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Investigation of *OXA-23*, *OXA-24*, *OXA-40*, *OXA-51*, and *OXA-58* Genes in Carbapenem-Resistant *Escherichia coli* and *Klebsiella pneumoniae* Isolates from Patients with Urinary Tract Infections

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Abstract

Background: *Escherichia coli* and *Klebsiella pneumoniae* are frequently responsible for urinary tract infections (UTIs). The high rate of carbapenem resistance in *Enterobacteriaceae* has become a global therapeutic concern.
Objectives: The study investigated *OXA-23*, *OXA-24*, *OXA-40*, *OXA-51*, and *OXA-58* genes in uropathogenic *E. coli* and *K. pneumoniae* isolates.
Methods: We isolated 500 uropathogenic isolates of *E. coli* and *K. pneumoniae* from patients at Milad Hospital, Tehran, Iran. Antibiotic susceptibility testing was performed using a strip-test method, and the carbapenem-nonsusceptoble isolates were confirmed with an automated antibiotic sensitivity testing system. The OXA genes were determined by multiplex PCR. Molecular typing was performed by multilocus variable-number tandem repeat (VNTR) analysis (MLVA).
Results: Out of 500 isolates, 40 (8%) were detected as carbapenem-resistant, including 13 *E. coli* and 27 *K. pneumoniae*. All carbapenem-resistant isolates were ESBL-producing and resistant to ceftriaxone, ciprofloxacin, meropenem, ceftazidime, and amoxicillin-clavulanate. Moreover, 46.1% and 26% of carbapenem-insensitive *E. coli* and *K. pneumoniae* isolates carried a beta-lactamase-producing gene associated with the OXA-23-like group. Finally, *E. coli* and *K. pneumoniae* isolates were divided into two and three MLVA patterns, respectively.
Conclusions: This is the first report of OXA-51, 58, and 24 carbapenemases in clinical isolates of *E. coli* and *K. pneumoniae* from UTI patients in Iran. Significant differences were seen in OXA-51, 58, and 24 genes between carbapenem-insensitive and carbapenem-sensitive *E. coli* and *K. pneumoniae* isolates. Molecular typing suggested the vertical transmission of resistance genes.

Keywords: *Escherichia coli*, *Klebsiella pneumoniae*, Carbapenem, *OXA* Group Genes

1. Background

The most common bacteria causing urinary tract infections (UTI) are *Escherichia coli* and *Klebsiella pneumoniae* (1). *Escherichia coli* is responsible for more than 85% of all UTIs in hospital and community settings (2). Based on statistics, 150 million people suffer from UTIs each year worldwide. Urinary tract infections occur about 30 times more frequently in females than males, and nearly 60% of them are infected with UTIs at least once in their lifetime (3). Indiscriminate use of antibiotics is a contributing factor that often leads to multi-drug-resistant strains. The remarkable ability of *E. coli* and *K. pneumoniae* to obtain plasmids, integrons, or transposons with clusters of resistant genes can result in multidrug resistance (MDR). The most severe medical concern is the control of MDR because the current therapeutic choices are limited. The resistance to carbapenems increased sharply in the past decade (4, 5).

The high level of carbapenem-insensitive Gram-negative bacteria has become a concern worldwide (6). Carbapenem resistance can be attributed to one of the mechanisms causing resistance in bacteria, such as the inactivation of enzymes, overproduction of efflux pumps, and modification of target sites. Nevertheless, among the reported mechanisms causing carbapenem resistance, the most important mechanisms are carbapenem-hydrolyzing-β-lactamases associated with the metallo-β-lactamases (Ambler class B) and oxacillinases (Ambler

class D). The ability to hydrolyze oxacillin can be an effective reaction (7, 8). The appearance of carbapenem-hydrolyzing class D β-lactamases depends on antimicrobial chemotherapy, particularly carbapenems. Major subgroups of acquisitive carbapenem-hydrolyzing class D β-lactamases include OXA-23-like, OXA-40-like, OXA-51-like, and OXA-58-like β-lactamases (9). The OXA-51-like lactamase has been discovered worldwide, e.g., in Argentina, Austria, England, France, Greece, Kuwait, Romania, Spain, Scotland, and Turkey (10).

El-Badawy et al. studied OXA-23, and OXA-51 β-lactamases in carbapenem-resistant *K. pneumoniae* isolates in Egypt and found that 5.3% and 10.5% of the isolates could produce OXA-23 and OXA-51 β-lactamases, respectively (11). In Cetinkol et al.'s study, OXA-23-like, OXA-40-like, OXA-51-like, and OXA-58-like β-lactamases were not observed in carbapenem-resistant *K. pneumoniae* isolates (12). Manohar et al. studied carbapenem resistance genes in Gram-negative bacteria in India and found the uncommon attendance of *OXA-23* in *E. coli* (n = 4) and *OXA-23* and *OXA-51* in *K. pneumoniae* (13).

2. Objectives

This research examined the antimicrobial susceptibility patterns and OXA-23, OXA-24, *OXA-40*, *OXA-51*, and *OXA-58* genes in clinical strains of *E. coli* and *K. pneumoniae*.

3. Methods

3.1. Isolates and Antimicrobial Susceptibility Tests

We collected 500 urine isolates from patients with UTI indications hospitalized at a public hospital in Tehran from 2019 to 2020. The reconfirmation of the isolates was performed by standard biochemical methods. The whole isolates were maintained at -80°C in trypticase soy broth with 15% glycerol for further processing. The minimum inhibitory concentration (MIC) was determined by the E-test method (Liofilchem® MIC Test Strips). The MIC was performed for ceftazidime (CAZ), ceftriaxone (CRO), meropenem (MEM), ciprofloxacin (CIP), piperacillin/tazobactam (TZP), gentamicin (GEN), amoxicillin-clavulanate (AMC), and trimethoprim/sulfamethoxazole (SXT). The data were interpreted as resistant, intermediate, and susceptible, conforming to the M100-CLSI 2021 instructions (14).

3.2. Confirmation of Carbapenemase Activity

The isolates were tested for carbapenemase activity with Carba-NP according to the CLSI guidelines and manufacturer instructions. The color change of the trial vial to perfect yellow or red-yellow confirmed carbapenem-resistant isolates, while the control vial stayed ruddy (15).

3.3. Recognition of OXA Group Genes

The genomic DNA of isolates was extracted by a High Pure PCR Template Preparation kit (Roche, Germany). Recognition of *OXA* group genes was done by formerly defined particular primer sets (Metabion, Germany) shown in Table 1. According to the manufacturer's guidelines, the DNA amplification by PCR was done using a Peqlab PCR thermal cycler and PCR Master Mix (Ampliqon Inc., Denmark). The experiment was done in a final volume of 25 μL. The reaction mix contained 12 μL of Master Mix Red (Ampliqon Inc., Denmark) and 1 μL of template DNA. Forward and reverse primers were adjoined according to Table 1. The total volume of the reaction mixture was adjusted to 25 μL. Primary denaturation was regulated at 95°C for 5 min, followed by 30 cycles of 94°C for 25 s, the ideal annealing temperature per gene for 40 s, and 72°C for 50 s. Afterward, the final extension was done at 72°C for 5 min. Amplicons of PCR were separated by 1.5% w/v agarose gels (16).

