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Prevalence of Enterobacteriaceae causing urine infections

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ABSTRACT **: Antibiotic resistance in Enterobacteriaceae, particularly Extended Spectrum β-lactam resistance, is increasingly dominated by the activation of constantly expressed genes encoding effective drug modifying enzymes. Forty three Gram-negative bacilli isolates were screened for their Extended Spectrum β-lactamase production. They were isolated from different clinical urine samples and identified by standard biochemical reactions. Antibacterial susceptibility testing including disc diffusion method using 13 antibiotics discs including (ceftriaxone, azithromycin, aztreonam, cefotaxime, ceftazidime, clindamycin, streptomycin, norfloxacillin, chloramphenicol, ciprofloxacin, sulphamethoxazole-trimethoprim, and ampicillin/clavulanic acid) was done for all isolates. The antibiotic susceptibility test, disk diffusion method, and double disc synergy test indicated that seven enteric uropathogenic isolates were ESBL producers during the present study. They recorded diameters of inhibition zones as ≤ 18, ≤ 8, ≤ 19, and ≤ 8 mm against cefotaxime (CTX), ceftazidime (CAZ), aztreonam (ATM), and ceftriaxone (CRO). Genotypically, bla_{TEM} genes are the most common, with (100%) occurrence in all five enteric tested uropathogens, followed by bla_{SHV} and bla_{CTX} genes (60%). Analyzing the 16S rRNA sequence confirmed that the most potent ESBL-producing bacteria (U60) isolate were identified as *Escherichia coli* U60.1 and with accession numbers MW173246, in GenBank.**

KEYWORDS: **Antibiotic resistance; Extended-spectrum β-lactamase; Virulence factors; Urinary tract infection; MIC, Minimal Inhibitory Concentration**

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I. INTRODUCTION

Urinary tract infections are of the serious prevalent illness in the wider society and the top causes are enteric bacteria in hospitalized patients (**Sabih and Leslie, 2023**). Some are basic UTIs that can be treated without antibiotics and result in virtually always positive outcomes. Complicated UTIs, on the other side, can result in florid urosepsis, which able to be fatal **(Habak and Griggs, 2022**). The most commonly detected bacteria in UTIs are Enterobacteriaceae, which include *Escherichia. coli, Enterobacter spp* and *Klebsiella pneumoniae* **(Flores-Mireles *et al*., 2015**). Resistance has increased in ESBL-generating infections (**Khety *et al*., 2017**). Antimicrobial resistance (AMR) in livestock bacteria is connected to AMR in bacterial populations that develop and attack humans (**Pruthvishree *et al*., 2018)**. AMR is a popular problem, with resistance in Enterobacteriaceae providing a severe danger to individuals' health.

Colonization is the initial step towards ESBL illness which is more likely to result in unsuccessful therapy and death more than other infections brought on by bacteria that have no ESBLs (**Anesi *et al*., 2018**). *E. coli* is a bacterium that regularly emerges in clinical laboratories. It has the potential to make a variety of problems both within and outside the gastrointestinal tract. It is also the main cause of UTIs (**Hossain *et al*., 2020**).Treatment of UTIs by uropathogenic *E.coli* (UPEC) has become difficult because of rising AMR (**Zangane *et al*., 2021**).

Uropathogenic E.coli strains can colonise and attack the urogenital tract, resulting in infections of the urinary system. Disease development is caused by virulence factors (VFs) of bacteria and host characteristics **(Abd El Ghany *et al*., 2018**). UPEC pathogenesis has been linked to adhesins, invasins, iron-acquisition systems, toxins and systemic resistance methods (**Khairy *et al*., 2019).** VFs can be located on chromosomes or picked up crosswise by transmissible genes like plasmids, transposons, and pathogenicity islands, leading to considerable variation in urine pathogens strains **(Hossain *et al*., 2020**). Ceftriaxone and other third-generation cephalosporins (3GC) are recommended as first-line therapy for a wide range of infections, including severe pneumonia and UTIs (**Malmros *et al*., 2019; Ferreira-Coimbra *et al*., 2020**). ESBLs can hydrolyze the

majority of beta-lactams, including fourth-generation cephalosporins. Antibiotic overuse is believed to assist in the development of antibiotic resistance (**Tamta *et al*., 2020**).

