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Phenotypic and Molecular Study of Extended-Spectrum β-lactamases Producing Enterobacteriaceae from Urinary Tract Infection in Zakho city, Kurdistan Region-Iraq

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ABSTRACT

The prevalence of ESBLs producing Enterobacteriaceae are increasing locally and globally. This study aimed to investigate the antibiotic susceptibility profile of Enterobacteriaceae isolates causing urinary tract infection and to assess the prevalence of ESBL genes among isolates. A total of 454 urine specimens were collected from outpatients with UTIs from two major hospitals in Zakho city, the Zakho General hospital and Emergency hospital. The enterobacterial isolates were identified using phenotypic and conventional biochemical tests. The antibiotic susceptibility of the isolated organisms was determined using the disk diffusion method, and ESBL production was detected by a double-disk synergy test. The suspected ESBL producers were further confirmed by the amplification of specific primers using PCR assay. Out of 454 specimens, 239 enterobacterial isolates were identified. The most common detected isolates and their rates were: Escherichia coli (65.20 %) and Klebsiella pneumoniae (25.49 %). Imipenem was the most effective antibiotic used while; amoxicillin and ampicillin were the most resistant. The highest level of ESBL production was determined among E. coli isolates (66.3 %) followed by K. pneumoniae (30.43%). The predominant detected genes were both CTX-M and SHV equally with a rate of 90.16% followed by TEM (34.43%). In conclusion, members of Enterobacteriaceae particularly, E. coli and K. pneumoniae are the predominant species causing UTIs. Imipenem was the most effective antibiotic. ESBL producers were high among isolates that were mediated by CTX-M, SHV, and less TEM genes due to the absence of restrictions on antibiotic uses, in addition to their abuse and overuse.

KEYWORDS: Enterobacteriaceae, UTIs, ESBL, TEM, SHV, CTX-M.

1. Introduction

Enterobacteriaceae is a family of gram-negative bacteria, common characteristics of the family members, are rod-shaped, facultative anaerobe, non-spore producing, motile by peritrichous flagella with the exceptions of *Shigella* and *Klebsiella* which are nonmotile, catalase-positive, oxidase negative, with the capability to reduce nitrate to nitrite and produce acid from glucose fermentation (Teklu *et al.*, 2019). Most of the nosocomial and community-acquired infections are caused by bacteria belonging to this family as well as they are the leading cause of urinary tract infections (UTIs), one of the world's most common bacterial infections (Tayh *et al.*, 2019).

Bacterial infections caused by Enterobacteriaceae are treated with β-lactam antibiotics, accounting for over 65% of used antibiotics (Patel *et al.*, 2018). Based on the chemical structure of the β-lactam ring, these antibiotics have been classified into six main groups which include Penicillins, Cephalosporins, Carbapenems, Monobactams, Cephamycins, and β-lactamase inhibitors. Such antibiotics inhibit cell wall synthesis since they play a key role in preventing Penicillin-binding protein (PBP) from working accurately, PBP has a critical role in bacterial cell wall formation and ultimately causes cellular death (Ali *et al.*, 2018).

Bacteria are becoming resistant to β-lactam antibiotics through different mechanisms but the most common is the production of β-lactamases. β-lactamases are enzymes able to inactive β-lactam antibiotics by attaching covalently to the carbonyl portion of β-lactam antibiotics and hydrolyzing the β -lactam ring

leading to β-lactam resistance (Eiamphungporn *et al.,* 2018; Tewari *et al.,* 2018).

Different microorganisms produce different types of β-lactamases, the reported β-lactamases to date include Penicillinases, Extended-spectrum β-lactamases (ESBLs), Cephalosporinases (AmpC), Metallo-β-lactamases (MBLs), and Carbapenemases (KPCs).

ESBLs are enzymes having the capability to hydrolyze a wide range of antibiotics such as the Penicillins, first-, second-, and third-generation cephalosporins, as well as aztreonam but not the cephamycins or carbapenems and, are inhibited by β-lactamase inhibitors such as clavulanic acid (Pitout *et al.,* 2005).

