



ANTIBIOTIC SUSCEPTIBILITY PROFILE AND MOLECULAR DETECTION OF EXTENDED-SPECTRUM BETA-LACTAMASES IN GRAM-NEGATIVE BACTERIA IN URINE SAMPLES FROM OLABISI ONABANJO UNIVERSITY TEACHING HOSPITAL, SAGAMU, OGUN STATE

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ABSTRACT

Gram-negative bacteria are believed to be important contributors to various forms of urinary tract infections. Therefore, this study aimed to determine the prevalence and resistance pattern of Gram-negative bacteria implicated in urinary tract infection and to detect extended-spectrum beta-lactamase (ESBLs) genes in them. A total of one hundred and twenty (120) clean catch urine samples were collected aseptically from patients attending Olabisi Onabanjo University Teaching Hospital, Sagamu. The urine samples were cultured on Cystine Lactose Electrolyte Deficient (CLED) agar. The isolates were identified using standard biochemical assays. The Kirby-Bauer disk diffusion method was employed to test the antibiotic susceptibility of the isolates. Phenotypic identification and genotypic detection of extended-spectrum beta-lactamase (ESBL) genes in the isolates were performed. Out of the 120 urine samples collected, 55(45.8%) were from females while 65 (54.2%) were from males (<1- >80 years). The prevalence of bacterial isolates in urine samples was 42.5%. The bacterial pathogens isolated were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Escherichia coli*. *K. pneumoniae* was the most common isolate. The highest resistance to ceftazidime was recorded against *S. aureus* (92.9%) while *K. pneumoniae* was the most resistant to imipenem (36.8%) and meropenem (42.1%). ESBL genes were found in 24/37 (64.9%). The *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes were present in 9 (24.3%), 6 (16.2%) and 8 (21.6%) of the isolates, respectively. As a result, increasing public awareness is critical to limiting the spread of antibiotic resistance and necessitates continuous surveillance.

Keywords: Antibiotic resistance, Extended Spectrum Beta-Lactamase, Gram-negative bacteria, Urinary tract infections

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INTRODUCTION

Urinary tract infections (UTIs) are an issue of public concern that affects both the community and the hospital, and they are among the most common illnesses worldwide [1]. These infections account for a considerable number of visits to the emergency section of the hospital [2]. UTIs continue to have significant prevalence and fatality rates, particularly in hospitals, despite improvements in their identification and treatment [3]. Up to 9.4% of individuals within healthcare facilities get UTIs and more than 80% of these infections are caused by urethral catheterization [4, 5]. The presence of a catheter is the most significant risk factor for bacteria in the urine [6]. Depending on a person's immunological condition, UTI may manifest in patients as acute, chronic, severe, or uncomplicated illness or, in some situations, go undetected [7]. UTIs are a prevalent cause of febrile sickness in young children all around the world [8]. They are linked to a reduction in

patients' standard of life and a major health and financial burden [9].

The pathogenic bacteria that cause UTI vary significantly over time, with notable variability between countries or locations [10]. Cases of UTIs are diverse and range from mild cystitis and urethritis to severe pyelonephritis, bacteremia, and septic shock [11, 12]. The majority of cases of simple cystitis are caused by *Escherichia coli*. Other bacteria implicated in UTI include *Staphylococcus saprophyticus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *P. aeruginosa* and *Enterococcus faecalis* [13-15]. Uropathogenic *Escherichia coli* (UPEC) is the major cause of UTIs, accounting for at least 80% of UTIs acquired in the community and 65% of hospital-acquired UTIs [16, 17]. The likelihood of developing a UTI rises with age, except for an elevated risk in young women between the ages of 14 and 24 [18]. Women are much more likely than men to get urinary tract infections (UTIs), which account for at least 8 million annual visits to different types of healthcare institutions in the United

States [18]. Increased sexual activity is a key risk factor for UTIs in younger women [19].