Table 1. Primer Sequences for OXA Group Genes

Studied Genes	Annealing Temperature °C	Amplicon Size	Reference
$Oxa_{51\text{-like}}$	53	392	(16)
F: TAATGCTTTGATCGGCCTTG			
R: TGGATTGCACTTCATCTTGG			
$Oxa_{23\text{-like}}$	53	465	(16)
F: GATCGGATTGGAGAACCAGA			
R: ATTTCTGACCGCATTTCCAT			
$Oxa_{24\text{-like}}$	53	668	(16)
F: GGTTAGTTGGCCCCCTTAAA			
R: AGTTGAGCGAAAAGGGGATT			
$Oxa_{58\text{-like}}$	53	404	(16)
F: AAGTATTGGGGCTTGTGCTG			
R: CCCCTCTGCGCTCTACATAC			

3.4. Multi Locus Variable-Number Tandem Repeat Analysis Technique in Escherichia coli

The overnight cultures of the isolates were applied for preparing total genomic DNA utilizing High Pure PCR Template Preparation kits (Roche, Germany). The multilocus variable number of tandem repeat analysis was performed utilizing seven loci (*CVN001*, *CVN002*, *CVN003*,

CVN004, *CVN007*, *CVN014*, and *CVN015*) as previously explained by Lindstedt et al. (17). The previously published primers (17) were used for multi locus variable-number tandem repeat (VNTR) analysis (MLVA) (Table 2). The loci were multiplied by PCR and assessed with 3% agarose.

The numbers of VNTR repeats per locus were assessed by the following equation (17): (NPS - OF)/RL, wherever PS = product size, OF = offset region (region of sequence not containing repeat), and RL = length of one repeat unit. CVN001: ((PS) - 250)/39, CVN002: ((PS) - 272)/18, CVN003: ((PS) - 404)/15, CVN004: ((PS) - 231)/15, CVN007: ((PS) - 314)/18, CVN014: ((PS) - 111)/6, and CVN015: ((PS) - 189)/6 (17, 21). The numbers of VNTR repeats were rounded to the closest whole repeat.

3.5. Multi Locus Variable-Number Tandem Repeat Analysis Technique in Klebsiella pneumoniae

Klebsiella pneumoniae MLVA was performed using eight tandem sequence repeats, including *VNTR10*, *VNTR27*, *VNTR45*, *VNTR51*, *VNTR52*, *VNTR53*, *VNTR58*, and *VNTR60*, as described by Lindstedt et al. (22). Previously published primers were used for MLVA analysis (Table 2). The loci were multiplied by PCR and assessed with 3% agarose. The VNTR repeat numbers for each locus were calculated (13): (NPS - OF)/RL. These allele definitions were applied to analyze the clinical isolates using the MLVA plugin of the BioNumerics program (Version 6.6, Applied Maths, Sint-Martens-Latem, Belgium) (21).

3.6. Data Analysis Method

We utilized SPSS v.22 (SPSS Inc., Chicago, IL, USA) for statistical analysis. A P value < 0.05 was assumed statistically significant.

4. Results

We collected 500 urinary bacterial isolates of *E. coli* and *K. pneumoniae*. Women had more urinary tract infections (190 cases of *K. pneumoniae* and 150 cases of *E. coli*) than men (80 cases of *K. pneumoniae* and 80 cases of *E. coli*). The UTIs with *E. coli* were most prevalent in 70 - 79-year patients, while those with *K. pneumoniae* were most prevalent in 51 - 59-year patients.

Out of 500 isolates, 40 (8%) were carbapenem-resistant, including 13 E. coli and 27 *K. pneumoniae*. Forty carbapenem-resistant isolates were used for the present study. The most common UTIs were observed in the 40 - 49-year age group (Figure 1). Interestingly, men had more UTIs (23 cases) than women (17 cases) (Figure 2).

4.1. Antimicrobial Susceptibility Testing

As shown in Figure 3, 60.6%, 58%, and 51.5% of *E. coli* isolates were resistant to SXT, CRO, and CIP, respectively. Also, 3% and 6.5% of *E. coli* isolates were resistant to MEM and TZP, respectively (Figure 3A). Also, 47.2%, 40.1%, 39.2%, and 36.4% of *K. pneumoniae* isolates were non-sensitive to CRO, CAZ, SXT, and AMC, respectively. Moreover, 10% and 12.3% of *K. pneumoniae* isolates were resistant to MEM and TZP, respectively (Figure 3B).

All carbapenem-resistant *E. coli* and *K. pneumoniae* isolates were ESBL-producing and resistant to CRO, CIP, MEM, CAZ, and AMP. Moreover, 33.3% of carbapenem-resistant *E. coli* isolates were susceptible to SXT, and 25%, 8.3%, and 4.2% of carbapenem-resistant *K. pneumoniae* isolates were susceptible to SXT, GEN, and TZP.

4.2. Frequency of OXA Group Genes in Escherichia coli and Klebsiella pneumoniae Isolates

In this research, 100 (43.4%) *E. coli* and 81 (30%) *K. pneumoniae* isolates contained a gene producing β-lactamases of the OXA-23-like group. In addition, nine (3.9%) and seven (2.6%) of the above isolates carried a gene encoding OXA-51-like enzymes, respectively. However, OXA-24, OXA-40, and OXA-58-like were not found in sensitive *E. coli*, and *K. pneumoniae* isolates.

Figure 4 summarizes the presence of the OXA group genes encoding carbapenemases in carbapenem-resistant *E. coli* and *K. pneumoniae*, including OXA-23, OXA-24, OXA-40, OXA-51, and OXA-58-like groups, indicating that six (46.1%) and seven (26%) carbapenem-insensitive *E. coli* and *K. pneumoniae* isolates possessed a gene producing β-lactamase of the OXA-23-like group. Moreover, one (7.7%) and three (23.1%) of the carbapenem-insensitive *E. coli* isolates had a gene encoding OXA-24-like and OXA-51-like enzymes, respectively. Five (18.5%) and three (11.1%) of the carbapenem-insensitive *K. pneumoniae* isolates had a gene coding OXA-24-like and OXA-51-like carbapenemases, respectively.

The OXA-40-like enzyme was not found in the mentioned isolates. One (7.7%) and four (14.8%) carbapenem-insensitive *E. coli* and *K. pneumoniae* isolates had a gene encoding β-lactamase belonging to OXA-58-like enzymes (Figure 5). Also, OXA-23, OXA-24, and OXA-51-like and OXA-23 and OXA-58-like were simultaneously found in two (15%) *E. coli* isolates. Also, the co-existence of OXA-23 and OXA-51-like, OXA-23 and OXA-58-like, and OXA-23 and OXA-24-like was observed in three (11.1%) *K. pneumoniae* isolates. Different carbapenems MIC ranges were observed in *E. coli*, and *K. pneumoniae* isolates harboring the OXA-58-like gene, and these isolates showed higher carbapenem MIC ranges.