ESBLs are found on plasmids that can be transferred from strain to strain and between different bacterial species (Rupp and Fey, 2003). The level of resistance is not applied only to β-lactams but rather extends to other regularly used antibiotics such as fluoroquinolones, sulphonamides, and aminoglycosides (Schwaber *et al.,* 2005; Chandel *et al.,* 2011). As a result, ESBL-producing enterobacterial isolates are now frequently having a very broad antibiotic resistance extending to multiple antibiotic classes (Rawat and Nair, 2010). Due to the high level of antibiotic resistance profile reported among isolates causing urinary tract infection and the distribution of high level of ESBL producing organisms it was necessary for us to identify the enterobacterial isolates associated with UTI as well as their antibiotic susceptibility pattern toward the prescribed antibiotic and to estimate the rate of ESBL production by the identified bacteria in order to determine the treatment of choice and to implement preventive measures to minimize the distribution of ESBL producing organisms in our locality.

2. MATERIALS AND METHODS

2.1 Specimens Collection and Bacterial Identification

From September to December 2021, a total of 454 urine specimens were collected from outpatients attending Emergency Hospital and Zakho General Hospital. All bacterial cultures were attended to the Microbiology lab at the Biology Department, Faculty of Science, University of Zakho. Primarily urine samples were inoculated on blood agar and MacConkey agar then purified colonies were confirmed by phenotypical and biochemical tests to the species level (Engelkirk and Duben-Engelkirk, 2008).

2.2 Antibiotic Susceptibility Test

All isolates were subjected to antibiotic susceptibility tests through the Kirby-Bauer disk diffusion technique using the CLSI recommendations (Hudzicki, 2009). Sixteen antibiotic disks were used in this study as shown in Table 1. The test preparation, inoculation, storing of antibiotic disks, and reading of the results were according to the manufacturer's recommendations (bioanalyses, Turkey) (CLSI, 2021) (Suravaram *et al.,* 2021).

Table 1: The antibiotics used for testing the susceptibility of isolated bacteria.

Antibiotics	Code	Potency (mcg)
Amoxicillin	AX	25 mcg
Amoxicillin/clavulanic aci	AMC	20/10 mcg
Ampicillin	AM	20 mcg
Cefixime	CFM	5 mcg
Cefotaxime	CTX	30 mcg
Ceftriaxone	CRO	10 mcg
Chloramphenicol	C	10 mcg
Ciprofloxacin	CIP	10 mcg
Norfloxacin	NOR	10 mcg
Gentamicin	CN	10 mcg
Amikacin	AK	10 mcg
Imipenem	IPM	10 mcg
Nalidixic acid	NA	30 mcg
Tetracycline	TE	10 mcg
Trimethoprim	TMP	10 mcg
Trimethoprim/ Sulfamethoxazole	SXT	1.25/23.75 mcg

2.3 Phenotypic Detection of ESBL Production

The isolates that were resistant to all three

cephalosporins were suspected for ESBL production and confirmed by the double-disk synergy test (DDST). Three antibiotic disks including cefotaxime (30 µg), ceftazidime (30 µg), and ceftriaxone (30 µg) were placed 25 mm (center to center) from an Amoxicillin-clavulanic acid disk (30 and 10 µg, respectively), incubated at 37°C overnight (Jarlier *et al.*, 1988).

2.4 Genomic DNA Extraction

Bacterial DNA was extracted from 61 isolates depending on their production of ESBLs by using a commercial Genomic DNA Mini extraction kit (Favorgen/Taiwan), according to the instructions supplied by the company. Following genomic extraction, the NanoDrop Spectrophotometer (Thermo Fisher Scientific) was used for measuring the concentration and the purity of the extracted DNA, and then PCR amplification was done.

2.5 Detection of ESBLs genes by Polymerase Chain Reaction

Genes coding for ESBL production were amplified using three pairs of specific primers provided by Macrogen (Korea). The primer's sequences and the weight of the amplified product are mentioned in Table 2.

Table 2: Primers used for ESBL detection.