Multidrug-resistant Gram-negative bacteria, such as extended-spectrum beta-lactamase (ESBL) and carbapenemase producers, are increasingly causing UTIs in individuals in healthcare facilities and those who have had regular antibiotic exposure [20]. The incidence of multidrug-resistant organisms, on the other hand, varies by location [4]. The relentless spread of ESBL-producing Gram-negative bacteria has major implications on the outcomes of treatment [21]. Several studies have reported treatment failure in patients infected with ESBL-producing bacteria due to limited therapeutic options [22, 23]. Bacteria that produce ESBL that are also resistant to carbapenems are widely distributed throughout the world [24]. According to reports, the outbreak of ESBL-producing microbes is worse in underdeveloped nations [24, 25]. ESBLs have been identified in the majority of Gram-negative bacteria, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Klebsiella oxytoca*, *Acinetobacter baumannii*, and *Proteus* species [26]. Gram-negative bacteria that produce ESBL were frequently discovered in community and hospital settings in Nigeria [21]. Observed prevalence ranged from 7.5% to 82.3% [21]. The most prevalent ESBL gene variants detected are *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV} and have been found in numerous researches conducted in Nigeria [27, 28]. However, because of insufficient or inefficient monitoring in certain healthcare settings, the scope of the ESBL problem is still possibly understated [24]. Several studies from Nigeria have reported the presence of ESBL genes in uropathogens [29, 30]. Understanding the frequency and resistance patterns of these pathogens is crucial for guiding effective treatment strategies and combating the spread of multidrug-resistant infections. This study was conducted to determine the frequency and antibiotic resistance patterns of ESBL-producing uropathogens at Olabisi Onabanjo University Teaching Hospital in Sagamu, Ogun state.

MATERIALS AND METHODS

Study design and sample size

This is a cross-sectional study. The formula $N = Z^2P(1-P)/d^2$ [31] was used to calculate the sample size. Using an 8.0% prevalence from Adebola and Adebola [32], 113.1 was the estimated sample size. However, 120 urine samples were collected.

Inclusion criteria

The patients were interviewed and individuals who voluntarily gave their consent and had been diagnosed with urinary tract infections but had not taken antibiotics

in the two weeks before their visits were included in the study

Exclusion criteria

Individuals who did not give their consent and had been diagnosed with urinary tract infections and had taken antibiotics in the two weeks prior were excluded from the study

Collection of samples

Ethical approval was obtained from the Olabisi Onabanjo University Teaching Hospital Ethics Committee (OOUTH/HREC/579/2023AP). Clean-catch midstream urine samples were collected from patients with urinary tract infection symptoms in Olabisi Onabanjo University Teaching Hospital between February and April, 2023.

Isolation and identification of bacterial isolates

The semiquantitative cultural method was used to culture the urine samples on Cysteine Lactose Electrolyte Deficient (CLED) agar. Inoculated plates were incubated overnight at 37°C. A urine culture that has a bacteria load of more than or equal to 10⁵ CFU/mL was regarded as significant growth. The colonies were sub-cultured on MacConkey agar, mannitol salt agar, and eosin methylene blue agar and incubated at 37°C for 24 hours. The bacterial colonies on agar plates were identified using common microbiological techniques such as colony morphology, microscopy, and biochemical assays [33, 34].

Antibiotic susceptibility testing

The disc diffusion method of Bauer and Kirby [35] was used to test isolates for their susceptibility patterns to antibiotics. Antibiotic discs used were cefepime (30 µg), amoxicillin-clavulanic acid (20/10 µg), ceftazidime (30 µg), cefotaxime (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), cefoxitin (30 µg), imipenem (10 µg), meropenem (10 µg), cefuroxime (30 µg) and carbenicillin (100 µg).

The isolates were sub-cultured on a nutrient agar plate and incubated for 24 hours. Then, 3-4 colonies of each isolate were picked using a sterile inoculating loop and suspended into sterile distilled water to give turbidity equivalent to McFarland standard of density 1.5 x 10⁸ CFU/mL. Molten Mueller Hinton Agar (MHA) was poured into a Petri dish and allowed to solidify. The surface of the Mueller Hinton agar was inoculated with the standardized bacterial culture using a sterile swab stick. The discs were placed on the agar plates using a sterile pair of forceps and allowed to stand for 30 minutes. The inoculated agar plates were incubated in an inverted position for 24 hours at 37°C. *E. coli* ATCC 25922 was used as a control. Zones of growth inhibition were measured in millimeters and interpreted as sensitive, intermediate, and resistant according to the guidelines of the Clinical Laboratory Standards Institute [36]. Isolates

that were resistant to three or more classes of antibiotics were classified as multidrug-resistant (MDR).

