$.

RESULTS

The cultural and morphological characteristics of the isolates (A's, B's, C's, D's, and E's) were determined, and it was revealed that the isolates appeared indifferent. Moreover, they all varied in colony sizes and elevations. All isolates were gram-negative rods arranged in single pairs, except for isolate C's, which was cocci in clusters (Table 1).

Table 2 show the biochemical characteristics of the isolates, presenting the results of the catalase test, oxidase test, coagulase test, indole test, citrate test, and sugar fermentation reactions.

The confirmed bacteria were *Escherichia coli* (60), *Klebsiella pneumoniae* (23), *Staphylococcus aureus*(90), *Proteus spp.* (17), and *Pseudomonas aeruginosa* (17).

Figure 1 shows the distribution of other bacterial organisms co-isolated with *Escherichia coli* from the urine culture plates. A total of 230 urine samples were obtained from adults diagnosed with UTI. Out of the 230 samples, 26.08% (60/230) were *E. coli*. The most frequently co-isolated organism was *Staphylococcus aureus* 39.13% (90/230), followed by *Pseudomonas aeruginosa* and *Proteus species*, which had 7.39% (17/230) each; while *Klebsiella pneumoniae* and others accounted for 10% (23/230). Therefore, the *E. coli* (60) isolates were used for further study.

Figure 2 illustrates the proportion of multidrug-resistant (MDR) and non-MDR *Escherichia coli* isolated from urine samples of adult patients with urinary tract infections. Out of a total of 60 *E. coli* isolates, 35 (58.33%) were non-MDR, while 25 (41.67%) were classified as multidrug-resistant. The drug susceptibility pattern of MDR *E. coli* (n=60) in the urine of adults with urinary tract infections is represented in Table 3.

Isolates showed high resistance to Penicillin and Streptomycin (86. 6% each), Nalidixic acid (85.0%), and Trimethoprim-Sulfamethoxazole and Amoxicillin-Clavulanate (83.3% each). Moderate resistance was observed for Cefuroxime (45.0%), Ofloxacin (23.3%), Ceftazidime (25.0%), and Ceftriaxone (20.0%), with low resistance to Pefloxacin (10.0%), Ciprofloxacin and Gentamycin (3.3%) respectively and Nitrofurantoin (1.66%).

Table 4 shows the prevalence and percentage of multidrug resistant *E. coli* isolates with demographic characteristics. Out of the 230 participants, 169 (73.47%) were females and 61 (26.52%) were males. Out of the sixty (60) isolated *E. coli*, forty-two (42) were from females, with eighteen 18 (42.85%) positive for multidrug resistance, while eighteen (18) were from males, with seven 7 (38.88%) positive for multidrug resistance. From the results, females had more positive urinary tract infections with a higher number of multidrug-resistant *E. coli*.

The participants ages ranged from 18 to 65 years, with the highest representation in the 36 – 45 years age group (31.14%), followed by 46 – 55 years (28.0%), 18 – 25 years

(24.0%), 26 – 35 years (21.27%), and 56 – 65 years (25.0%). Regarding the duration of illness, 23.07% had UTIs for 1 – 6 months, 36.36% for 7 to 12 months, and 50% for more than 12 months. In term of the level of education of the participants, primary education accounted for 30.76%, secondary for 23.39%, while tertiary education accounted for 34.7%. The majority of the sample types were midstream urine.

Table 5 shows the association of demographic clinical factors with MDR *E. coli* in adult urine. The results show that there was no statistically significant ($p>0.05$) association between gender and age in non- MDR *E. coli.* Moreover, the duration of illness shows a higher percentage (50.0%) in those with illness lasting over 12 months. There was no statistically significant ($p>0.05$) association between the duration of illness and MDR *E. coli*.

Figure 3 illustrates the genetic analysis of the MDR *E. coli* isolates. The gel bands show that all samples tested positive for the blaTEM gene except for samples 1 and 6, which did not exhibit visible bands at the 459 bp region. The presence of the bla_{TEM} gene in the majority of isolates suggests a high prevalence of ESBL-producing *E. coli*, which contributes significantly to antibiotic resistance.

Figure 4 presents the gel electrophoresis image showing the amplification results for the blaCTX-M (560 base pairs) and blaSHV (398 base pairs) genes among the samples. The image reveals that only samples 3, 4, and 5 showed positive bands for the blaCTX-M gene, indicating the presence of this ESBL gene in those isolates. In contrast, none of the samples exhibited bands at the 398 bp region, suggesting that all tested isolates were negative for the blaSHV gene.