Primer	DNA sequence 5'- 3'	Amplified product (bp)	Reference
TEM	F: ATAAAATTCTTGAAGAAGA CGAAA R: GACAGTTACCAATGCTTAA TC	1080	Kim *et al.*, 2006
SHV	F:TCGTTATGCGTTATATTC GCC R:GGTTAGCGTTGCCAGTG CT	861	
CTX-M	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	551	

The amplification reaction was performed in a 20 µl reaction tube consisting of 5 µl of Taq Master mix 2X, 4 µl of (10 pmol/µl) primer (2 µl of forward and 2 µl reverse), 2 µl of DNA template, and 9 µl of deionized distilled water for each of the primers (TEM, SHV, and CTX-M). These primers were used for the detection of the three beta-lactamase genes *bla*TEM, *bla*SHV, and *bla*CTX-M. The PCR condition for each primer is mentioned in Table 3. The amplification was performed using a thermocycles PCR. The PCR cycling protocol for TEM, SHV, and CTX-M are illustrated in Table 3. PCR products were run on 1.5% (w/v) agarose gel dissolved in 100 ml of 1X TBE buffer and stained with RedSafe Dye. The gel was run for 15 min at 45 Volt then raised to 85 Volt for 60 min. A DNA ladder of 100-1500 bps was added as a reference ladder for determining the band size. The gel was viewed using UV Transilluminator to confirm amplification (Ibrahim *et al*., 2020)

Table 3: The amplification condition of different ESBL genes.

PCR setting	Gene	*bla*TEM	*bla*SHV	*bla*CTX-M
	Initial denaturation	94 ºC /5min.	94 ºC /5min.	94 ºC /5min.
Number of cycles (30)	Denaturation	94 ºC /30sec.	94 ºC /30sec.	94 ºC /30sec.
	Annealing	45 ºC /1min.	58ºC /30sec.	58 ºC /1min.
	Extension	72 ºC /1min.	72 ºC /1min.	72 ºC /1min.
	Final extension	72 ºC /10min.	72 ºC /10min.	72 ºC /10min.
Reference	Kim *et al.,* (2006)			

3. RESULTS

In this study 56.17% (255/454) of bacterial isolates were identified from the collected samples of both hospitals, using phenotypic and biochemical tests. Of the identified bacteria, 93.73% (239/255) of the isolates were found to be members of Enterobacteriaceae family (Figure.1), with *Escherichia coli* constituting the highest rate 69.46% (166 /239), followed by *Klebsiella pneumoniae* in 27.20% (65 /239). While other species were reported at low rates as indicated in Figure.1.

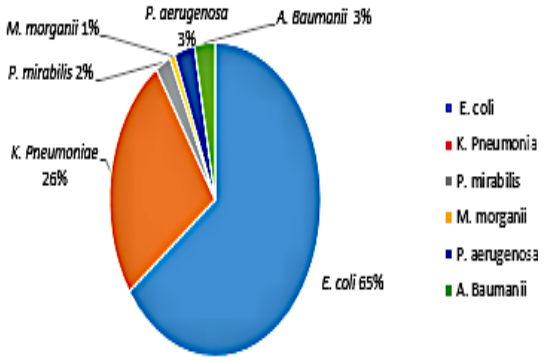

Figure 1: Distribution of Enterobacteriaceae

3.1 Antibiotics Resistance Pattern of Enterobacteriaceae

All the 239 enterobacterial isolates were tested for their antibiotic sensitivity profile against 16 antibiotic disks as shown in Table 4. *E. coli* showed the highest (92.17 %) sensitivity to Imipenem, followed by Chloramphenicol (86.75 %), Gentamicin (77.71 %), and Norfloxacin (63.25 %). While the highest level of resistance was detected against Amoxicillin and Ampicillin equally (87.35 %) for each, followed by amoxicillin/ clavulanic acid (86.14 %) and Nalidixic acid (75.90 %). *K. pneumoniae* showed absolute sensitivity (100 %) to Imipenem, (86.15 %) to Chloramphenicol, (81.54 %) to Gentamicin, and (78.46 %) to both Amikacin and Norfloxacin.

On the other hand, *K. pneumoniae* expressed high resistance (98.46%) to both Amoxicillin and Ampicillin, followed by Tetracycline (69.23 %) and Nalidixic acid (61.54 %).

P. mirabilis showed absolute resistance (100 %) to Amoxicillin, Ampicillin, Trimethoprim/ sulfamethoxazole, and Tetracycline. While the highest level of sensitivity was detected towards Imipenem and Gentamicin (83.33 %).

Furthermore, *M. morganii* isolates showed absolute sensitivity (100 %) toward Ceftriaxone, Amikacin, Gentamicin, Norfloxacin, Ciprofloxacin, Trimethoprim, Imipenem, and Chloramphenicol. While it was (100 %) resistant to Amoxicillin, Amoxicillin/ clavulanic acid, Ampicillin, and Tetracycline.