Screening for ESBLs production

Gram-negative isolates were screened for ESBL production using a modified double disc synergy test as described by the Clinical Laboratory Standards Institute [37]. Cefepime (30 µg), ceftazidime (30 µg), and cefotaxime (30 µg) antibiotics were placed 20 mm center to center from amoxicillin-clavulanic acid (20/10 µg) disc on inoculated Mueller Hinton agar plates and incubated at 37 °C for 24 hrs. ESBL-producing strains were identified by confirmation of an increase of ≥5 mm in the zone of inhibition for any of the three cephalosporins towards amoxicillin clavulanic acid compared to cephalosporins alone. *E. coli* ATCC 25922 was used as a control.

DNA extraction

The boiling method was used to extract the bacterial DNA. A pure colony of each isolate was inoculated in 2 mL Luria Bertani broth (LB) and incubated for 24 h and then centrifuged at 10,000 RPM/min for 10 min. The bacterial cells were suspended in 500 µL of phosphate buffer (100 mM, pH 7) to weaken the membranes and then immersed in a boiling water bath at 100°C for 15 minutes to release

the DNA. After that, the DNA was precipitated with 250 µL of absolute alcohol, centrifuged again and resuspended in 100 µL of sterile water [38]. The primer sequences used are listed in Table 1.

Molecular detection of ESBL genes in multidrug-resistant Gram-negative uropathogens

The ESBL genes, *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} were detected by PCR using the thermal cycler (Applied Biosystems, USA). A 25 µL total PCR volume was prepared with 1 µL of DNA, 1 µL of each primer (0.2 pmol/µL), 12.5 µL of WizPure™ PCR 2X Master (Inqaba, South Africa), and 9.5 µL molecular grade water. The amplification condition for *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M-9} consisted of initial denaturation at 94°C for 10 min, then 30 cycles of denaturation 94°C for 40 s, annealing at 60°C for 40 s and elongation at 72°C for 1 min with a final elongation step at 72°C for 7 min [39, 40].

To visualize PCR products, an electrophoretic migration at 100 volts for 1 hour was performed on a 2% agarose gel stained with ethidium bromide (0.5 µg/ml). As a reference, a molecular weight marker of 100 bp was used. The distinct bands were observed under UV illumination after migration, and the results were photographed and documented.

Table 1: Primer sequences for ESBL gene detection

Genes	Primer sequence	Amplicon sizes (bp)	Reference
TEM	F-5'GAGTATTCAACATTTTCGT-3' R-5' ACCAATGCTTAATCAGTGA-3'	857	Maynard <i>et al.</i> [40]
SHV	F-5'TCGCCTGTGTATTATCTCCC-3' R-5' CGCAGATAAATCACCACAATG-3'	768	Maynard <i>et al.</i> [40]
CTX-M	F-5'TTTGCGATGTGCAGTACCAGTAA-3' R-5'CGATACGTTGGTGGTGCCATA-3'	544	Edelstein <i>et al.</i> [39]

Statistical analysis

The Chi-square test was used to determine the prevalence among the age groups and gender (*p* = 0.05)

RESULTS

Prevalence of bacteria in urine samples in relation to age and sex of the patients

Out of 120 urine samples collected from both inpatients and outpatients, 65 (54.2%) were from males. Fifty-one samples showed significant bacterial growth (42.5%). The prevalence of bacteria in urine samples was greater in

females 25/55 (45.5%) than in males 26/65 (40.0%), but not statistically significant (*p* = 0.55). The highest occurrence of bacteria (100%) was obtained from both males and females between the ages of 81 years and above while the least occurrence of bacteria (16.7%) was from male patients of between the ages of 21 and 40 years (Table 2).

Table 2: Prevalence of bacteria in urine samples in relation to age and sex of the patients

Ages (years)	Male		Female	
	No. of samples	Positive samples n (%)	No. of samples	Positive samples n (%)
<1	3	1 (33.3)	5	2 (40.0)
21 – 40	24	4 (16.7)	27	10 (37.0)
41 – 60	17	3 (17.7)	14	5 (35.7)

61 – 80	16	13 (81.3)	4	3 (75.0)
>81	5	5 (100)	5	5 (100)
Total	65 (54.2)	26 (40.0)	55 (45.8)	25 (45.5)

Percentage distribution of bacteria isolated (uropathogens) from patients with UTI in Olabisi Onabanjo University Teaching Hospital

The bacterial species identified were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Escherichia coli*. *K. pneumoniae* was the most common 19 (37%), followed by *S. aureus* 14 (27%) and *P. mirabilis* was the least 1 (2%) (Figure 1). Table 3 shows the distribution of isolated bacteria according to age group. Highest number of isolates was found within the age group 61 and 80 years 16/51(31.4%).