Table 1: Cultural and Morphological Characteristics of the Isolates

PARAMETERS	**A's**	**B's**	**C's**	**D's**	**E's**
Appearance	Pink/Red	Cream	Golden yellow	Colourless	Greenish
Motility	Motile	Non-mobile	Non-motile	Highly motile	Motile
Shape	Rods	Rods	Cocci	Rods	Curved rods
Arrangement	Single/Pairs	Single/Pairs	Cluster	Single/Pairs	Single/Pairs
Edge	Smooth	Mucoid	Smooth	Swarming	Metallic
Size	1-3µm long by 0.4 – 0.7 µm wide	1 - 2 µm long by 0.5 – 0.8 µm wide	0.8 - 1 µm	0.4 – 0.6 µm wide by 1-3 µm long	1.5 - 3 µm long by 0.5-0.8 µm wide
CLED	Yellow	Yellow/muoid	Yellow	Blue/green	Green
MacConkey	Pink/Red (hf)	Pink/Mucoid	No growth	Colourless/Pale(hf)	Colourless
Chrom Agar	Pink/Magenta	Blue-green, Mucoid	Golden-Yellow	Brownish/light amber	Greenish/Grey
Bacterium	***E. coli***	***Klebsiella Pneumonia***	***Staphlococcus aureus***	***Proteus spp***	***Pseudomonas Aeruginosa***

Table 2: Biochemical Characteristics

Parameter	**A's**	**B's**	**C's**	**D's**	**E's**
Gram reaction	Gram negative rod	Gram negative rod	Gram positive cocci (cluster)	Gram negative rod	Gram negative rod
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	+
Urease	weakly+	+	-	Strong+	-
Indole	+	-	-	+	-
Methyl red	+	-	-	+	-
Voges-Proskauer	-	+	-	+	-
Citrate	-	+	-	+	+
H_2S production	-	-	-	-	+
Coagulase	-	-	+	-	-
Glucose	+	+	-	+	-
Maltose	+	+	+	+	-
Xylose	+	+	-	+	-
Dulcitol	-	+	-	Variable	-
Inositol	-	+	-	Variable	-
Sorbitol	+	+	Variable	Variable	-
Bacterium	***E. coli***	***Klebsiella Pneumonia***	***Staphyloccus aureus***	***Pseudomonas aeruginosa***	***Proteus spp***

Key: Variable= reaction depend on strain/specie. Positive (+) =reaction, Acid(medium twin yellow) and/or gas (bubble in durham tube). Negative(-)= No reaction, No change or alkaline(medium remain red/pink)

