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Antibiotic Resistance Pattern and Prevalence of *tetA*, *tetB*, *tetR*, *OXA-10* and *OXA-48* Resistance Genes among *Escherichia coli* Isolates from Toilets in Mashhad Azad University (Iran) in 2020

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ABSTRACT

Background and Aim: *Escherichia coli* is the normal flora of the human and animal intestine that sometimes causes pathogenesis within the intestine and urinary tract. The current study investigates resistance genes in *Escherichia coli* isolated from the toilets at the Islamic Azad University of Mashhad, Iran, and to perform genome sequencing in tetracycline-resistant strains.

Materials and Methods: This cross-sectional evaluation was carried out on 200 samples collected over three months. Samples were cultured and isolated using eosin methylene blue agar medium (EMB). *E. coli* Samples were identified and used to evaluate the resistance genes by polymerase chain reaction (PCR). The *E. coli* isolates were used for an Antibiogram test to identify resistant strains. For the statistical analysis of antibiotics pattern, SPSS-22 and the Kolmogorov-Smirnov test were used.

Results: Out of 200 samples, 41 isolates were identified as *E. coli* with the highest resistance rate to cefotaxime (74.60%) and the highest sensitivity rate to gentamicin (58.74%). Among the 20 isolates, 30%, 20%, 25% carried bla_{TEM-1}, bla_{OXA-10} and bla_{OXA-48} genes, respectively. Among 38 tetracycline-resistant isolates, three isolates (7.89%) had *tetA* gene, and two (5.26%) had the *tetR* gene.

Conclusion: In this experiment, most of the isolates were identified as *E. coli* and were resistant to antibiotics. To control the spread of *E. coli* infectious disease and arbitrary use of antibiotics, it is recommended to conduct some educational programs and social activities with the aim of increasing health awareness.

Keywords: *Escherichia coli*, resistance genes, tetracycline-resistant isolates, antibiotics

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1. Introduction

The appearance and prevalence of antimicrobial-resistance (AR) among human pathogens have been observed in recent decades and have led to the development of antibiotic-resistant bacteria **(1)**. Microorganisms are capable of developing resistance to antibiotics, and this occurs when a bacterium acquires a resistance gene that reduces or eliminates the drug's effect, in which case the mutated gene survives. Any antimicrobial agent can cause resistance

in the microbial population and thus produce resistant genes **(2)**.

Regrettably, improper use of antimicrobial drugs kills some sensitive microorganisms and creates favorable living conditions for resistant bacteria. So the effectiveness of some antibiotics has decreased **(3)**. In developing countries, the transmission of antibiotic-resistant bacteria often occurs person-to-person through contaminated food, drinking water, and insects. Drug resistance can mean that people infected with such bacteria do not respond to conventional antibiotic drugs **(4, 5)**.

For the first time in 1885, Theodor Escherich, a German-Austrian pediatrician, isolated a motile gram-negative bacterium from an infant´s gut that grew well in synthetic culture media. Escherich named the *bacterium coli* commune, known as *Escherichia coli* in 1919 **(6)**. The genus *E. coli* is a member of the *Enterobacteriaceae* family, which exists as part of the normal flora in the large intestine of humans and animals. *E. coli* is a gram-negative bacillus usually with mobile flagella. Each gram of human feces contains more than10^8 *E. coli* bacteria **(7, 8)**. *E. coli* resistant strains can be transmitted through human, animal, and food products and can also transfer their resistance genes to other pathogens. Among antibiotic-resistant *E. coli*, the prevalence of tetracycline-resistant strains is high **(9)**.

Tetracycline is not commonly used to treat *E. coli* infections in humans; however, resistance to this antibiotic is widespread in *E. coli* infections. The amino acid sequence of tetracycline pumps is divided into six groups, including *tet A*, *tet B*, *tet C*, *tet D*, and *tet E*, in group 1 due to amino acid similarities. Tetracycline efflux pumps are specifically involved in developing tetracycline resistance **(10)**. Plasmids and transposons are the main factors in the rapid spread of tetracycline resistance genes among *E. coli* strains **(9)**. Resistance to tetracycline in bacteria is associated with reflux, ribosomal protection, enzyme inactivation, and target modification. Tetracycline efflux was first identified in *Shigella dysentery* in 1953 **(11)**.