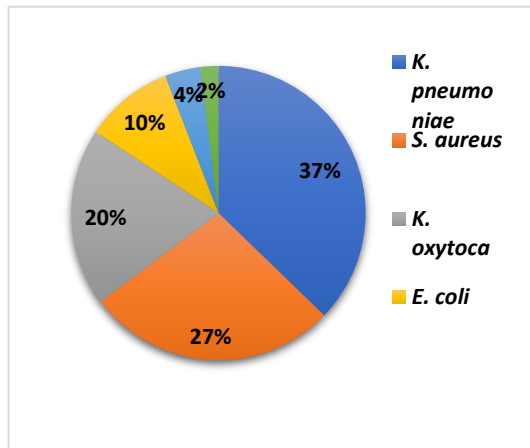


Figure 1: Distribution of bacteria isolates from UTI

Table 3: Distribution of the isolates according to age group

Isolates (No)	Age group no (%)				
	<1 - 20	21 - 40	41 - 60	61 - 80	>81
<i>S. aureus</i> (14)	2	5	3	4	3
<i>E. coli</i> (5)	-	3	1	-	1
<i>P. aeruginosa</i> (2)	-	1	-	-	1
<i>P. mirabilis</i> (1)	-	-	1	-	-
<i>K. oxytoca</i> (10)	-	2	4	4	-
<i>K. pneumoniae</i> (19)	-	3	4	8	-
Total (51)	2 (3.9)	14 (27.5)	13 (25.5)	16 (31.4)	5 (9.8)

Antibiotic resistance profiles of uropathogenic bacterial isolates

S. aureus was highly resistant to ceftazidime (92.9%) followed by cefotaxime (78.6%) and ciprofloxacin (71.4%). *K. pneumoniae* was moderately resistant to imipenem (36.8%) and meropenem (42.1%). *P. mirabilis* was resistant (100%) to cefepime, cefotaxime, ceftazidime, aztreonam, ciprofloxacin, cephalixin and cefoxitin. *P. aeruginosa* was highly resistant (100%) to ciprofloxacin, amoxicillin-clavulanic acid, carbenicillin, cephalixin, and cefoxitin. All *E. coli*, *P. aeruginosa* and *P. mirabilis* were susceptible to imipenem (Table 3).

Table 3: Antibiotic resistance patterns of uropathogenic isolates

Antibiotics	<i>S. aureus</i> (14)	<i>E. coli</i> (5)	<i>P. aeruginosa</i> (2)	<i>P. mirabilis</i> (1)	<i>K. oxytoca</i> (10)	<i>K. pneumoniae</i> (19)
CFP	8 (57.1)	0 (0.0)	1 (50.0)	1 (100.0)	8 (80.0)	12 (63.2)
AUG	5 (35.7)	3 (60.0)	2 (100.0)	0 (0.0)	6 (60.0)	11 (57.9)
CTX	11 (78.6)	1 (20.0)	1 (50.0)	1 (100.0)	8 (80.0)	14 (73.7)
CAZ	13 (92.9)	0 (0.0)	1 (50.0)	1 (100.0)	8 (80.0)	12 (63.2)
AK	3 (21.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	4 (21.1)
CIP	10 (71.4)	4 (80.0)	2 (100.0)	1 (100.0)	9 (90.0)	14 (73.7)
FOX	6 (42.9)	2 (40.0)	2 (100.0)	0 (0.0)	3 (30.0)	10 (52.6)
IMI	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	7 (36.8)
CRX	9 (64.3)	2 (40.0)	2 (100.0)	1 (100.0)	8 (80.0)	16 (84.2)
CAR	8 (57.1)	5 (100.0)	2 (100.0)	1 (100.0)	10 (100.0)	17 (89.5)
MEM	2 (14.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	8 (42.1)
ATM	ND	1 (20.0)	0 (0.0)	1 (100.0)	3 (30.0)	7 (36.8)