Fig 1: The prevalence of other bacteria Isolated with *E. coli*

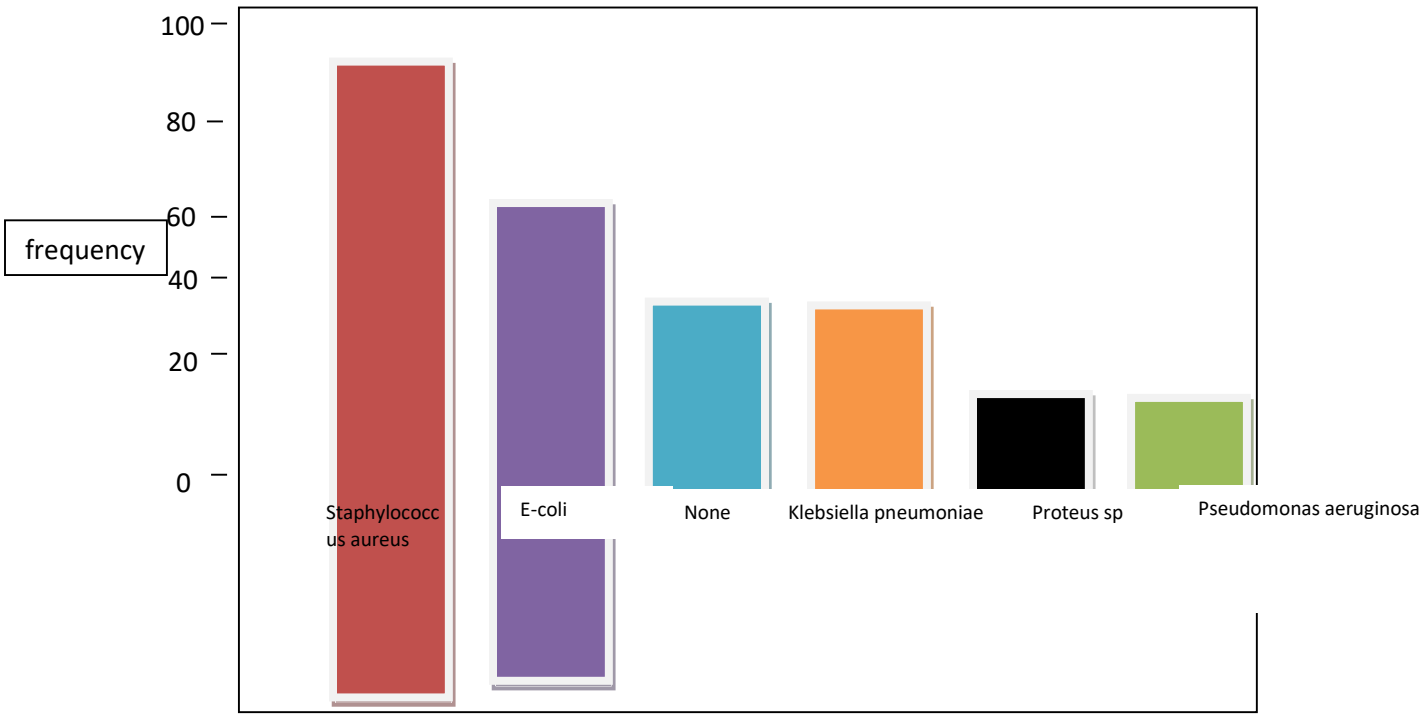


Fig 2: The prevalence of MDR *E. coli* in urine of Adults with Urinary Tract Infections

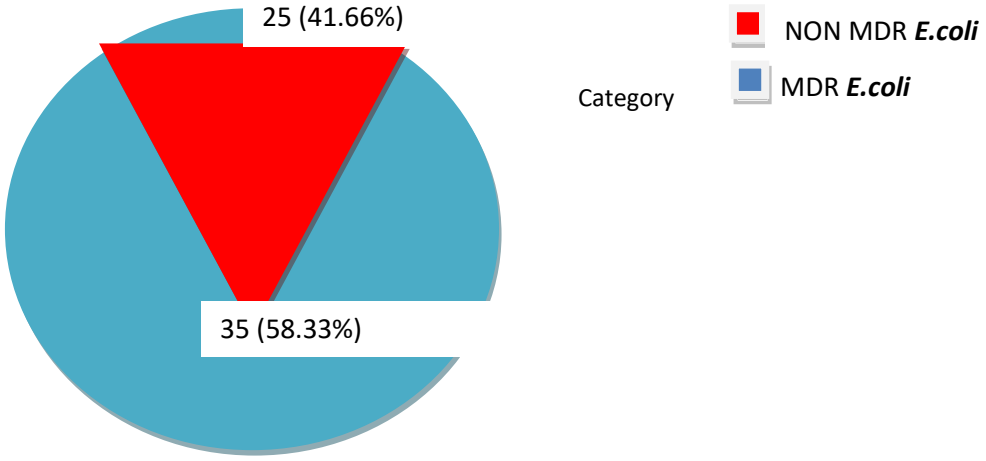


Table 3: **Drug Susceptibility Pattern Of MDR *E. coli* In Urine Of Adults With Urinary Tract Infections.**

Antibiotics	Number Of *E. coli* Isolated (n=60)			
	Sensitive	Intermediate	Resistant	% Resistance
CPX	57	1	2	3.3
N	59	-	1	1.66
NA	1	2	57	85
CN	57	1	2	3.3
S	6	2	52	86.6
SXT	2	8	50	83.3
PN	5	3	52	86.6
AU	6	4	50	83.3
PEF	53	1	6	10.0
OFX	46	-	14	23.3
CEP	33	-	27	45.0
CEF	38	10	12	20.0
CFZ	35	11	15	25.0

Key: CPX= Ciprofloxacin, N=Nitrofurantoin, NA= Nalidixic acid, CN=Gentamicin, S=Streptomycin, SXT=Trimethoprim-Sulfamethoxazole, PN=Penicillin, AU=Augmentin-Clavulanate, PEF= Pefloxacin, OFX=Ofloxacin, CEP=Cefuroxime, CEF=Ceftriaxone, CFZ=Ceftazidine.