Various gene variations encode ESBL hydrolytic enzymes. The primary groupings are TEM (Temoniera), CTX-M (Cefotaximase-Munich), SHV (Sulfhydryl Variable), and OXA (Oxacillin), which have all been used to detect ESBL genes at the molecular level. Because these genes are typically mobile and situated on plasmids, they are horizontally transmitted (**Ur Rahman *et al*., 2018**). Mobile elements carrying resistance genes for additional drug classes, including sulfonamides, aminoglycosides, and fluoroquinolones, are frequently found in these plasmids. As a consequence, multidrug-resistant bacteria often include these plasmids (**Zeynudin *et al*., 2018**).The effective treatment of infections caused by ESBL-generating bacteria is limited, which contributes to the problem.

In our study we focused on the prevalence of enteric bacteria especially ESBL producing bacteria isolated from lower UTIs, as well as their resistance patterns against commonly used antimicrobial agents, treatment outcomes in patients, and the prevalence of hospital-acquired extended-spectrum beta-lactamases.

1.1. Our contributions to this work are as follows:

i) We investigated 13 antibiotics for antibacterial susceptibility against 43 gram-negative uropathogenic isolates. Our findings revealed a high prevalence of resistance to the majority of antibiotics examined. Amikacin was the best effective antibiotic, with a 74% success rate.

ii) ESBL isolates accounted for 16% of the total, with *E. coli* U60 producing the highest ESBLs. Bla_{TEM} genes are the most prevalent, appearing in all five enteric-investigated uropathogens (100%), then bla_{SHV} and bla_{CTX} genes (60%).

II. MATERIALS AND METHODS

Apparatus

2.1. Collection and characterization of Uropathogens: From November 2017 to September 2018, 66 isolates of uropathogens were detected in urine samples from UTI patients at Zagazig University Hospitals (**Murray *et al*., 2007**).

2.2. Isolation, purification, and identification of isolated bacteria: For enteric bacterial isolation, nutrient agar, blood agar, and MacConky agar media were used. All bacterial isolates were streaked over the appropriate media multiple times in a row (2–5) until pure single colonies had been manufactured. For 24 hours, plates were incubated aerobically at 37°C. The purified bacterial isolates were then kept in saline glycerol. All isolates were kept in a freezer at zero. These uropathogens were identified according to their physical and biochemical characteristics (**Holt *et al*., 1994**). *Escherichia coli* U60 MW173246.1 was obtained from urine of UTI patients at Zagazig University Hospitals, Sharkia, Egypt (**El-Mekkawy *et al*., 2023**).

2.3. Antibiotic susceptibility test: Antibiotic discs were placed on freshly made lawns of each isolate on Mueller-Hinton agar (MHA) plates and incubated for 24 hours at 37 °C, as recommended by the Clinical and Laboratory Standards Institutes (**CLSI, 2012**). The inhibition zones' diameters were measured, and the isolates were categorized using the standard antibiotic disc chart. Standard antibiotic discs have been obtained from 'HiMedia' which includes azteronam (30 mcg), ciprofloxacin (5 mcg), ceftriaxone (30mcg), sulphamethoxazole /trimethoprim (25 mcg), Chloramphenicol (30mcg), ciprofloxacin, norfloxacin (10mcg), amikacin (30mcg), amoxicillin / clavulanic acid (30mcg), azithromycin (15mcg), ceftazidime (30mcg), streptomycin (10mcg), cefotaxime (30mcg) and Clindamycin (10mcg).