Note: AUG = Amoxicillin clavulanic acid; CAZ = Ceftazidime; CFP = Cefepime; CTX = Cefotaxime; ATM = Aztreonam; AK = Amikacin; CIP = Ciprofloxacin; FOX = Cefoxitin; IMI = Imipenem; CRX = Cefuroxime; MEM = Meropenem; CAR = Carbenicillin

Detection of ESBL in Gram-negative isolates

Among the Gram-negative isolates, 19/37 (51.4%) were positive for ESBL production by phenotypic methods including seven *K. oxytoca*, nine *K. pneumoniae* and one each of *P. aeruginosa*, *P. mirabilis*, and *E. coli* (Table 4). Figures 2-4 show the gel electrophoresis of amplified *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes. Both ESBL-producing and non-ESBL-producing Gram-negative uropathogens were subjected to singleplex PCR to detect ESBL genes.

Among these, 24/37 (64.9%) had ESBL genes. The *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes were found in 9 (24.3%), 6 (16.2%) and 8 (21.6%) of the isolates, respectively. One *K. pneumoniae* had both *bla*_{TEM} and *bla*_{SHV} genes (Table 5). Seven phenotypically negative isolates had ESBL genes while two phenotypically positive isolates did not have any of *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes. *bla*_{TEM} was the most prevalent of ESBL genes occurring in 10 (27.0%) of the isolates.

Table 4: Phenotypic detection of ESBL in Gram negative bacteria

S/N	Code	Isolate	Diameter of zone of inhibition (mm)						ESBL
			CAZ	CAZ-AUG	CTX	CTX-AUG	CFP	CFP-AUG	
1	Ko003	<i>K. oxytoca</i>	17	9	0	15	10	17	+
2	Kp004	<i>K. pneumoniae</i>	0	0	0	0	20	20	-
3	Ko007	<i>K. oxytoca</i>	12	19	10	17	16	24	+
4	Ec012	<i>E. coli</i>	18	23	12	19	19	26	+
5	Ko014	<i>K. oxytoca</i>	0	0	0	10	0	11	+
6	Kp015	<i>K. pneumoniae</i>	12	10	0	7	1	11	+
7	Pm017	<i>P. mirabilis</i>	0	21	10	14	12	13	+
8	Kp023	<i>K. pneumoniae</i>	15	9	15	19	19	27	-
9	Kp028	<i>K. pneumoniae</i>	0	17	8	16	8	18	+
10	Kp034	<i>K. pneumoniae</i>	14	19	8	16	19	22	+
11	Ko044	<i>K. oxytoca</i>	26	22	32	26	30	27	-
12	Kp046	<i>K. pneumoniae</i>	0	0	0	2	0	4	-
13	Kp049	<i>K. pneumoniae</i>	16	14	13	12	15	17	-
14	Ko051	<i>K. oxytoca</i>	0	8	0	8	0	12	+
15	Kp053	<i>K. pneumoniae</i>	8	18	7	16	14	20	+
16	Kp059	<i>K. pneumoniae</i>	0	4	0	0	0	4	-
17	Ko061	<i>K. oxytoca</i>	12	20	8	16	14	20	+
18	Ko062	<i>K. oxytoca</i>	0	0	0	4	0	0	-
19	Ko065	<i>K. oxytoca</i>	13	19	10	16	14	14	+
20	Kp067	<i>K. pneumoniae</i>	23	20	15	12	18	16	-
21	Ec070	<i>E. coli</i>	30	28	32	30	32	30	-
22	Kp073	<i>K. pneumoniae</i>	0	3	0	0	9	9	-
23	Kp075	<i>K. pneumoniae</i>	10	20	8	17	13	22	+
24	Kp079	<i>K. pneumoniae</i>	16	23	16	22	10	19	+
25	Kp082	<i>K. pneumoniae</i>	28	13	32	16	32	17	+
26	Ko086	<i>K. oxytoca</i>	28	20	32	20	30	20	-
27	Kp088	<i>K. pneumoniae</i>	8	17	8	18	11	21	+
28	Ko092	<i>K. oxytoca</i>	10	13	0	14	7	12	+
29	Pa093	<i>P. aeruginosa</i>	8	16	0	15	7	12	+
30	Pa096	<i>P. aeruginosa</i>	23	11	26	11	26	26	-
31	Ec098	<i>E. coli</i>	28	25	30	18	32	18	-
32	Kp103	<i>K. pneumoniae</i>	24	16	30	15	26	17	-
33	Kp105	<i>K. pneumoniae</i>	0	0	0	0	9	0	+
34	Ec108	<i>E. coli</i>	27	15	28	16	24	12	-
35	Ec110	<i>E. coli</i>	22	14	27	19	28	20	-