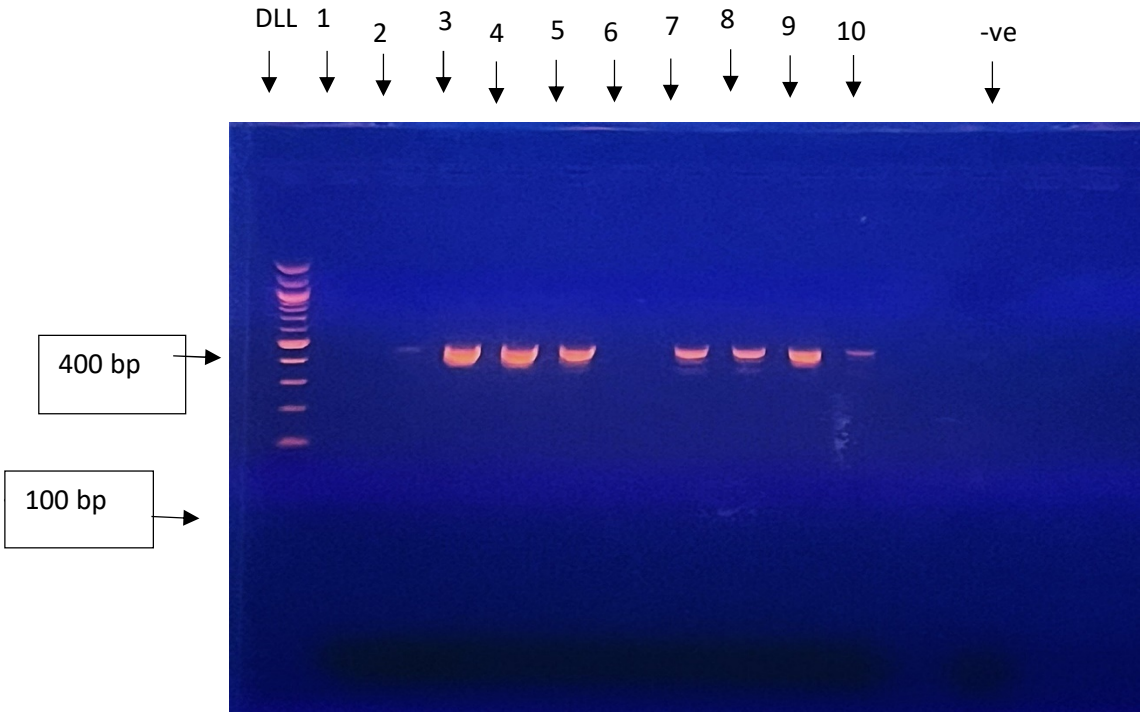
Table 4: PREVALENCE OF MULTIDRUG RESISTANCE *E. COLI* ISOLATES WITH DEMOGRAPHIC CHARACTERISTICS.

Parameter (Total Number)	*E. coli* Isolated(%)	MDR E. coli (%)
Gender		
Female	42 (24.9)	18 (42.85)
Male	18 (29.51)	7 (38.88)
Total	60 (26.08)	25 (41.66)
Age		
18 – 25 years	12 (24.0)	6 (42.85)
26 – 35 years	10 (21.27)	5(41.66)
36 – 45 years	18 (31.14)	5 (30.0)
46 – 55 years	14 (28.0)	7 (57.14)
56 – 65 years	3 (25.0)	2 (40.0)
Duration of illness		
1 – 6 months (182)	42 (23.07)	18 (42.85)
7 – 12 months (44)	16 (36.36)	6 (37.5)
Above 12 months (4)	2 (50.0)	1 (50.0)
Sample type		
Midstream (228)	59 (25.43)	24 (41.37)
Catheterization (2)	2 (100)	1 (50.0)
Level of Education		
Primary (13)	4 (30.76)	3 (75.0)
Secondary (171)	40 (23.39)	16 (40.0)
Tertiary (46)	16 (34.7)	6 (37.5)
Mean duration +/- SD		

Table 5: Association of Demographic and Clinical factors with MDR *E. coli* in Adult Urine Samples