2.4. Determining Minimal Inhibitory Concentration of antibiotic against selected bacteria

To determine MICs, a modified approach of **Schwalbe *et al*., (2007)** was employed. The antibiotic Amoxicillin/clavulanic Acid (AMC) stock solution (200/28 mg/5mL) was diluted in 70 mL pure H_2O and added to each tube at varying concentrations (10, 20, 40, 80, and 160 µl). To prepare bacterial suspension, isolated colonies (2-4) from an overnight culture were diluted in nutrient broth to turbidity comparable to a 0.5 McFarland turbidity standard. The MH broth was inoculated with several isolates (10, 27, 52, 60, and 65). The control (MH broth) was taken into account at every stage of the experiment. At 37°C, all tubes were incubated for 24 hours. The lowest concentration that inhibits visible growth is known as the minimum inhibitory concentration (MIC).

2.5. ESBLs production detection

2.5.1. ESBLs production was performed phenotypically using technique of disc diffusion by employing the antibiotics ATM, CTX, CAZ, CRO, and AMC, and the double-disc synergy test was used to confirm it (**CLSI, 2021).**

2.5.2. Molecular detection of ESBLs was performed by searching for (bla_{TEM}, bla_{SHV}, and bla_{CTX}) genes. Isolates no. (U 10, 27, 52, 60, and 65) have been submitted to PCR for genotypic verification of bla_{TEM}, bla_{SHV}, and bla_{CTX} gens. The QIAamp DNA Instructions for the Mini Kit were used to extract the DNA. Tested isolates were submitted to ESBL detection by PCR using the oligonucleotide primer sequences provided in table 1.

Table (1): Critical oligo primers.

	Primer sequences (5'-3')	Length of amplified product	Reference
Bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	**(Colom *et al*., 2003)**
Bla $_{SHV}$	AGGATTGACTG.C.CTTTTTG ATTTGCTGATTTCGCTCG	392 bp	
Bla $_{C.T.X}$	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593 bp	**(Archambault *et al*., 2006)**

III. RESULTS

3.1. Patients with Urinary Tract Infection:

One hundred urine specimens were collected from patients suspected of having a UTIs, 66 (66%) showed substantial growth, confirming the infection. Females (48, 72.7%) were the most common category of UTI patients while males were (18, 27.2 %) only, with the majority being between the ages of 21 and 50 as showed in table (2).

Table (2): Shape, arrangement and Gram stain test of bacterial isolates.

Source of isolation	**Gram positive isolates** No.	%	**Gram negative isolates** No.	%	**Total** No.	%
Male	15	22.72	3	85.3	18	27.27
Female	8	65.4	40	34.6	48	72.72
Total	23	34.8	43	65.15	66	100

3.2. Bacterial pathogens. Bacterial pathogens were 66 isolates collected from 100 patients with probable UTIs. Gram-negative bacteria (65.2%) were the most common, *Escherichia coli* **(26, 60%)** remaining the most common pathogen linked with UTIs across all age categories. *Klebsiella pneumoniae* (8, 18%), *Citrobacter fundi*. (6, 14.0%), and *Pseudomonas aeruginosa* (3, 6.9%) were also isolated from our UTI cases as showed in table (3).

Table (3): Biochemical tests and physical characteristics for identifying tested enteric bacteria isolates.

GROUP	Bacteria species	Total no.	(%)
Group I	***Escherichia coli***	**26**	**60.4**
Group II	*Klebsiella pneumonia*	8	18.6
Group III	*Citrobacter fundi*	6	13.9
Group IV	*Pseudomonas aeruginosa*	3	6.9
Total		43	100

3.3 Antibiotic susceptibility test of pathogenic enteric bacteria: It was observed that among the 43 enteric bacteria isolates , 32 (74.4 %) were susceptible to Amikacin, 26 (60.4%) were susceptible to chloramphenicol, 22 (51.1%) were susceptible to Ciprofloxacillin, 16 (37.2 %) were susceptible to aztreonam, 14 (32.5%) were susceptible to norfloxacillin, azithromycin and ceftriaxone, 11 (25.5 %) were susceptible to sulphamethoxazole-trimethoprim . However most resistance was to clindamycin and ceftazidim 43 (100%) while the least resistance was to Amikacin (0%) as illustrated in figure (1).