Table 4: The pattern of antimicrobial susceptibility of enterobacterial isolated from UTI patients

Organisms	*E. coli*		K. pneumoniae		P. mirabilis		M. morganii	
Antibiotics	S %	R %	S %	R %	S %	R %	S %	R %
Amoxicillin	12.65	87.35	1.54	98.46	0	100	0	100
Amoxicillin/ clavulanic acid	13.86	86.14	73.38	24.62	66.67	33.33	0	100
Ampicillin	12.65	87.35	1.54	98.46	0	100	0	100
Cefixime	41.57	58.43	44.62	55.38	66.67	33.33	50	50
Ceftriaxone	40.96	59.04	47.69	52.31	50	50	100	0
Cefotaxime	36.75	63.25	43.08	56.92	50	50	50	50
Amikacin	43.37	56.63	78.46	21.54	66.67	33.33	100	0
Gentamicin	77.71	22.29	81.54	18.46	83.33	16.67	100	0
Nalidixic acid	24.1	75.90	38.46	61.54	33.33	66.67	50	50
Norfloxacin	63.25	36.75	78.46	21.54	50	50	100	0
Ciprofloxacin	42.77	57.22	47.69	52.31	50	50	100	0
Trimethoprim	44.58	55.42	47.69	52.31	16.67	83.33	100	0
Trimethoprime / sulfamethoxazole	42.17	57.83	53.85	46.15	0	100	50	50
Imipenem	92.17	7.83	100	0	83.35	16.67	100	0
Tetracyclin	42.17	57.83	30.77	69.23	0	100	0	100
Chloramphenicol	86.75	13.25	86.15	13.85	50	50	100	0
Total No. tested	166		65		6		2	

3.2 Multi-drug Resistant Isolates Among Enterobacteriaceae

Among the tested isolates to the selected used antibiotics disks, some of them were found to be resistant to at least 3 antibiotics belonging to different antibiotics categories and they were considered multidrug resistance (MDR) as shown in Table 5. The highest level of MDR was detected within *E. coli* isolates since 72.29% (120/166) of the isolates were found to be multi-drug resistant. While 66.15% (43/65) of *K. pneumoniae* isolates were MDR. Regarding *P. mirabilis* and *M. morganii* isolates the rate of MDR was found to be 100 % for both. Furthermore, isolates that showed sensitivity to only one or two classes of the tested antibiotic were considered as extensive drug resistance (EDR). Among *E. coli*, 16.87% (28/166) were EDR. while only 3.08% (2/65) *K. pneumoniae* were found to be EDR. Regarding the total resistance referred to as pan drug resistance (PDR), only one *E.*

coli isolate exhibited resistance to all tested antibiotics.

Table 5: Frequency of MDR, XDR, and PDR of Enterobacteriaceae isolates for selected antimicrobial classes.

`	MDR No. (%)	EDR No. (%)	PDR No. (%)
E.coli (166)	120 (72.29%)	28 (16.87%)	1 (0.60%)
K.pneumoniae (65)	43 (66.15%)	2 (3.08%)	0
P.mirabilis (6)	6 (100%)	0	0
M.morganii (2)	2 (100%)	0	0
Total (239)	171 (71.55%)	30 (12.55%)	1 (0.422%)

3.3 Phenotypic ESBL detection

Figure.2 shows the results of ESBL producers using DDST. They comprised 66.30 % (61/92) positive *E. coli*, 30.43% (28/92) positive *K. pneumoniae,* and 3.26% (3/92) *P. mirabilis.*

Figure 2: Positive double-disk synergy test.

3.4 Molecular Detection of ESBL genes

The obtained results from the molecular detection of *bla* ESBL genes were as follows; TEM was detected in 34.43% (21/61) isolates with a rate of 34.43%, while high rates of both SHV and CTX-M were found in 90.16% (55/61) of the isolates as shown in Figures (3, 4 and 5). It is obvious from these results that the most predominant genes detected were found to be both CTX-M and SHV equally followed by TEM. The distribution of *bla* genes was variable among different bacterial isolates.