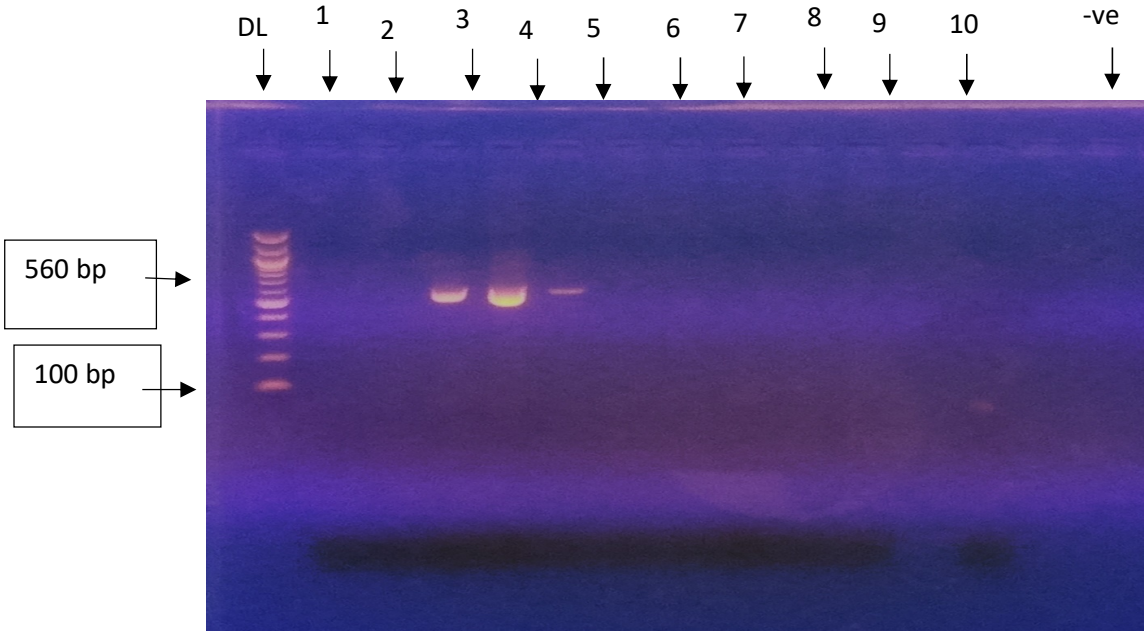
Par ammeters	Non-MDR *E. coli*	MDR *E. coli*	Total	χ^2	p-value
Gender					
– Female	24 (40.0%)	18 (30.0%)	42	0.03	0.866
– Male	11 (18.33%)	7 (11.66%)	18		
Age Range					
– 18–25 Years	6 (17.1%)	2 (8.0%)	8	7.75	0.051
– 26–35 Years	5 (14.3%)	4 (16.0%)	9		
– 36–45 Years	13 (37.1%)	12 (48.0%)	25		
– 46–55 Years	8 (22.9%)	4 (16.0%)	12		
– 56– 65 Years	3 (8.6%)	3 (12%)	6		
Sample Type					
– Catherterization	2(3.1%)	2 (5.0%)	4		
– Midstream	33 (96.9%)	23(95.0%)	56		
Duration Of Illness					
– 1–6 Months	6 (23.07%)	6 (25.0%)	12	29.35	0.000*
– 7–12 Months	12 (36.36%)	6 (25.0%)	18		
– Above 12 Months	18 (50.0%)	13 (50.0%)	30		

Fig 3: blaTEM Gene (459bp) Gel Image



Key:
DL= DNA Ladder
-ve= Negative Control.

Fig 4: blaCTX-M Gene(560bp) and blaSHV gene (398bp) gel image



Key:
DL= DNA Ladder
-ve= Negative Control

DISCUSSION

Escherichia coli are very important pathogens of public health importance affecting both children and adults worldwide.[17] A total of 230 adult urine samples were processed to determine the prevalence, molecular types, and susceptibility rate of multidrug resistance patterns of *Escherichia coli* in the study area. One hundred and sixty-nine (73.47%) were female and sixty-one (26.52%) were male, aligning with prior studies that documented that urinary infections (UTIs) are more common in females due to anatomical predisposition, such as a shorter urethra and proximity to the anal region.[18,19] The work also agrees with,[20] which also reported a high prevalence rate of *E. coli* in females.

The age distribution was broad with most participants falling within the age brackets of 36 – 45 years (31.14%) and 46 - 55 years (28.0%). This middle-aged group is often exposed to factors such as hormonal changes, chronic illness, and hospital visitations that increase UTI risk.[21]

The study highlighted that 42 (23.07%) participants with *E. coli* isolates had UTI cases that persisted between 1 and 6 months, and 16 (36.36%) persisted between 7 and 12 months, while 2 (50.0%) persisted for more than 12 months. This chronicity may reflect delayed healthcare-seeking behavior, empirical antibiotic use, or recurrent infections linked to resistant

organisms. According to,[22] recurrent UTIs, particularly in women, are often exacerbated by self-medication and inappropriate antibiotic usage, factors prevalent in low-resource settings like Nigeria. The isolation of *E. coli* from 26.08% of adult urine samples reinforces its role as one of the common uropathogens, as substantiated by previous findings that *E. coli* accounts for the majority of UTIs globally.[23,24]

This study also revealed that participants whose level of education was secondary school had a higher number of *E. coli* isolates, followed by those with tertiary education, with sixteen (16) isolated *E. coli.* Out of the sixty (60) *E. coli* isolates, 25 (41.66%) were multidrug-resistant.

In this study, the highest drug resistance (86.6%) in *E. coli* was recorded for penicillin and streptomycin, respectively. Higher resistance (81% - 100%) of *E. coli* to penicillin has been reported by many researchers.[20,25,26,27,28 and 29] It has been suggested that high resistance to penicillin may have been caused by the presence of the beta-lactamase enzyme in the bacteria or as a result of the reduction of affinity of existing penicillin binding proteins.[30] Another possible reason for the high resistance to penicillin is the frequent use or misuse due to its easy availability and affordability.[31] Also implicated in antibiotic resistance is non-adherence to standard treatment procedures, especially in sub-Saharan Africa, the use of fake or substandard drugs, and the unauthorized sale of antibiotics without appropriate prescriptions.[32,33]

A relatively low resistance level was observed with nitrofurantoin (1.66%), and ciprofloxacin (3.3%). This result is in line with the studies of[34 and 35] who also recorded a low resistance rates against these antibiotics.