A limited number of bacteria receive tetracycline resistance through mutations. This can happen by altering the permeability of outer membrane purines, outer membrane lipopolysaccharides, changes in ribosomal 16S rRNA component, or mutations in genes associated with the ion pump mechanism swells **(3)**. Regarding tetracycline resistance among *E. coli* in clinical and animal samples and the lack of genetic sequences of this bacterium, we decided to sample from the toilets and identify the resistant genes by molecular techniques. The aim of this study was to determine antibiotic resistance pattern and prevalence of *tetA*, *tetB*, *tetR*, *OXA-10* and *OXA-48* genes among *E. coli* isolates from Toilets in Mashhad Azad University in Iran.

2. Materials and Methods

2-1. Collection and Identification of Isolates

This cross-sectional study involved 200 samples that were collected randomly from the toilets of faculty of Basic Sciences, Islamic Azad University in Mashhad, over three months from October to December 2020 (during the Covid-19 pandemic) and under sterile conditions, sent to the laboratory. Tests for isolation and identification of bacteria were performed up to 5 h after sampling. To separate *E. coli*, sterile swabs soaked in sterile distilled water then sampling was done from bathroom parts, including stones, hoses, faucets, trash cans, toilets, washbasin, handles, and floors. Twenty-four samples were taken from each part. Samples were separated from men's, women's, and employees' toilets.

2-2. Culture of Isolates

Swabs were put in the tube containing nutrient broth medium, and the tubes were incubated for 24 h at 37°C under aerobic conditions. Next, to dilute the microbial suspension, 10µl of suspension was inoculated into the surface of eosin methylene blue (EMB) agar and nutrient agar by streak culture and plates were incubated at 37°C for 24 h. In the next step, colonies were tested for gram staining and differential biochemical tests include oxidase, catalase, indole, methyl red, Voges-Proskauer test, citrate, fermentation of sugar, production of H_2S and gas in Kligler's Iron (KIA) agar **(12)**. Gram staining, catalase and coagulase test, esculin hydrolysis in bile esculin agar, fermentation of mannitol in mannitol salt agar and novobiocin test were used to identify gram-positive bacteria **(12)**.

2-3. Antibiotic Susceptibility Test

An antibiogram test was performed by the disk diffusion method according to the Clinical & Laboratory Standards Institute (CLSI) **(13)**.The antibiotic resistance test was assessed in Mueller-Hinton agar for the eighth standard antibiotic discs (Padtan Teb, Iran), including Ampicillin (10µg), Tetracycline (30µg), Gentamicin (10µg), Cefixime (5µg), Cefotaxime (30µg), Ceftazidime Clavulanat (10µg), Ceftazidime (30µg) and Ciprofloxacin (5µg). After measuring the diameter of the growth inhibition zone and according to the standard table, each isolate's sensitivity and resistance status were reported. *E. coli* PTCC 1330 was used as a reference strain for antibiotic susceptibility testing.

2-4. DNA Extraction

For this purpose, pure colonies of *E. coli* isolates were inoculated in 3 mL of brain heart infusion broth and incubated at 37℃ for 18 hours. DNA extracted by using a Sinaclon DNA extraction kit for gram-negative bacteria and stored in a freezer at -20 °C. To confirm *E. coli*, a PCR test was performed using 16S rRNA specific primer (Table 1). *E. coli* PTCC 1330 was used as a positive control, and sterile distilled water was used as a negative control.