Table 2. Primer Sequences for MLVA Technique for *Escherichia coli* and *Klebsiella pneumoniae*

Studied Loci	Annealing Temperature (°C)	Reference
CVN001	50	(17, 18)
F: AACCGGCTGGGGCGAATCC		
R: GGCGGCGGTGTCAGCAAATC		
CVN002	50	(17, 18)
F: AACCGTTATGAARGRAAGTCCT		
R: TCGCCCAGTAAGTATGAAATC		
CVN003	50	(17, 18)
F: AAAAATCCGGATGAGWTGGTC		
R: TTGCGTTGTCAGTAATTTGTTCAG		
CVN004	50	(17, 18)
F: MGCTGCGGCRCTGAAGAAGA		
R: CCCGGCAGGCGAAGCATTGT		
CVN007	50	(17, 18)
F: ACCGTGGCTCCAGYTGATTTC		
R: ACCAGTGTTGCGCCCAGTGTC		
CVN014	50	(17, 18)
F: TCCCCGCAATCAGCAAMACAAAGA		
R: GCAGCRGGGACAACGGAAGC		
CVN015	50	(17, 18)
F: TAGGCATAGCGCACAGACAGATAA		
R: GTACCGCCGAACTTCAACACTC		
VNTR10	58	(19, 20)
F: AGCGCGCAGACGATGAGCAG		
R: AGCCCCGCAGTGGGGTTACT		
VNTR27	58	(19, 20)
F: CAGCGTCAGCGCCAGACCAA		
R: CCATGGCCGGCCTGTGGTTT		
VNTR45	58	(19, 20)
F: CGCTGACACATTGACGAAAACAGAGA		
R: ATGAATATTGCCCAGTTTCTGGAACAA		
VNTR51	58	(19, 20)
F: CCGCCGCGCCATCGTTAGAT		
R: TCAACGCGCCCAGCTGAACC		
VNTR52	58	(19, 20)
F: TTTGGCGGCAGCGGTTTCCC		
R: GCCAGAAAAAGGCGCGCAGC		
VNTR53	58	(19, 20)
F: CGCAGAAGAAAGCGGAAG		
R: TGTTTTAGGCGCATTCTTACC		
VNTR58	58	(19, 20)
F: CTATCTGGCGAACCAGACG		
R: ATTATGACGGGCGATATAATAGGC		
VNTR60	58	(19, 20)
F: CGGTACGAATCTGTTGGATTAAG		
R: GGCCTTCTTCCGGGTCTAT		

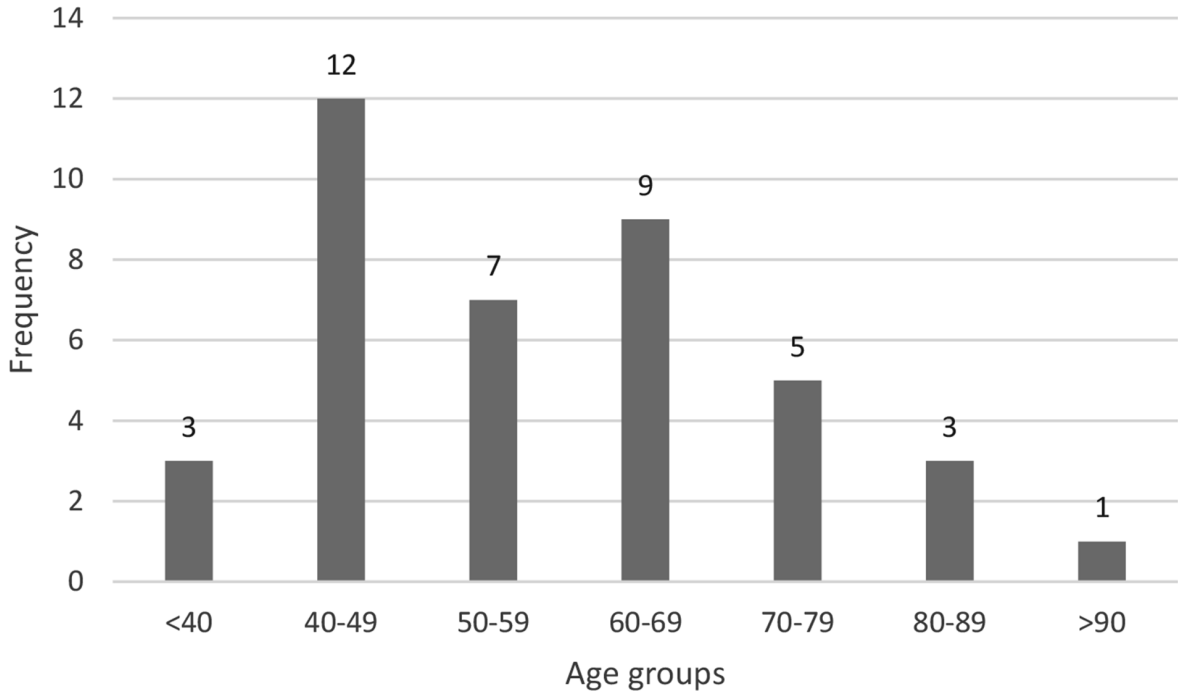

Figure 1. Distribution of urinary tract infections by gender

4.3. Molecular Typing of Carbapenem-Resistant Klebsiella pneumoniae and Escherichia coli

A dendrogram was formed based on MLVA for both strains using BioNumerics software ver.6.6. Figures 6A and B show that *K. pneumoniae* isolates were divided into three MLVA patterns and a singleton (Figure 6A), while *E. coli* isolates displayed two MLVA patterns (Figure 6B). According to the dendrograms, the resistance profile exhibited no significant relationship with MLVA patterns in both strains of *K. pneumoniae* and *E. coli*.

5. Discussion

In this study, in both genders and all age groups, the most common bacteria were *E. coli* and *K. pneumoniae*. The most prevalent UTIs were observed in 40 - 49 years of age group. This is probably because most patients in this sample were referred to the hospital for remedy. In a study of *E. coli* isolates from urine specimens in Egypt, resistance to antibiotics such as ampicillin, amoxicillin, cephalexin, and chloramphenicol was 100%. Moreover, resistance to trimethoprim-sulfamethoxazole and imipenem was 45.7% and 10.64%, respectively (23). The rates of antimicrobial resistance were slightly different. In the present study, 3% and 60.6% of *E. coli* isolates were resistant to meropenem and trimethoprim-sulfamethoxazole, respectively. Therefore, the location and time of study could have affected the pattern of antibiotic resistance and antibiotic use (16, 24-30). In a study of positive urine cultures for *K. pneumonia* in Pakistan, high resistance rates to ampicillin (100%) and trimethoprim-sulfamethoxazole (93%) were found. Also, above 85% of isolates were sensitive to carbapenem antibiotics (29). In this research, the sensitivity rate to SXT (60.8%) and MEM (83.1%) was above 60%. In addition, in this research, the sensitivity to MEM and SXT was shown to be declining, and their management must be performed with caution.