Out of the twenty-five (41.66%) MDR *E. coli* isolates, ten (10) were selected for further analyses to identify the genes responsible for the resistance using multiplex and linear PCR.

The molecular findings from the gel electrophoresis images showed that among the Extended Spectrum Beta-Lactamases (ESBL) genes screened, blaCTX-M (560 bp) was present in three isolates, while no bands were observed for blaSHV (398 bp).

This corroborates findings by[36] who documented a surge in blaCTX-M-15 among Nigerian isolates, especially in multidrug-resistant (MDR) strains.

Interestingly, the blaTEM gene was also detected in the isolates, with a band at 459 bp, confirming its role in beta-lactam resistance, as observed by.[37] These ESBL genes are often plasmid-mediated and associated with horizontal gene transfer, contributing to the rapid spread of resistance among Enterobacteriaceae.[38,7] The predominance of these genes in a specialized tertiary hospital population—such as an orthopedic hospital—highlights the risk posed by nosocomial infections and prolonged catheterization.[39]

Furthermore, the lack of detection of blaSHV aligns with observations in other Nigerian studies, where CTX-M has increasingly displaced older ESBL genotypes such as SHV and TEM in terms of frequency and distribution.[8, 23] This evolutionary shift may be attributed to selective antibiotic pressure in the hospital

environment and the clonal expansion of successful lineages such as ST131, which are commonly associated with CTX-M-15 production.[40]

CONCLUSION

This study concludes that *Escherichia coli* remains the most prevalent causative agent of urinary tract infections among adults attending the National Orthopedic Hospital, Enugu, with a concerning level of multidrug resistance. The bacterial isolates demonstrated high carriage rates of resistance genes such as blaCTX-M and blaTEM, confirming the spread of extended-spectrum beta-lactamase (ESBL)-producing strains within the hospital environment. The absence of the blaSHV gene suggests a possible regional decline in its prevalence, in contrast to the growing dominance of CTX-M genes. The predominance of these ESBLs, especially blaCTX-M, reflects the global trend of the rapid emergence of resistant clones like ST131, known for their clinical severity, persistence, and limited therapeutic options. The findings of this study underscore the threat that multidrug-resistant *E. coli* poses to the effective management of UTIs in both hospital and community settings.

The study also reinforces the need for the integration of molecular diagnostic tools into clinical microbiology laboratories for early detection of resistance genes and targeted therapy. In conclusion, this research emphasizes the urgent necessity for institutional and national strategies that include antimicrobial stewardship, public health education, improved diagnostic infrastructure, and infection control protocols to contain the threat of antimicrobial resistance, particularly in specialized hospital settings like orthopedic units.

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REFERENCES

1. Medugu N, Aworh M K, Iregbu K, Nwajiobi-Princewill P, Abdulraheem K, Hull DM, Harden L, Singh P, Obaro S, Egwuenu A, and Thakur S. Molecular characterization of multi drug resistant *Escherichia coli* isolates at a tertiary hospital in Abuja, Nigeria. *Scientific Reports*, 2022;*12*(1), 14822. https://doi.org/10.1038/s41598-022-19289-z
2. Naylor N, Pouwels K, Hope R, Green N, Henderson K, Knight G, Atun R, Robotham J, and Deeny S. The health and cost burden of antibiotic resistant and susceptible *Escherichia coli* bacteraemia in the English hospital setting : A national retrospective cohort study. *PLos One*, 2019;*14*(9), e0221944.
3. Walker MM, Roberts J A, Rogers BA, Harris PNA, and Sime FB. Current and Emerging Treatment Options for Multidrug Resistant *Escherichia coli* Urosepsis: A Review. In *Antibiotics* 2022;(Vol. 11, Issue 12, p. 1821). https://doi.org/10.3390/antibiotics11121821