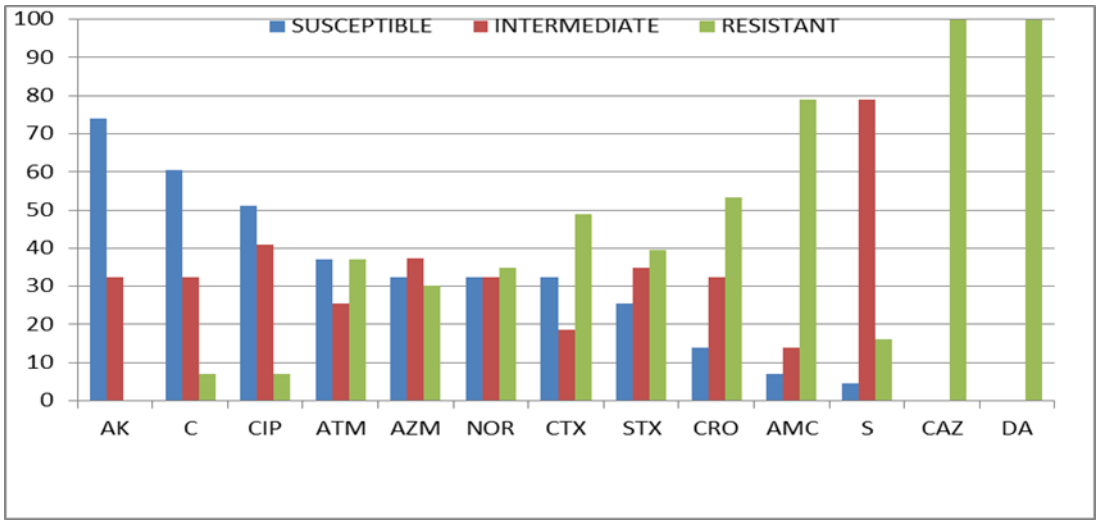

Figure (1): Susceptibility of bacterial isolates against different antibiotics.

3.4 Phenotypically, The disc diffusion method and the double disc synergy tests were used to determine the resistance profile of ESBLs. Table (4) explains the phenotypic detection of tested intestinal bacteria. Isolates displayed diameters of inhibition zones of 19 mm, 18 mm, 8 mm, and 8 mm for ATM, CTX, CAZ and CRO, respectively, according to the data. Only 7 isolates (U 2, 10, 25, 27, 52, 60, and 65) were positive for AMC. As a result, they are supposed to be ESBL producer.

Table (4): ESBL Phenotypic confirmation test

Bacterial isolates no.	ATM	CTX	CAZ	CRO	AMC
2	**5**	**5**	**5**	**5**	**16**
5	5	27	5	5	18
7	5	5	5	5	5
8	5	5	5	5	5
10	**5**	**5**	**5**	**5**	**18**
20	30	27	5	15	5
25	**20**	**6**	**5**	**14**	**14**
26	28	30	5	15	10
27	**17**	**5**	**15**	**5**	**16**
28	31	5	5	18	5
41	5	5	5	5	5
42	27	25	5	18	5
45	28	26	5	13	**5**
47	30	0	5	15	**5**
52	**15**	**16**	**5**	**5**	**15**
54	14	17	5	13	5
55	17	15	5	15	5
56	14	13	5	15	5
57	27	28	5	20	5
59	30	23	5	22	5
60	**5**	**5**	**5**	**5**	**18**
61	18	15	6	5	5
62	5	6	7	5	5
63	25	27	7	18	5
64	24	25	6	23	5
65	**7**	**6**	**6**	**5**	**18**
66	17	5	5	5	5