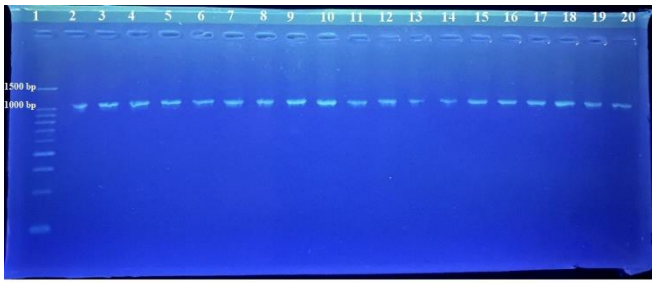

Figure 3: The PCR amplification of TEM gene, using 1.5% agarose gel electrophoresis showing positive bands of 1080 bp. Lane 1 contains a DNA ladder of 100-1500 bp, lane 2-10 *E. coli*, lane 11-19 *K. pneumoniae*, and lane 20 *P. mirabilis*.

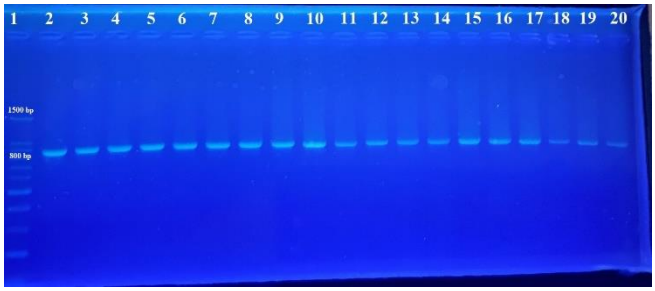

Figure 4: The PCR amplification of SHV gene, using 1.5% agarose gel electrophoresis showing positive bands of 861 bp. Lane 1 contains a DNA ladder of 100-1500 bp, lane 2-10 *E. coli*, lane 11-19 *K. pneumoniae*, and lane 20 *P. mirabilis*.

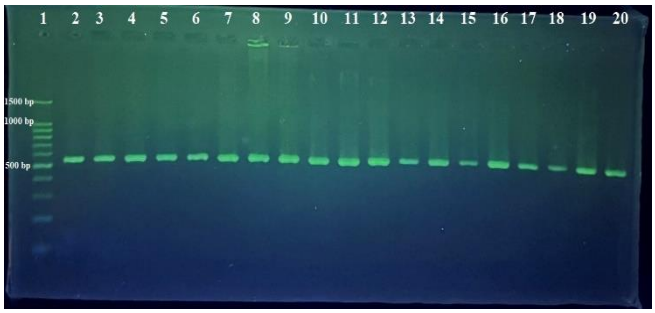

Figure 5: The PCR amplification of CTX-M gene, using 1.5% agarose gel electrophoresis showing positive bands of 551 bp. Lane 1 contains a DNA ladder of 100-1500 bp, lane 2-10 *E. coli*, lane 11-19 *K. pneumoniae*, and lane 20 *P. mirabilis*.

Among *E. coli* isolates, the most frequent gene encountered was SHV at a rate of 86.67% (26/30) of tested isolates. While 80% (24/30) of isolates were found to carry the CTX-M gene, and only 33.33% (10/30) of the isolates harbored the TEM gene. Furthermore, 100% (28/28) of the isolates of *K. pneumoniae* contained both SHV and CTX-M genes and only 35.71% (10/28) of the isolates carried TEM gene.

Whereas all the 3 (100%) of *P. mirabilis* isolates were positive for the CTX-M gene, and only 33.33% (1/3) isolate of TEM, and SHV contained genes.

3.5 Genotypic Patterns

The TEM, SHV, and CTX-M genes in the present study were not detected only as a single gene, but they were found in a combination pattern of two or more genes in the same isolate. The genotypic patterns recorded were as follow; the predominant gene combination was SHV+CTX-M, and they were in 83.61% (51/61) of the isolates. While *K. pneumoniae* was found to be harboring the highest rate 100% of this combination since all of the (28/28) isolates harbored them, followed by 73.33% (22/30) and 33.34% (1/3) among *E. coli* and *P. mirabilis*, respectively. On the other hand, TEM + CTX-M combination occurred in 31.15% (19/61) of all the isolated species with different rates as follows; the highest rate seen in *K. pneumoniae* 35.71% (10/28), followed by *E. coli* with 26.67% (8/30) rate and *P. mirabilis* with (1/3) 33.33%. Furthermore, another coexistence of two genes included the TEM+SHV detected in 29.51% (18/61) of isolates, 35.71% of *K. pneumoniae,* and 26.67% of *E. coli*. Finally, the combination of three ESBL genes (TEM+SHV+CTX-M) was 29.51% (18/61) at rates of 35.71% and 26.67% in *K. pneumoniae* and *E. coli*, respectively.