4. Moxon CA, and Paulus S. Beta-lactamases in Enterobacteriaceae infections in children. *Journal of Infection*, *72*, S41–S49. https://doi.org/10.1016/j.jinf.2016.04.021
5. Poirel L, Madec YY, Lupo A, Schink AK, Kieffer N, Nordmann P and Schwarz S. Antimicrobial resistance in *Escherichia coli.* Microbiology Spectrum,2018; 6(4): ARBA-0026-2017.
6. Liu U, Zhu M, Fu X, Cai J, Chen S, Lin U,Jiang N, Chen S and Lin Z. *Escherichia coli* causing neonatal meningitis during 2001-2020: A study in Eastern China. International Journal of General Medicine, 2021;14:3007-3016.
7. LI Q, Chang W, Zhang H, Hu D, and Wang X. The role of Plasmids in the multiple antibiotic resistance transfer in ESBLs-producing *Escherichia coli* isolated from waste water treatmen plants. Frontiers in Microbiology, 2019;10:633.
8. Bischoff S, Walter T, Gerigk M, Ebert M, and Vogelmann, R. Empiric antibiotic therapy in urinary tract infection in patients with risk factors for antibiotic resistance in a German emergency department. *BMC Infectious Diseases*,2018;*18*(1), 56. https://doi.org/10.1186/s12879-018-2960-9
9. Micali, S., Isgro, G., Bianchi, G., Miceli, N., Calapai, G., and Navarra. Cranberry and Recurrent Cystitis: More than Marketing? *Critical Reviews in Food Science and Nutrition*,2014; *54*(8), 1063–1075. https://doi.org/10.1080/10408398.2011.625574
10. Flores-Mireles AL, Walker JN, Caparon M, and Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*, 2015; *13*(5), 269–284. https://doi.org/10.1038/nrmicro3432
11. Bonkat G, Bartoletti R, Bruyere F, Cai T, Geerling SE, Kove B, Schubert S,Pilatz A, Kranz J, Veeratterapillay R, Wagenlehner F . EAU guidelines on urological infections. European association of urology, 2017; 18,22-26
12. Mahony M, McMullan B, Brown J, and Kennedy SE. Multidrug-resistant organisms in urinary tract infections in children. *Pediatric Nephrology*, 2020; *35*(9), 1563–1573. https://doi.org/10.1007/s00467-019-04316-5
13. Founou RC, Founou LL, and Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. *PLOS ONE*, 2017;*12*(12), e0189621. https://doi.org/10.1371/journal.pone.0189621
14. Cheesebrough, M. (2010). District laboratory practice in tropical countries (Part 2, 2nd ed.). Cambridge University Press.
15. Clinical and Laboratory Standard Intitute. Breakpoint Implementation

Toolkit. 2024; 34th Edition. CLI, Wayne PA, USA.

16. Okafor JU, and Nweze EI. Antibiotic Suceptibility of *Escherichia coli* isolated in cases of urinary tract infection in Nsukka, Nigeria. Journal of Pre-Clinical and Clinical Research.2020; 14(1),1-7,10.26444/jpccr/118949
17. Pormohammad A, Nasiri MJ, and Azimi T. Prevalence of antibiotic resistance in *Escherichia coli* strains simultaneously isolated from humans, animals, food and the environment: a systematic review and meta-analysis. Infection Drug Resistance,2019; 12:1181-1197.
18. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infectious Disease Clinics, 2014; 28(1), 1–13. https://doi.org/10.1016/j.idc.2013.09.003
19. Hooton T M. Clinical practice. Uncomplicated urinary tract infection. The New England Journal of Medicine,2012; 366(11), 1028–1037.
20. Omolajaiye SA, Afolabi KO and Iweriebor BC. Pathotyping and antibiotic resistance profiling of *Escherichia coli* isolates from children with acute diarrhea in Amatole district municipality of Eastern Cape, South Africa. Biomedical Research International, 2020;10:4250165.
21. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG and Soper DE. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clinical Infectious Diseases, 2011; 52(5), e103–e120. https://doi.org/10.1093/cid/ciq257
22. Micali S, Isgro G, Bianchi G, Miceli N, Calapai G, and Navarra, M. (2014). Cranberry and Recurrent Cystitis: More than Marketing? *Critical Reviews in Food Science and Nutrition*, *54*(8), 1063–1075. https://doi.org/10.1080/10408398.2011.625574
23. Ajayi AO, Shonekan AO, and Ajayi OO. Characterization of fluoroquinolone-resistant Escherichia coli isolated from urinary tract infections in Nigeria. *African Journal of Clinical and Experimental Microbiology*, 2014;15(3), 127–134.
24. Allocati N, Masulli M, Alexeyev M F, and Di Ilio C. (2013). *Escherichia coli* in Europe: An Overview. In *International Journal of Environmental Research and Public Health,* 2013; Vol. 10, Issue 12, pp. 6235–6254. https://doi.org/10.3390/ijerph10126235
25. David EE, Yameen MA, Igwenyi IO, Okafor AC, Obeten UN, Obasi DO, Ezeilo UR and David CN. The frequency of virulent genes and

antimicrobial resistance patterns of diarheagenic *Escherichia coli* isolated from stools of children presenting with diarrhea in a tertiary institution in Abakaliki, Nigeria. International Journal of One Health, 2020;6 (2):147 - 152.