Gomes et al. detected that the multidrug resistance rate in *E. coli* and *K. pneumoniae* isolated from UTI cases was about to 86% (31). Besides, *E. coli* isolates displayed resistance rates of 85%, 72%, 84%, and 50% to ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, and gentamicin, respectively, while *K. pneumoniae* isolates showed the insensitivity rates of 100% to ampicillin, 54% to SXT, 54% to CIP, and 27% to GEN. Also, *E. coli* and *K. pneumoniae* isolates were 100% susceptible to imipenem. In our study, resistance rates of *E. coli* strains were 60.6% to SXT, 51.5% to CIP, and 33% to GEN, and *K. pneumoniae* isolates showed the insensitivity rates of 33.1%, 39.2%, and 26.5% to ciprofloxacin, trimethoprim-sulfamethoxazole, and gen-

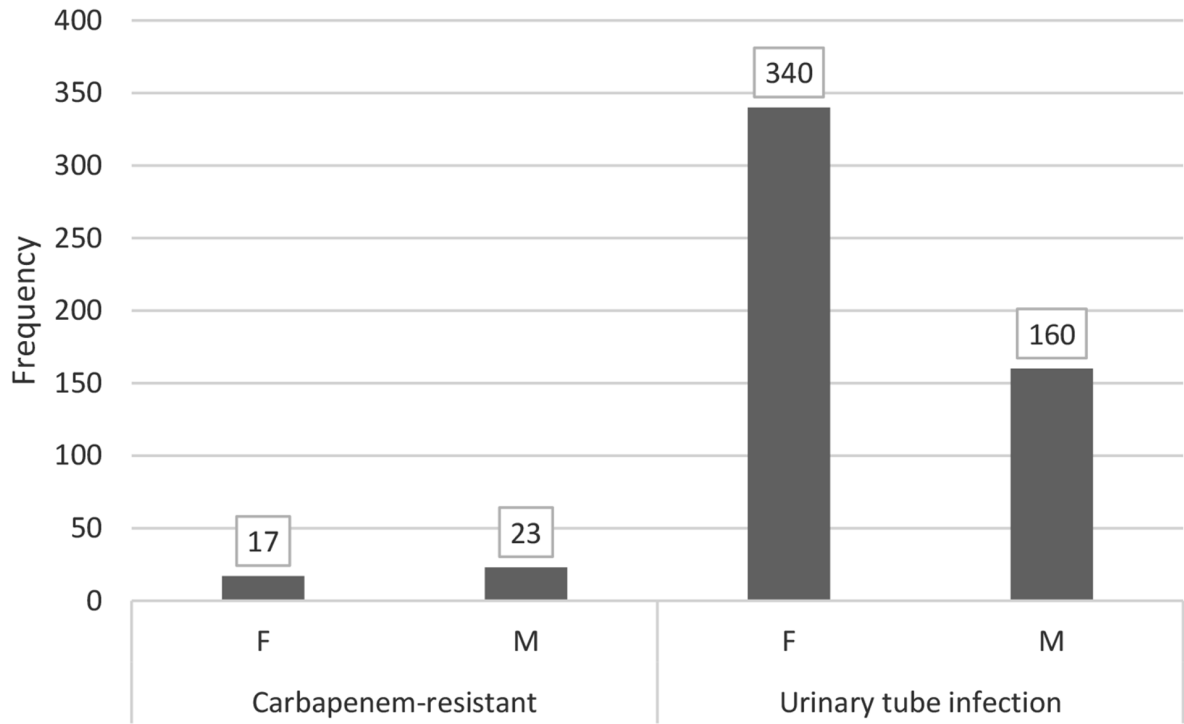

Figure 2. Distribution of carbapenem-resistant cases within age groups

tamicin, respectively. The susceptibility of *E. coli* and *K. pneumoniae* strains was 95.7% and 83.1% to meropenem as a representative of carbapenems, respectively. In Gomes et al.'s study, the resistance rates of both strains to trimethoprim-sulfamethoxazole and ciprofloxacin were more than those of our study (31). A survey on 7,098 *E. coli* positive cultures was done by Oteo et al. (32) in Spain that reported high resistance rates to trimethoprim-sulfamethoxazole (32.6%) and ciprofloxacin (19.3%). In our study, *E. coli* isolates showed higher resistance rates to trimethoprim-sulfamethoxazole and ciprofloxacin.

Comparing our results with the mentioned research indicates that diverse antibacterial resistance rates in the third world can depend on the irrational use of antimicrobial drugs, sampling biases, geographic variations, social factors, and patient characteristics. The carbapenem resistance among *E. coli* and *K. pneumoniae* isolates is a common challenge in Iran. Increasing resistance to the mentioned antibacterial drugs has made the remedy of different UTIs created by *E. coli* and *K. pneumoniae* problematic. Our study evaluated the relationship of the non-susceptibility rates of *E. coli*, and *K. pneumoniae* isolates to OXA-23-like, OXA-24-like, OXA-40-like, OXA-51, and OXA-58-like genes in Tehran. Based on the findings, all carbapenem-resistant strains were ESBL-producing and non-susceptible to CRO, CIP, MEM, CAZ, and CTX. These antibiotics can be helpful in the treatment of UTIs caused by *E. coli* and *K. pneumoniae* isolates in hospital settings.

The carbapenem resistance is mainly due to creating two β-lactamases: MBLs enzymes and oxacillinases (33). Zowawi et al. showed that the main carbapenemases in *Acinetobacter baumannii* (30) were carbapenem-hydrolyzing class D β-lactamases. Therefore, carbapenem-hydrolyzing class D β-lactamases were studied for *E. coli*, and *K. pneumoniae* isolates in this study. This study showed oxacillinases such as OXA-23, OXA-24 in *E. coli* and OXA-23 and OXA-51 in *K. pneumoniae* are more common in Iran. Some research has evaluated OXA group genes in *E. coli* and *K. pneumoniae*. The results of our study are consistent with the above research. Although the capability of OXA-51 and OXA-23 to hydrolyze carbapenem antibiotics is weak, the addition of the ISAba1 element upstream of the blaOXA-51/23-like gene can weaken the sensitivity rate to carbapenem antibiotics in *Enterobacteriaceae* (34). Budak et al. found the OXA-51-like gene in *K. pneumoniae* isolates in hospital samples (35). El-Hendawy et al. showed that 73.68% (14/19), 10.53% (2/19), and 21.05% (4/19) of carbapenem-insensitive *K. pneumoniae* isolates