36	Kp116	<i>K. pneumoniae</i>	22	8	14	0	20	6	-
37	Kp118	<i>K. pneumoniae</i>	20	18	32	6	40	38	-

Note: + = ESBL positive; - = ESBL negative; CAZ = ceftazidime; CAZ-AUG = ceftazidime + amoxicillin clavulanic acid; CTX = cefotaxime; CTX-AUG = cefotaxime + amoxicillin clavulanic acid; CFP = cefepime; CFP-AUG = cefepime + amoxicillin clavulanic acid

Table 5: Detection of extended spectrum beta-lactamase genes in bacterial isolates

Isolate	ESBL genes			Total
	<i>bla_{TE}</i> M	<i>bla_{SH}</i> V	<i>bla_{CT}</i> X-M	
<i>K. oxytoca</i> (10)	3	1	4	8
<i>K. pneumoniae</i> (19)	4	4	4	13
<i>E. coli</i> (5)	-	1	-	1
<i>P. mirabilis</i> (1)	1	-	-	1
<i>P. aeruginosa</i> (2)	1	-	-	1
Total	9	6	8	24

Note: - = negative

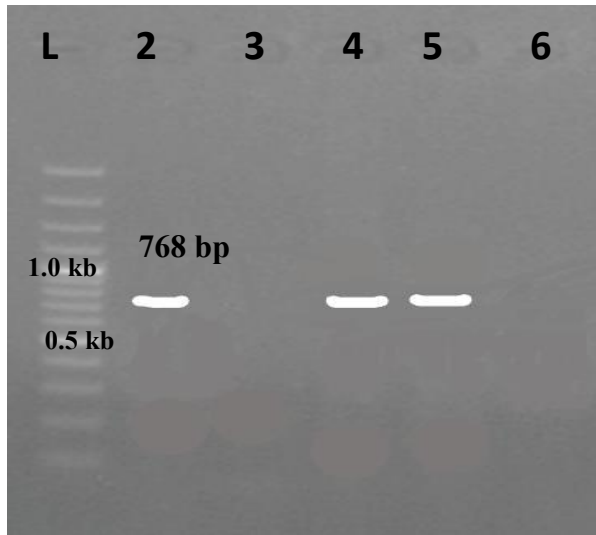


Figure 2: Agarose gel electrophoresis of amplified *bla_{SHV}* genes (768 bp). L = 100bp ladder; Line 1= 100 bp ladder; Lines 2-5, Kp023, Kp034, Ko044, Kp073 Ec070

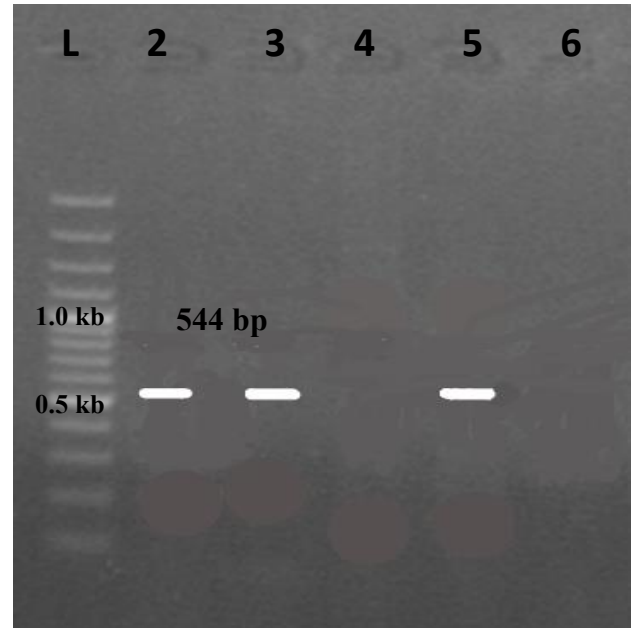


Figure 3: Agarose gel electrophoresis of amplified *bla_{CTX-M}* genes (544 bp). L = 100bp ladder; Line 1= 100 bp ladder; Lines 2-8, Ko003, Ko007, Ec012, Ko014, Kp088, Pa096