3.5 ESBL genotypic detection (bla_{TEM}, bla_{SHV}, and bla_{CTX} genes) .The isolates (U 10, 27, 52, 60, and 65) were suspected of ESBLs producing bacteria and were submitted to PCR for genotypic confirmation by looking for the **bla_{TEM}, bla_{SHV}, and bla_{CTX}**. Bla_{TEM} was present in all five isolates. However, as demonstrated in table (5) and figures (2, 3, and 4), isolates no. (U 10, 60, and 65) also exhibited bla_{SHV}, and bla_{CTX}. Furthermore, the data showed that the bla_{TEM} gene was the most common, with 100% incidence in all examined ESBL-producing bacteria, followed by the bla_{SHV}, and bla_{CTX} genes (60%).

Table (5): PCR detection of ESBLs in ESBL-producing bacteria

Bacterial isolate no.	E.S.B.L genes		
	bla_{TEM}	**bla_{SHV}**	**bla_{CTX}**
U 10	+	+	+
U 27	+	-	-
U 52	+	-	-
U 60	+	+	+
U 65	+	+	+
% of occurrence	100	60	60

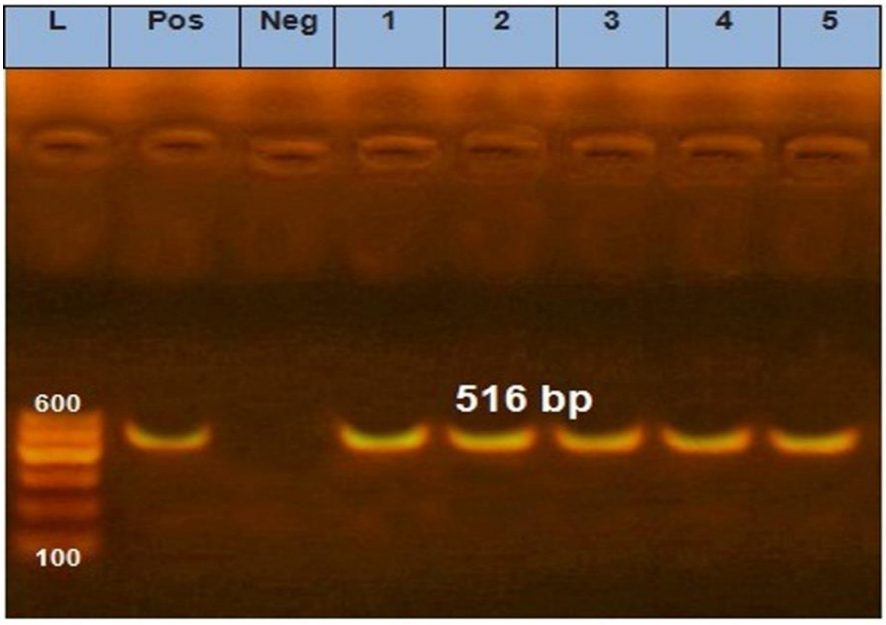

Figure (2): Detection of the ESBL gene bla_{TEM} using PCR. Lane L has the DNA ladder marker 100–600 bp. Pos. and Neg. lanes are for positive and negative controls, Isolate no. 10, 27, 52, 60, and 65 are represented by lanes 1, 2, 3, 4, and 5 respectively.