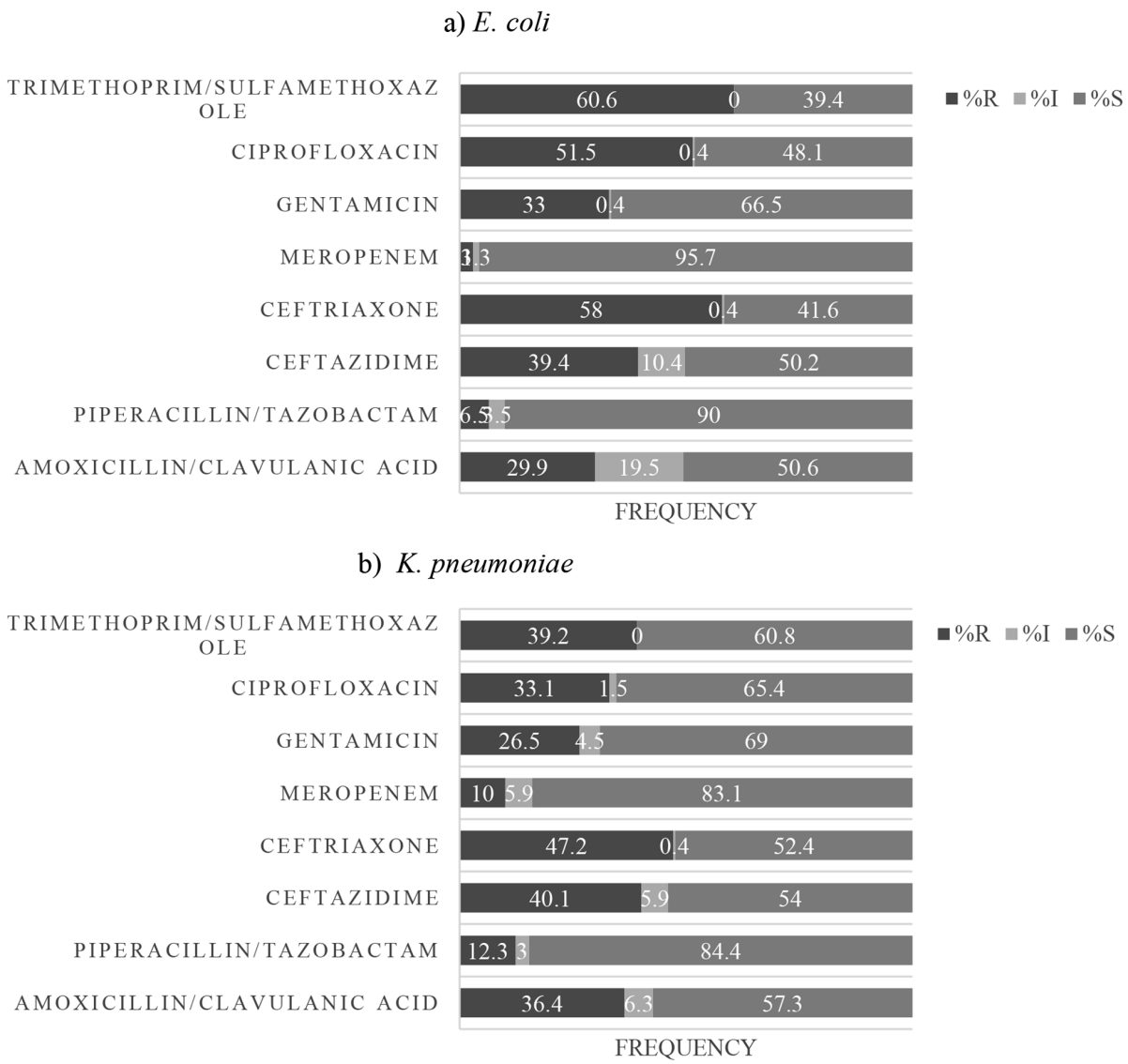

Figure 3. Antimicrobial susceptibility results in isolates (A) *Escherichia coli* and (B) *Klebsiella pneumoniae*

from hospital specimens in Egypt had *OXA-48*, *OXA-51*, and *OXA-181*, respectively (23).

We found significant differences in OXA group genes between carbapenem-resistant and carbapenem-susceptible *E. coli* and *K. pneumoniae* isolates. However, these differences were insignificant for the OXA-23 gene ($P > 0.05$). A significant association was observed between OXA group genes in *E. coli* and *K. pneumoniae* and carbapenem resistance ($P < 0.05$). Also, *E. coli* and *K. pneumoniae* isolates harboring the OXA-58-like gene showed higher carbapenem MIC ranges. Higher carbapenem MIC ranges indicate the role of OXA-58-like in reducing carbapenem susceptibility in the studied isolates. The OXA enzymes may become extensive quickly among Gram-negative bacteria. These gene epidemics result in the distribution of plasmids, transposons, and integrons among bacterial species. Due to the ability of integrons for the recruitment, spread, and expression of resistance genes, integrons are disseminated among Gram-negative bacteria (34, 36). Chromosomal intermediation of blaOXA-23 has been formerly demonstrated for *Proteus mirabilis* (37).

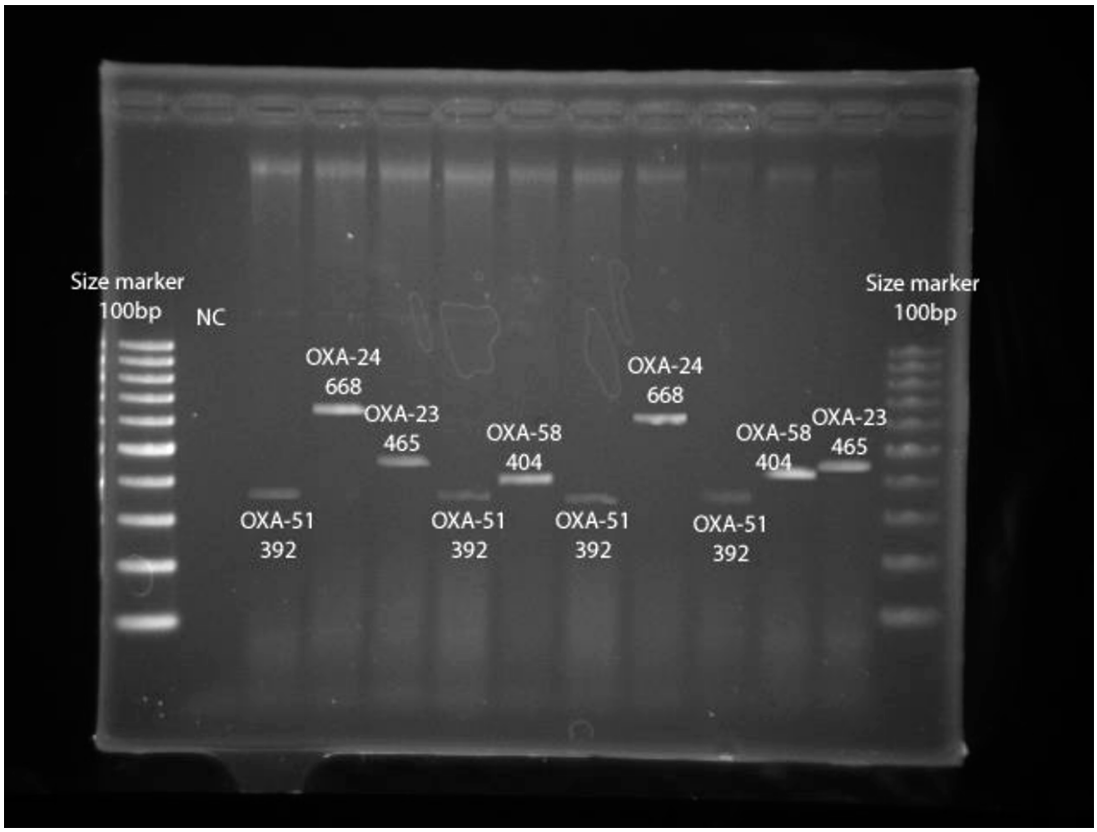

Figure 4. OXA group genes in carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates. From left to right: The first lane: Size marker (100 bp), the second lane: Negative control, lanes 3 to 12: Studied OXA genes, last lane: Size marker (100 bp)