26. Patil S, Chen H, Chen Y, Dong S, Mai H, Lopes BS, Liu S, and Wen F. Trends in antibiotic pesistance Patterns and burden of *Escherichia coli* infections in young children: A retrospective cross-sectional study in Shenzen, China From 2014 - 2018. Infection Drug Resistance,2023; 16: 5501-5510.
27. Afiukwa CA, Iroha IR, Afiukwa N, and Onwa C. Presence of antibiotic resistant coliforms in sachet water sold in some parts of Southeastern Nigeria. Journal of Microbiology and Antimicrobials,2010; 2(5): 51-54.
28. Adesoj AT, and Liadi AM. Antibiogram studies of *Escherichia coli* and Salmonella species isolated from diarrheal patients attending Malam Mande General Hospital Dutsin-Ma, Katsina State, Nigeria. Pan African Medical Journal,2020; 37:110
29. Singh AK, Das S, Singh S, Gajamer VR, Pradhan N, Vangchen DL, and Tiwari KH. Prevalence of antibiotic resistance in commensal *Escherichia coli* among the children in rural communities of East India. PLOS ONE, 2018;13(6): e0199179.
30. Anyamene CO, Godwin VC and Ezebialu CU. Isolation and characterization of multidrug resistant bacterial pathogens present in hospital environment within Awka. metropolis. IDOSR Journal of Biology, Chemistry and Pharmacy,2023; 8 (2): 63 – 91.
31. Akande-Sholabi W, Oyesiji E, Adebisi YA, . Antimicrobial Stewardship: Community Pharmacists'Antibiotic Dispensing Practices, knowledge and Perception Regarding Antibiotics and Antibiotic Resistance.Journal of Pharmaceutical Health Services, 2023;14(4).
32. Rodrigues IA, Ferrari RG, Panzenhagen PH, Mano SB, and Conte- Junior CA. Antimicrobial resistance genes in bacteria from animal- based foods. Advances in Applied Microbiology,2020; 112: 143 – 183.
33. Elton L, Thomason MJ, Tembo J, Valava TP, Pallerla SR, Arruda LB. Antimicrobial resistance preparedness in Sub - Saharan African countries. Antimicrobial Resistance and Infection Control, 2020; 9(1):145.
34. Olaniran O, Japheth OM, Asinwa HJ, Olajokun- Hasssan RE and Adekunle OT. Isolation and evaluation of *E.coli* in diarrhoeic stool samples from children in Ile-Ife, Nigeria. Journal of Disease Global Health,2015; 4(4): 145 -151.
35. Bartoloni A, Pallecchi L, Rodriguez H, Fernandez C, Mantella A. Antibiotic resistance in a very remote Amazonas community. Int J Antimicrob Agents, 2009; 33,125-129.

36. Peirano G Pitout JDD. Extended-Spectrum beta-Lactamase-Producing Enterobacteriaceae: Update on Molecular Epidemiology and Treatment Options. Drug, 2019; 79, 1529-1541. doi:10.1007/s40265-019-01180-3
37. Ahmed S, Hasan MM, and Khan MA. Rising antimicrobial resistance in *E. coli* UTIs: A global health concern. *Journal of Medical Microbiology*, 2020; 69(4), 567-575.
38. Basak S, Singh P, and Rajurkar M. Multidrug Resistant and Extensively Drug Resistant Bacteria: A Study. *Journal of Pathogens*, *2016*(1), 4065603. https://doi.org/https://doi.org/10.1155/2016/4065603
39. Johnson, A. P., Woodford, N., and Livermore, D. M. (2018). Resistance trends among *E. coli* from community-acquired UTIs. *Antimicrobial Agents and Chemotherapy*, 62(3), e01576-17.
40. Price LB, Johnson JR, Aziz M, Clabots C, Johnston B, Tchenokova V, Nordtrom L, Billig M, Chattopadhyay S, Stegger M, Anderon PS, Pearon T, Riddell K, Rogers P, Scholes D, Kahl B, Keim P, Sokurenko EV.The Epidemic of Extended-Spectrum-Lactamase-Producing *Escherichia coli* ST131 Is Driven by a Single Highly Pathogenic Subclone, H30-Rx. Journal.am.org, MMBio 2013; 4(6), 10.1128/mbio.00377-13.
41. Daniel WW, and Cross CL. (2013). Biostatistics: A foundation for analysis in the health sciences. 10th ed. New York: John Wiley & Sons.
42. Chaudhary M K, Jadhav I, and Banjara MR. Molecular detection of plasmid mediated blaTEM, blaCTX-M, and blaSHV genes in Extended Spectrum β-Lactamase (ESBL) *Escherichia coli* from clinical samples. Annals of Clinical Microbiology and Antimicrobials, 2023; 22(1), 33. doi: 10.1186/s12941-023-00584-0