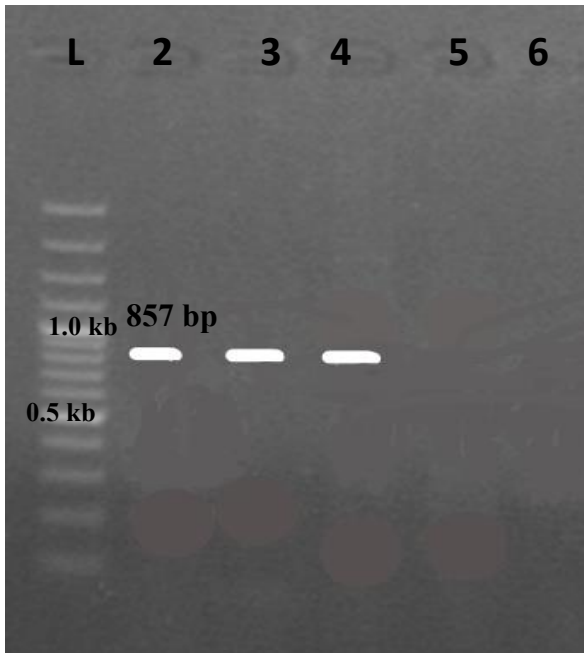


Figure 4: Agarose gel electrophoresis of amplified *bla*_{TEM} genes (857 bp). L = 100bp ladder; Line 1= 100 bp ladder; Lines 2-5 = Ko061, Ko065, Kp067, Ec070, Kp073

DISCUSSION

Gram-negative bacteria are the most often documented causes of UTIs and are typically resistant to multiple antibiotics, leading to recurrent UTIs in the high-risk group in particular [41, 42]. They pose a public health risk and hence require adequate medical treatment [30]. Women are more likely to get urinary tract infections (UTIs) due to anatomic factors, poor hygiene, sex activities, and contraception use. Hormonal changes, including menopause and estrogen depletion, contribute to the increased prevalence of urinary tract infections in older women [43]. Previous research findings indicating females were more prone to UTIs than males due to anatomical variations between the sexes [1, 44], this study also found a slightly greater prevalence of UTIs in females (45.5%) than in males (40%). However, UTIs were more common in people over the age of 80 years (100%), both males and females in this study. This corresponds to the report by other researchers that the prevalence of UTI rises with age and is equally widespread among older men and women [19, 45].

The increased occurrence of UTIs with age can be due to a variety of reasons, including diminishing immunity. As people age, their immune systems weaken, making them more vulnerable to infections like UTIs. Waning immunity is not the only cause that leads

to an increased incidence of UTIs in the elderly; additional contributing variables include altered bladder function, hormonal changes in women postmenopausal, and underlying medical disorders like kidney stones or diabetes [46,47]. The prevalence of bacterial pathogens in cases of urinary tract infections reported in this study (42.5%) was higher than the 15.5% prevalence reported by Ejerssa *et al.* [48]. It has been observed that *E. coli* is the most usually isolated bacteria in UTI cases [48-50] while this study found *K. pneumoniae* to be most prevalent. The prevalence of certain bacteria in UTI cases can also fluctuate over time due to factors such as antibiotic usage, changes in bacterial resistance patterns, or shifts in microbial ecosystems, lack of proper hygiene practices and UTI prevention strategies, delay in seeking medical help for UTI symptoms may lead to complications and recurrent infections could increase susceptibility. Additionally, variations in sample populations and geographical locations may have influenced the difference in results [51].

This study found that *E. coli* and *K. pneumoniae* isolates were 80% and 73.7% resistant to ciprofloxacin. This is similar to the findings of Mofolorunsho *et al.* [50], who reported resistance rates to ciprofloxacin of 83.3% and 75% against *E. coli* and *K. pneumoniae*, respectively. A low resistance rate was observed to cefepime among *E. coli* isolates but high in *S. aureus*, *P. aeruginosa*, *P. mirabilis*, *K. oxytoca*, and *K. pneumoniae* isolates. A low resistance rate (0-22%) was also observed for amikacin among all isolates. This is similar to the report by Chander and Shrestha [52] who also documented a lower resistance rate to amikacin among *E. coli* (6.7%) and *K. pneumoniae* (12.5%). Lower resistance to amikacin and cefepime observed in this study may be due to limited use compared to other antibiotics.