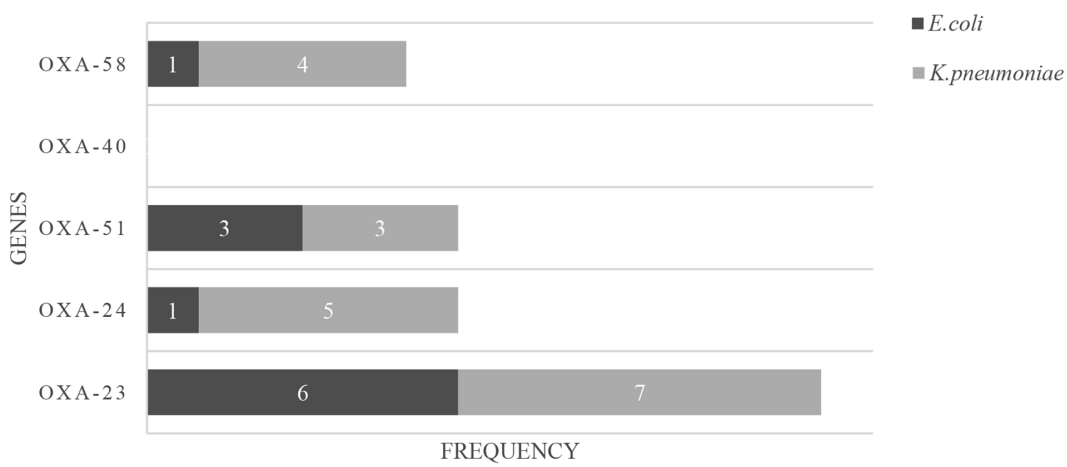

Figure 5. The abundance of OXA group genes in carbapenem-insensitive *Escherichia coli* and *Klebsiella pneumoniae*

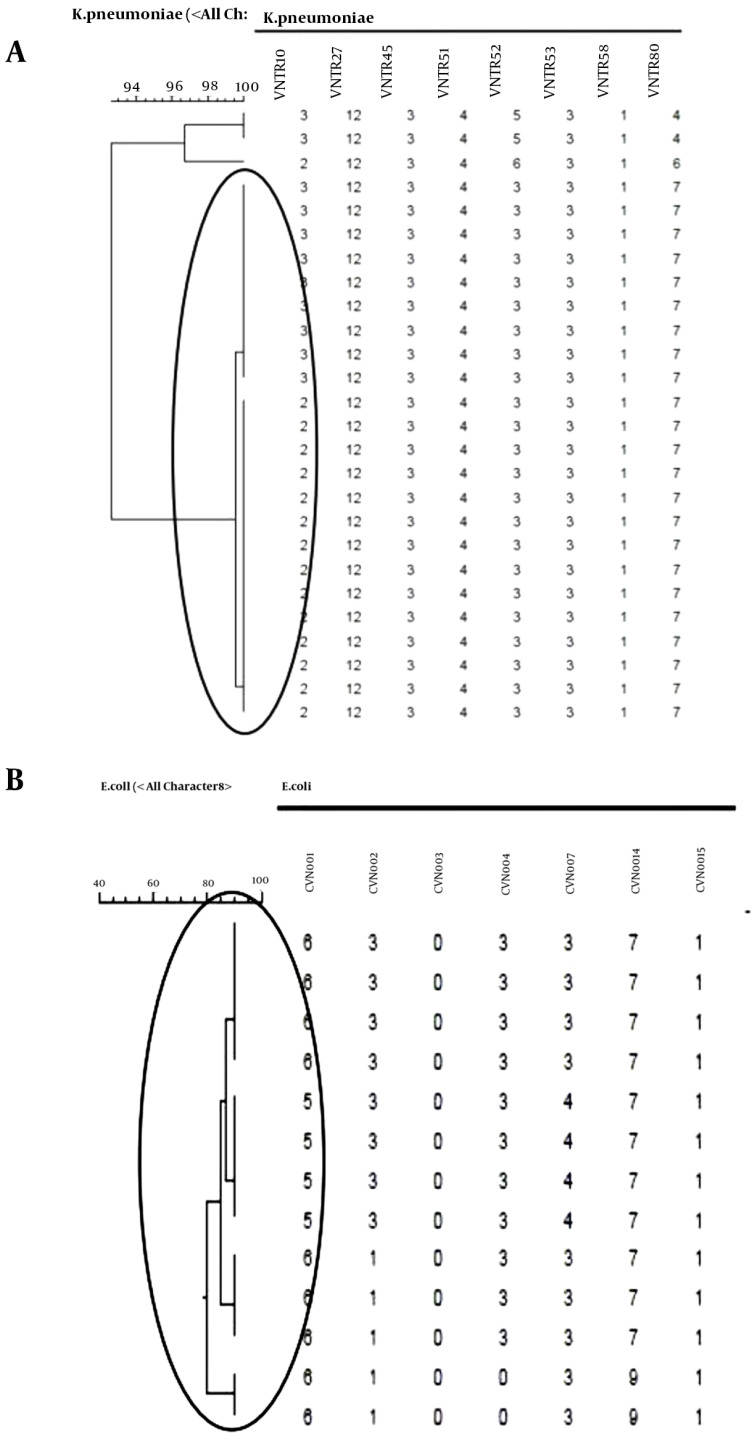

Figure 6. Clustering results of carbapenemase-producing isolates by MLVA (A) *Klebsiella pneumoniae*, (B) *Escherichia coli*

In Budak et al.'s research, a patient infected with multidrug-resistant *A. baumannii* was treated with an extended-spectrum beta-lactam and beta-lactamase inhibitor combination (35). However, two weeks later, an ertapenem-resistant *K. pneumoniae* isolate was discovered in the same patient. Their study suggests that the main reason for carbapenem-hydrolyzing oxacillinases is OXA-genes. Genetic events such as recombination, co-integration, and transposition *in vivo* (35) probably insert these genes into the chromosome. According to dendrograms (Figure 6A and 6B), carbapenem-resistant isolates possibly originated from one main clone and spread in the hospital via the vertical transmission of resistant genes. The dissemination of some genes was probably due to the transfer of mobile genetic elements.