Table 6: Distribution of bla gene types in ESBL producing isolates.

Patterns of ESBL genotype	*E .coli* n(%)	*Kl. pneumoniae*n(%)	*P. mirabilis* (%)	Total n (%)
TEM only	10 (33.33%)	10 (35.71%)	1 (33.33%)	21 (34.43%)
SHV only	26 (86.67%)	28 (100%)	1 (33.33%)	55 (90.16%)
CTX-M only	24 (80.0%)	28 (100%)	3 (100%)	55 (90.16%)
TEM + SHV	8 (26.67%)	10 (35.71%)	0 (%)	18 (29.51%)
TEM + CTX-M	8 (26.67%)	10 (35.71%)	1 (33.33%)	19 (31.15%)
SHV + CTX-M	22 (73.33%)	28 (100%)	1 (33.33%)	51 (83.61%)
TEM+SHV+CTX-M	8 (26.67%)	10 (35.71%)	0 (%)	18 (29.51%)
Total	30	28	3	61 samples

4. DISCUSSION

Urinary tract infections (UTIs) are very common infectious diseases occurring in a high proportion of the population and are a serious concern in the healthcare system (Moustafa *et al.*, 2018). Members of Enterobacteriaceae are major causative agents of urinary tract infections (Foxman, 2010). In the present study, *E. coli* isolates were the predominant bacteria 65.20%, followed by *K. pneumoniae* (25.49 %) among other Enterobacteriaceae isolated from out-patients in two Zakho hospitals. The present findings are in line with the findings of Abdulrahman (2018) in Duhok city, and Osman (2019) in Erbil city, they stated that *E. coli* was the most common isolate associated with UTI followed by *K. Pneumonia.* Possessing several factors such as adhesion, pili, fimbriae, and P1 genotype receptor for blood group by members of the Enterobacteriaceae family particularly by *E. coli* and *Klebsiella* spp. helps these bacteria to attach to the urothelium and make them major causative agents of UTIs (Shrestha *et al.,* 2016). All the isolated Enterobacteriaceae were tested for their susceptibility against 16 antibiotics that are usually prescribed by physicians in this area. The isolates showed different rates of sensitivity and resistance to these antibiotics, Imipenem was the most effective antibiotic against *E. coli* exhibiting a sensitivity rate of 92.17 %. On the other hand, the most resistant antibiotic tested was penicillin. Similar findings were reported by Ibrahim *et al.* (2020) in Zakho city as they found *E. coli* was 97.16% sensitive to imipenem and resistant to penicillins such as Amoxicillin, Ampicillin, and Amoxicillin/ clavulanic acid with different rates. While *K. pneumoniae* showed absolute 100% sensitivity to Imipenem, and high resistance 98.46% to Ampicillin. Similar results were reported in previous studies conducted by Haji *et al.* (2018) in Erbil city. Of which all isolates 100% were susceptible to imipenem,

while most of the isolates 91.6% showed a high degree of resistance to ampicillin. Regarding *P. mirabilis* 83.33% of the isolates were sensitive to Imipenem, findings higher than the present study were reported by Shabeeb *et al.* (2018) in Holy Karbala province, Iraq. In which *P. mirabilis was* 100% sensitive to Imipenem. Also, high levels of resistance toward Amoxicillin, Ampicillin, and Trimethoprim/ sulfamethoxazole were reported by Khalid and Yassin (2017) for the same species in Duhok city. Whereas *M. morganii* isolates were found to be 100 % sensitive to Imipenem, Amikacin, Ciprofloxacin, and 100% resistant to Amoxicillin, and Ampicillin. Similarly, Auda and Al-Grawi (2009) reported that *M. morganii* isolates were 100 % sensitive to Amikacin and Ciprofloxacin but 100 % resistant to Penicillin and Gentamicin in Baghdad, Iraq.