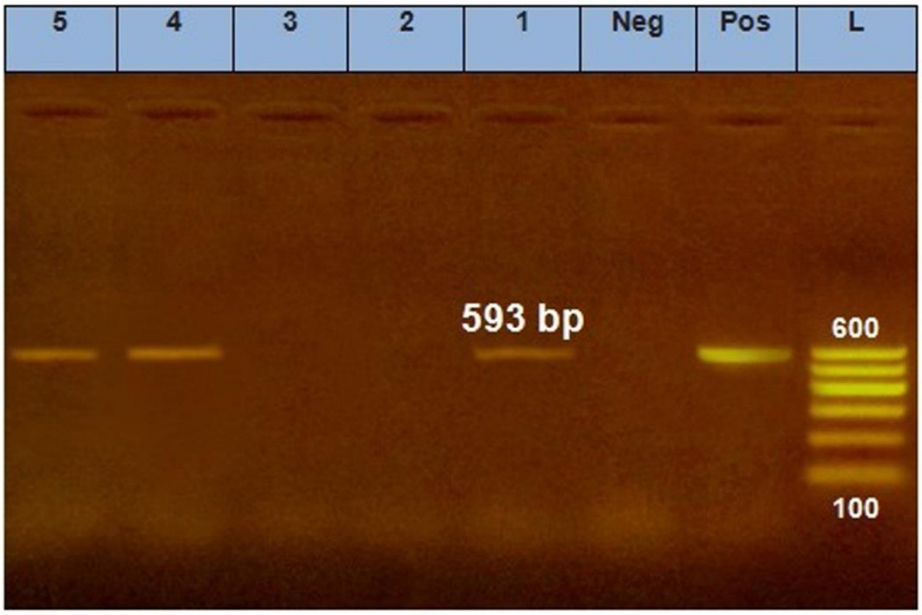

Figure (3): Detection of the ESBL gene blaCTX by PCR.

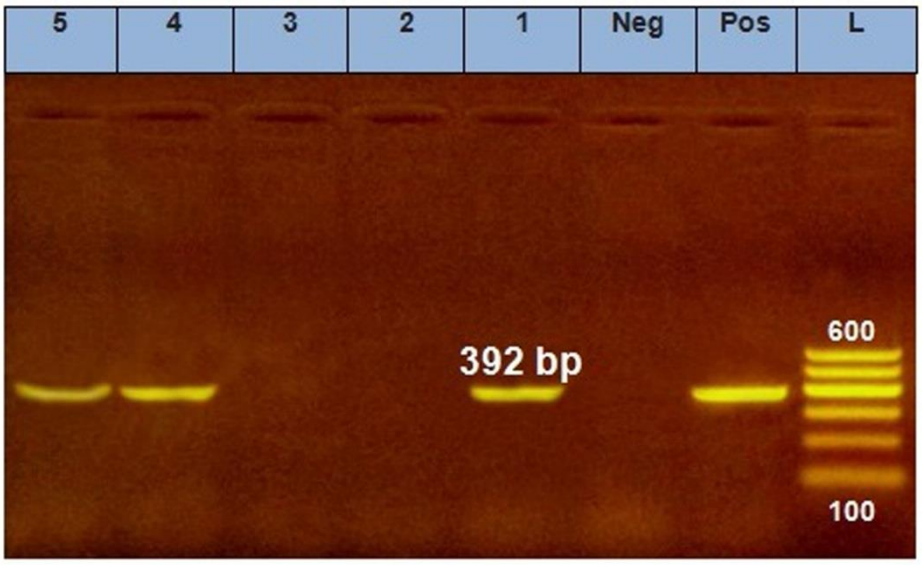

Figure (4): Detection of the ESBL gene blaSHV by PCR.

3.6 Determination of MIC: AMC antibiotic is used for determination of MIC against ESBL producing bacteria by turbidity method. The MIC for all tested strains was at 20 u.