5.1. Conclusions

Our study showed that all carbapenem-resistant *E. coli* and *K. pneumoniae* isolates were ESBL-producing and resistant to CRO, CIP, MEM, CAZ, and AMP. Also, OXA-51, 58, and 24 carbapenemases were firstly reported in the clinical strains of *E. coli* and *K. pneumoniae* isolated from volunteers with UTIs in Iran. Carbapenamases have global distribution, but there is considerable diversity at the continental, national and regional levels. A vital issue in preventing resistant strains and selecting appropriate treatment options is the awareness of the prevalence and occurrence of specific carbapenem resistance mechanisms in *E. coli* and *K. pneumoniae*. Also, OXA-23-like is the predominant gene responsible for carbapenem resistance. Further studies on large scales and in several areas are suggested for epidemiologic analyses.

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Footnotes

Authors' Contribution: All authors contributed equally to the aspects of the article, including study design, data collection and analysis, drafting, proofreading, and editing of the manuscript. All authors reviewed and approved the final draft of the manuscript.

Conflict of Interests: The authors declare no conflict of interest.

Data Reproducibility: The data presented in this study are uploaded during submission as a manuscript file and are openly available for readers upon request.

Ethical Approval: This study and all procedures performed were approved by the Ethics Committee of Islamic Azad University of Iran (registration number IR.IAU. PS.REC.1399.178). (link: ethics.research.ac.ir/EthicsProposalViewEn.php?id=159463)

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References

1. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med*. 2002;**113 Suppl 1A**:5S–13S. doi: 10.1016/s0002-9343(02)01054-9. [PubMed: 12113866].
2. Bergeron CR, Prussing C, Boerlin P, Daignault D, Dutil L, Reid-Smith RJ, et al. Chicken as reservoir for extraintestinal pathogenic Escherichia coli in humans, Canada. *Emerg Infect Dis*. 2012;**18**(3):415–21. doi: 10.3201/eid1803.111099. [PubMed: 22377351]. [PubMed Central: PMC3309577].
3. Al-Badr A, Al-Shaikh G. Recurrent Urinary Tract Infections Management in Women: A review. *Sultan Qaboos Univ Med J*. 2013;**13**(3):359–67. doi: 10.12816/0003256. [PubMed: 23984019]. [PubMed Central: PMC3749018].
4. Poulou A, Grivakou E, Vrioni G, Koumaki V, Pittaras T, Pournaras S, et al. Modified CLSI extended-spectrum beta-lactamase (ESBL) confirmatory test for phenotypic detection of ESBLs among Enterobacteriaceae producing various beta-lactamases. *J Clin Microbiol*. 2014;**52**(5):1483–9. doi: 10.1128/JCM.03361-13. [PubMed: 24574283]. [PubMed Central: PMC3993656].
5. Bhat MA, Sageerabanoo S, Kowsalya R, Sarkar G. The Occurrence of CTX-M3 Type Extended Spectrum Beta Lactamases among Escherichia Coli Causing Urinary Tract Infections in a Tertiary Care Hospital in Puducherry. *Journal of Clinical and Diagnostic Research*. 2012;**6**(7).
6. Doi Y, Murray GL, Peleg AY. Acinetobacter baumannii: evolution of antimicrobial resistance-treatment options. *Semin Respir Crit Care Med*. 2015;**36**(1):85–98. doi: 10.1055/s-0034-1398388. [PubMed: 25643273]. [PubMed Central: PMC4465586].
7. Sartelli M, Weber DG, Ruppe E, Bassetti M, Wright BJ, Ansaloni L, et al. Erratum to: Antimicrobials: a global alliance for optimizing their rational use in intra-abdominal infections (AGORA). *World J Emerg Surg*. 2017;**12**:35. doi: 10.1186/s13017-017-0147-0. [PubMed: 28785301]. [PubMed Central: PMC5541698].
8. Mitchell JM, Clasman JR, June CM, Kaitany KC, LaFleur JR, Taracila MA, et al. Structural basis of activity against aztreonam and extended spectrum cephalosporins for two carbapenem-hydrolyzing class D beta-lactamases from Acinetobacter baumannii. *Biochemistry*. 2015;**54**(10):1976–87. doi: 10.1021/bi501547k. [PubMed: 25710192]. [PubMed Central: PMC4476283].
9. Higgins PG, Poirel L, Lehmann M, Nordmann P, Seifert H. OXA-143, a novel carbapenem-hydrolyzing class D beta-lactamase in Acinetobacter baumannii. *Antimicrob Agents Chemother*. 2009;**53**(12):5035–8. doi: 10.1128/AAC.00856-09. [PubMed: 19770279]. [PubMed Central: PMC2786334].
10. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. *Clin Microbiol Infect*. 2006;**12**(9):826–36. doi: 10.1111/j.1469-0691.2006.01456.x. [PubMed: 16882287].