Overall, of all the tested antibiotics, Imipenem was the most effective against all the isolated enterobacteria, while Penicillins including amoxicillin and ampicillin were the most resistant antibiotics tested. Since *E. coli* which is the causative agent of 90% of community acquired UTIs is becoming resistant to ampicillin and amoxicillin, these two antibiotics are no longer considered reliable antibiotics for the treatment of UTIs (den Heijer *et al.,* 2012; Habeeb *et al.,* 2014). The carbapenems such as Imipenem are known to be stable against ESBL enzymes and are effective in the treatment of infections caused by ESBL-producing bacteria (Sana *et al.,* 2011). In this study, 71.55% (171/239) of tested Enterobacteriaceae, were found to be multidrug resistant and 12.55% (30/239) were found to be EDR. Among the multidrug resistance isolates, *E. coli* was the predominant constituting 72.29% (120/166). Slightly higher findings were reported by Assafi *et al.* (2022) in Zakho city, in which 80.56 % (174/216) of isolated *E. coli* were multidrug resistant. While the magnitude of MDR in *K. Pneumoniae* isolates was observed at a rate of 66.15%. Higher results were reported by Aljanaby and Alhasnawi, (2017) in AL-Najaf province, Iraq, in which 74.41% of *K. pneumoniae* isolates were MDR. The Plasmid-mediated production of beta-lactamase is frequently obtained by the transmission of genetic information between organisms. These transferable plasmids often carry resistant determinants to different antimicrobial agents. As a result, multidrug resistance is expected to be more widespread in organisms that produce ESBL (Livermore, 2001). Regarding ESBL, 38.49% (92/239) of the tested Enterobacteriaceae were ESBL positive, with *E. coli* isolates constituting the highest rate (66.3%) of ESBL-producing isolates followed by *K. pneumoniae* (30.43%). Similar findings were reported by Gharavi *et al.* (2021) who observed that among the ESBL producers, the highest rate was observed in *E. coli* followed by *K. pneumoniae*. Several factors such as species, hospital/ward, geographic region, infection type, patient group, and extensive antibiotic usage affect the incidence of ESBL-producing isolates (Shakya *et al.,* 2017; McNulty *et al.,* 2018).

The results obtained from the molecular detection of the three ESBL genes showed that the predominant gene detected among all enterobacterial species in this study were both SHV and CTX-M with equal rates of 90.16% and the least detected was TEM with 34.43%. Regarding *E. coli*, isolates, the gene SHV was the predominant type with a rate of 86.67% followed by CTX-M and TEM with rates of 80.0% and 33.33%, respectively. In contrast, Polse *et al.* (2016) reported that CTX-M genes were the most dominant genes (87.2%) in *E. coli* isolates while TEM and SHV genes were less dominant (54.5% and 21.8%), respectively in a study conducted in Zakho city. In *K. pneumoniae* however, both SHV and CTX-M were found to be 100% predominant, followed by TEM (35.71%). Whereas Al-Hashimy and Al-Musawy (2020) in Najaf, Iraq observed that the presence of ESBL genes in *K. pneumoniae* isolates were 65.8% of the ESBL genotypes expressed SHV genes followed by 52.6% TEM and

42.1% for CTX-M. In the case of *P. mirabilis* according to the present study results CTX-M was 100% the predominant gene, while the other two genes (SHV and TEM) were less common (33.33%). These findings are close to the results reported by Khalid and Yassin (2017) in which CTX-M was also the predominant gene with a rate of 81%, followed by TEM (57%) and SHV (24%). The present results showed that the prevalence of ESBL-producing Enterobacteriaceae are increasing compared to earlier studies. As well as the members tested are showing a high level of multidrug resistance to most prescribed antibiotics used to treat UTIs leaving behind a limited treatment choice.

5. CONCLUSION

Members of Enterobacteriaceae family are major causative agents for community acquired UTIs. *E. coli* was the most predominant bacteria isolated from urine specimens of patients with UTIs followed by *K. pneumonia*. The isolated bacteria were resistant to most used antibiotics with imipenem being the most effective. ESBL genes were predominant among all enterobacterial species in this study notably SHV and CTX-M with less TEM due to the overuse and abuse of antibiotics in the absence of guidelines and proper instruction regarding antibiotic consumption.

6. REFERENCE

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