IV. DISCUSSION

ESBLs are a diverse collection of bacterial enzymes carried on plasmids that have been shown to break down and remove a wide range of beta-lactam antibiotics **(El-Mekkawy *et al.*, 2023).** The emergence and spread of Multidrug resistant mutations have reduced number of the available medicines. Current antimicrobial treatments are incapable of curing all infections (**Lynch *et al.*, 2021**). Antimicrobial resistance is associated with increased morbidity and mortality, longer hospital stays, need for critical care, lost work time, and significant economic costs (**Luepke *et al.*, 2017; Bartsch *et al.*, 2017**). The annual mortality toll from antibiotic resistance, which is expected to rise sharply as a threat to world health (**Adzitey, 2020**).In current study there is unique relation between age, gender and type of patients with infections. In similarity **Pandit *et al.*, (2020)** studies approved that. In our study found that females (48, 72.7%) were the most common category of UTIs patients while males were (18,27.2 %) only as previously mentioned by **Pandit *et al.* (2020),** substantially more females

(66.9%) were found to have UTIs, with the majority of them being between the ages of 21 and 50. The prevalence of UTIs varies depending on individual's age, sex, and type.

Gram-negative bacteria (65.2%) were the most prevalent, while *Escherichia coli* (26, 60%) remained the most common pathogen linked with UTIs in all age categories, according to the current study. *Klebsiella pneumoniae* (8, 18%), *Citrobacter* sp. (6, 14.0%), and *Pseudomonas aeruginosa* (3, 6.9%) were also isolated from our UTIs cases. Furthermore, **Gharavi *et al.*, (2021)** discover that *E. coli* was the most prevalent bacterial strain causing UTIs (72.16%), followed by *K. pneumoniae* (10.3%) and *Streptococcus agalactiae* (5.7%).

Moreover, the results showed that Amikacin was highly susceptible to the tested isolates with 74 %, followed by Chloramphenicol. However, most resistance was to Clindamycin and Ceftazidim (100%). A comparable study, approved that ceftazidime (88.9%) and cefepime (82.2%) almost had no effect against *Pseudomonas aeruginosa* strains while the most efficient drugs were imipenem and amikacin; with the lowest percentage of resistance (28.9%).Amikacin's high sensitivity rate may be due its great activity against more resistant gram-negative of the family Enterobacteriaceae (**Endo *et al.*, 2019; Sizar *et al.*, 2022).**

In our study, It was found that (16 %) of bacterial isolates were ESBL producers. *E. coli* (U 25, 27) and 60) (37.5 %) was the predominant, followed by *K. sp* (U 52, 65) (25 %) and then *P .sp* (U 10) 12%. As a similar study by **Erdem *et al.* (2018)** reported that ESBL production was in 29 (32.2%).Other study found that the great percent (62.2%) of the isolates were found to be ESBLs producing bacteria (**Mahazu *et al.*, 2022**). ESBL producing isolates no. U (10, 27, 52, 60, and 65) were subjected to PCR for genotypic confirmation of bla_{TEM}, bla_{SHV}, and bla_{CTX} gens. Bla_{TEM} genes were the most prevalent, appearing in all five enteric-investigated uropathogens (100%), followed by bla_{SHV} and bla_{CTX} genes (60%). Previous studies found that ESBL-producing *E. coli* isolates represented high percent (54.8%) carried bla_{TEM} and bla_{CTX-M} (**Jena *et al.*, 2017**) and in agreement also with **Pandit *et al.* (2020).**

V. CONCLUSIONS

Antibiotic resistance, particularly β -lactam resistance, is increasingly dominated by the activation of continually expressed genes producing efficient drug-modifying enzymes in Enterobacteriaceae. The goal of the present research is to look into ESBL uropathogenic producers' antibiotic resistance. Antibiotic resistance is rapidly spreading, according to the research. Because of the consequently poor precision induced by the high rate of multidrug-resistant drugs, a phenotypic approach to ESBL identification should be developed. The best ESBL detection methods are found using genotypic techniques.

Declaration of competing interests:
None.

Contributors

NEH gathered information, conducted the inquiry, and carried out the practical work. The data were designed and analyzed by RME, WAH, and AAA. The final document was created, written, rewritten, and edited by NEH, RME, and WAH. The final version has been authorized by all authors.

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