11. El-Badawy MF, El-Far SW, Althobaiti SS, Abou-Elazm FI, Shohayeb MM. The First Egyptian report showing the co-existence of bla NDM-25, bla OXA-23, bla OXA-181, and bla GES-1 among carbapenem-resistant K. pneumoniae clinical isolates genotyped by BOX-PCR. *Infect Drug Resist*. 2020;**13**:1237–50. doi: 10.2147/IDR.S244064. [PubMed: 32425561]. [PubMed Central: PMC7196799].
12. Cetinkol Y, Yildirim AA, Telli M, Calgin MK. The investigation of oxacillinase/metallo-beta-lactamase genes and clonal analysis in carbapenem-resistant Klebsiella pneumoniae. *Infez Med*. 2016;**24**(1):48–53. [PubMed: 27031897].
13. Manohar P, Leptihn S, Lopes BS, Nachimuthu R. Dissemination of carbapenem resistance and plasmids encoding carbapenemases in Gram-negative bacteria isolated in India. *JAC Antimicrob Resist*. 2021;**3**(1):dlab015. doi: 10.1093/jacamr/dlab015. [PubMed: 34223092]. [PubMed Central: PMC8210035].
14. Weinstein MP, Clinical; Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. Albany, NY, USA: National Committee for Clinical Laboratory Standards; 2018.
15. Rudresh SM, Ravi GS, Sunitha L, Hajira SN, Kalaiarasan E, Harish BN. Simple, rapid, and cost-effective modified Carba NP test for carbapenemase detection among Gram-negative bacteria. *J Lab Physicians*. 2017;**9**(4):303–7. doi: 10.4103/JLP.JLP_138_16. [PubMed: 28966495]. [PubMed Central: PMC5607762].
16. Hou C, Yang F. Drug-resistant gene of blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58 in Acinetobacter baumannii. *Int J Clin Exp Med*. 2015;**8**(8):13859–63. [PubMed: 26550338]. [PubMed Central: PMC4613023].
17. Lindstedt BA, Brandal LT, Aas L, Vardund T, Kapperud G. Study of polymorphic variable-number of tandem repeats loci in the ECOR collection and in a set of pathogenic Escherichia coli and Shigella isolates for use in a genotyping assay. *J Microbiol Methods*. 2007;**69**(1):197–205. doi: 10.1016/j.mimet.2007.01.001. [PubMed: 17291612].
18. Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem resistant Klebsiella pneumoniae isolated from bloodstream infection: Indian experience. *Pathog Glob Health*. 2017;**111**(5):240–6. doi: 10.1080/20477724.2017.1340128. [PubMed: 28670975]. [PubMed Central: PMC5560201].
19. Lobersli I, Haugum K, Lindstedt BA. Rapid and high resolution genotyping of all Escherichia coli serotypes using 10 genomic repeat-containing loci. *J Microbiol Methods*. 2012;**88**(1):134–9. doi: 10.1016/j.mimet.2011.11.003. [PubMed: 22088357].
20. Arana DM, Rubio M, Alos JI. Evolution of antibiotic multiresistance in Escherichia coli and Klebsiella pneumoniae isolates from urinary tract infections: A 12-year analysis (2003-2014). *Enferm Infecc Microbiol Clin*. 2017;**35**(5):293–8. doi: 10.1016/j.eimc.2016.02.018. [PubMed: 27056582].
21. Dolatyar Dehkharghani A, Haghighat S, Rahnamaye Farzami M, Douraghi M, Rahbar M. Subtyping beta-lactamase-producing Escherichia coli strains isolated from patients with UTI by MLVA and PFGE methods. *Iran J Basic Med Sci*. 2021;**24**(4):437–43. doi: 10.22038/ijbms.2021.49790.11372. [PubMed: 34094024]. [PubMed Central: PMC8143711].
22. Lindstedt BA, Vardund T, Kapperud G. Multiple-locus variable-number tandem-repeats analysis of Escherichia coli O157 using PCR multiplexing and multi-colored capillary electrophoresis. *J Microbiol Methods*. 2004;**58**(2):213–22. doi: 10.1016/j.mimet.2004.03.016. [PubMed: 15234519].
23. El-Hendawy G, Melake NA, Salama AA, Eissa NA, Zahran WA, Elaskary S. Distribution of class 1 integrons among multidrug-resistant Escherichia coli in Menoufia University Hospitals and commensal Escherichia coli isolates. *Menoufia Medical Journal*. 2016;**29**(4):772–82.
24. Schechner V, Temkin E, Harbarth S, Carmeli Y, Schwaber MJ. Epidemiological interpretation of studies examining the effect of antibiotic usage on resistance. *Clin Microbiol Rev*. 2013;**26**(2):289–307. doi: 10.1128/CMR.00001-13. [PubMed: 23554418]. [PubMed Central: PMC3623381].
25. Ranjbar R, Ghazi FM, Farshad S, Giammanco GM, Aleo A, Owlia P, et al. The occurrence of extended-spectrum beta-lactamase producing Shigella spp. in Tehran, Iran. *Iran J Microbiol*. 2013;**5**(2):108–12. [PubMed: 23825726]. [PubMed Central: PMC3696844].
26. Ranjbar R, Memariani H, Sorouri R. Molecular epidemiology of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae strains isolated from children with urinary tract infections. *Arch Pediatr Infect Dis*. 2016;**5**(2). doi: 10.5812/pedinfect.39000.
27. Ranjbar R, Mirsaeed Ghazi F. Antibiotic sensitivity patterns and molecular typing of Shigella sonnei strains using ERIC-PCR. *Iran J Public Health*. 2013;**42**(10):1151–7. [PubMed: 26060624]. [PubMed Central: PMC4436544].
28. Ashayeri-Panah M, Feizabadi MM, Eftekhar F. Correlation of multidrug resistance, integron and blaesbl gene carriage with genetic fingerprints of extended-spectrum beta-lactamase producing Klebsiella pneumoniae. *Jundishapur J Microbiol*. 2014;**7**(2). e8747. doi: 10.5812/jjm.8747. [PubMed: 25147670]. [PubMed Central: PMC4138679].
29. Ullah F, Malik SA, Ahmed J. Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiella pneumoniae from urinary tract infections in the North-West of Pakistan. *Afr J Microbiol Res*. 2009;**3**(11):676–80.
30. Zowawi HM, Sartor AL, Balkhy HH, Walsh TR, Al Johani SM, AlJindan RY, et al. Molecular characterization of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in the countries of the Gulf cooperation council: dominance of OXA-48 and NDM producers. *Antimicrob Agents Chemother*. 2014;**58**(6):3085–90. doi: 10.1128/AAC.02050-13. [PubMed: 24637692]. [PubMed Central: PMC4068443].
31. Gomes DJ, Rahman SR, Lina TT. Multiple-Antibiotic Resistance Mediated by Plasmids and Integrons in Uropathogenic *Klebsiella pneumoniae*. *Banglad J Microbiol*. 1970;**24**(1):19–23. doi: 10.3329/bjm.v24i1.1231.
32. Oteo J, Lazaro E, de Abajo FJ, Baquero F, Campos J, Spanish members of E. Antimicrobial-resistant invasive Escherichia coli, Spain. *Emerg Infect Dis*. 2005;**11**(4):546–53. doi: 10.3201/eid1104.040699. [PubMed: 15829192]. [PubMed Central: PMC3320321].
33. Aksoy MD, Cavuslu S, Tugrul HM. Investigation of metallo beta lactamases and oxacilinases in carbapenem resistant Acinetobacter baumannii strains isolated from inpatients. *Balkan Med J*. 2015;**32**(1):79–83. doi: 10.5152/balkanmedj.2015.15302. [PubMed: 25759776]. [PubMed Central: PMC4342142].
34. Chen TL, Lee YT, Kuo SC, Hsueh PR, Chang FY, Siu LK, et al. Emergence and Distribution of Plasmids Bearing the blaOXA-51-like gene with an upstream ISAba1 in carbapenem-resistant Acinetobacter baumannii isolates in Taiwan. *Antimicrob Agents Chemother*. 2010;**54**(11):4575–81. doi: 10.1128/AAC.00764-10. [PubMed: 20713680]. [PubMed Central: PMC2976157].
35. Budak S, Aktaş Z, Oncul O, Acar A, Ozyurt M, Turhan V, et al. Detection of OXA-51 carbapenemase gene in klebsiella pneumoniae: a case report and a new dimension on carbapenemase resistance. *J Mol Genet Med*. 2013;**7**(63):1747–862.1.
36. Walsh TR. Emerging carbapenemases: a global perspective. *Int J Antimicrob Agents*. 2010;**36 Suppl 3**:S8–14. doi: 10.1016/S0924-8579(10)70004-2. [PubMed: 21129630].
37. Bonnet R, Marchandin H, Chanal C, Sirot D, Labia R, De Champs C, et al. Chromosome-encoded class D beta-lactamase OXA-23 in Proteus mirabilis. *Antimicrob Agents Chemother*. 2002;**46**(6):2004–6. doi: 10.1128/AAC.46.6.2004-2006.2002. [PubMed: 12019126]. [PubMed Central: PMC127228].