The main mechanism of beta-lactam resistance in Enterobacteria, especially *E. coli*, is often caused by the production of ESBL or AmpC enzymes [53]. Using a double disc synergy test, this investigation identified 51.4% of the Gram-negative isolates as ESBL-producers, whereas Mofolorunsho *et al.* [50] in a study from Nigeria found 50% of their isolates to be ESBL-producers. The reported high rates are in contrast to earlier studies with prevalence of 5.9% [29] and 12.9% [54]. This study found that 70% of *K. oxytoca*, 47.4% of *K. pneumoniae*, and 20% of *E. coli* were positive for ESBL production, with *K. oxytoca* being the highest ESBL-producer, in contrast to Mofolorunsho *et al.* [50], who found that *E. coli* was the highest ESBL-producer (69% of *E. coli* and 31% of *K. pneumoniae*). This study revealed that 47.4% of *K. pneumoniae* produced ESBLs whereas Chinedu *et al.* [54] found that 12.9% of *K. pneumoniae* produced ESBLs, while Yarima *et al.* [55]

reported a higher percentage (54.1%) in a study from Gombe, Nigeria. The high ESBL presence in this study indicates a potential issue of antibiotic resistance. ESBL-producing bacteria can hydrolyze a broader range of antibiotics, making them difficult to treat. This finding highlights the importance of infection control measures and the need for appropriate antibiotic stewardship to combat the spread of ESBL-producing bacteria.

The incidence of ESBL genes (*bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M}) among Gram-negative bacteria varies by region. The prevalence of ESBL genes (64.9%) was higher in this study than the previously reported prevalence of 45.5 – 54.0% from Gaza [56]. This study found *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M} to be prevalent in 21.1% of *K. pneumoniae* compared to 5.0% for each gene reported by Yarima et al. [56]. While this study found only *bla*_{CTX-M} to be present in 20% of *E. coli* isolates, Yarima et al. [55] found *bla*_{SHV} and *bla*_{CTX-M} to be prevalent in 10% and 5% of *E. coli* isolates, respectively. It also found *bla*_{TEM} to be the most frequently occurring gene (24.3%) of the three, which is comparable to the findings of Iseghohi et al. [29] and Chinedu et al. [54], who found *bla*_{TEM} to be the most prevalent gene (100%). While this study revealed a greater incidence of 20% among *E. coli* isolates, Iseghohi et al. [29] reported a prevalence of 5.9% in *E. coli*. Iseghohi et al. [29] found *bla*_{TEM} and *bla*_{CTX-M} but not *bla*_{SHV} in *E. coli* isolates. A study by Saisi et al. [57] showed co-occurrence of ESBL genes- *bla*_{CTX-M}/*bla*_{TEM} and *bla*_{CTX-M}/*bla*_{SHV} in 9.5% and 2.4% of the isolates, respectively, while our study confirmed co-occurrence of *bla*_{TEM}/*bla*_{SHV} in 2.7% of the isolates. The co-existence of *bla*_{TEM} and *bla*_{SHV} in Gram-negative isolates has also been documented [58]. The disparity in phenotypic and genotypic methods for detecting ESBL can be attributable to the differences in the techniques used to identify ESBL-producing bacteria. Phenotypic methods rely on observing the actual biological activity of enzymes, which is dependent on expression levels and may not always accurately reflect the presence of ESBL genes due to differences in substrate specificity and the presence of non-ESBL beta-lactamases, whereas genotypic methods look for genes associated with ESBL production.

CONCLUSION

The presence of ESBL genes in 64.9% of multi-drug resistant Gram-negative uropathogens is worrisome. The high ESBL content in our study indicates a significant presence of antibiotic resistance among the sampled population. This could potentially impact treatment outcomes and increase the complexity of

managing infections caused by ESBL-producing bacteria. Further research and antibiotic stewardship efforts may be needed to address this growing public health concern.

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