2-5. Detection of bla_{OXA-10}, bla_{OXA-48}, bla_{TEM-1}, tetA, tetB, and tetR genes

Specific primers were used to differentiate strains containing beta-lactamase enzymes *OXA-10*, *OXA-48*, and *TEM*-1 in beta-lactam resistant isolates and to detect *tetA*, *tetB*, and *tetR* genes in tetracycline-resistant isolates. PCR was used to detect genes. Specific primers of bla_{OXA-10}, bla_{OXA-48}, bla_{TEM-1}, and *tetA* were selected from the papers (as shown in Table 1) and for the *tetB* and *tetR* primers were designed using AlleleID v 6.01 software **(14)**. In order to check the specificity for the desired sequence, all the primers were blasted in the NCBI site.

Table 1. Primers used in the present research

primer	forward sequences (5'→3')	reverse sequences ('5→'3)	Product Size	Reference
16S rRNA	AGAGTTTGATCATGGCTCAG	GGACTACCAGGGTATCTAAT	798	**(15)**
OXA-10	TCAACAAATCGCCAGAGAAG	TCCCACACCAGAAAAACCAG	276	**(16)**
OXA-48	GCGTGGTTAAGGATGAACAC	CATCAAGTTCAACCCAACCG	438	**(16)**
TEM-1	CATTTCCGTGTCGCCCTTATTC	CGTTCATCCATAGTTGCCTGAC	800	**(17)**
tetR	GGCAGGCAGAGCAAGTAGAG	CAGGCAGGTGGATGAGGAAC	174	-
tetB	CTACTTCGGTATCTGTATTATCAC	GCATCGCTGGATTACTTATTG	340	-
tetA	GACAATCAACTACTTCACTG	CTTTCTCGGTCCTTCAAC	234	**(18)**

The final volume of the PCR reaction (25 µL) in was as follows: 12.5 µL of Mastermix (2X) (Pars Toos, Iran), 1 µL of each forward and reverse primer (10 mM) (Sinaclon, Iran), 2 µL of DNA template (33.0 ng) and 8.5 µL of distilled water. PCR of each gene was performed using a thermocycler (Kyratec, Korea) with the time and temperature program as listed in Table 2.

Table 2. Optimal conditions for PCR of 16S rRNA, bla_{OXA-10}, bla_{OXA-48}, bla_{TEM-1}, *tet-A, tet-B* and *tet-R* genes

	Initial denaturation		Denaturation		Annealing		Extension		Final extension	
	Temp	Time	Temp	Time	Temp	Time	Temp	Time	Temp	Time
16S rRNA	94	5 min	94	45s	51	45s	72	45s	75	5 min
bla_{OXA-10}	95	5 min	95	30s	52	30s	72	30s	75	5 min
bla_{OXA-48}	95	5 min	95	30s	53	30s	72	30s	75	5 min
bla_{TEM-1}	94	9 min	94	45s	57	45s	72	60s	72	10 min
tetA	95	5 min	94	30s	57	30s	72	30s	75	3 min
tetB	95	5 min	94	45s	56	45s	72	45s	75	5 min
tetR	95	5 min	94	30s	50	30s	72	30s	75	3 min

2-6. Gene Sequencing

PCR products were electrophoresed on a 1% agarose gel containing green viewer for 60 min at a voltage of 90 volts. Finally, gel agarose and DNA bands were photographed using a gel documentation device.

The PCR products of bla_{OXA-10}, bla_{OXA-48}, bla_{TEM-1}, *tetA*, *tetB,* and *tetR* genes were sent to the Genetics Laboratory of Razavi Hospital in Mashhad for sequencing. The obtained results were analyzed via Gene Codes Sequencher v5.4.6 software to check the sequence on NCBI website.

2-7. Statistical Analysis

SPSS Statistics V22.0 (IBM, Chicago, IL, USA) and Kolmogorov-Smirnov tests were used to perform statistical analysis and p-values of less than 0.05 ($p<0.05$) were considered statistically significant.

3. Results

Results being studied out of 200 samples collected in this study showed that 159 isolates were identified as gram-negative bacteria based on microscopic characteristics and, 41 isolates (20.5%) were identified as *E. coli* based on colony characteristics and biochemical tests (Figure 1).

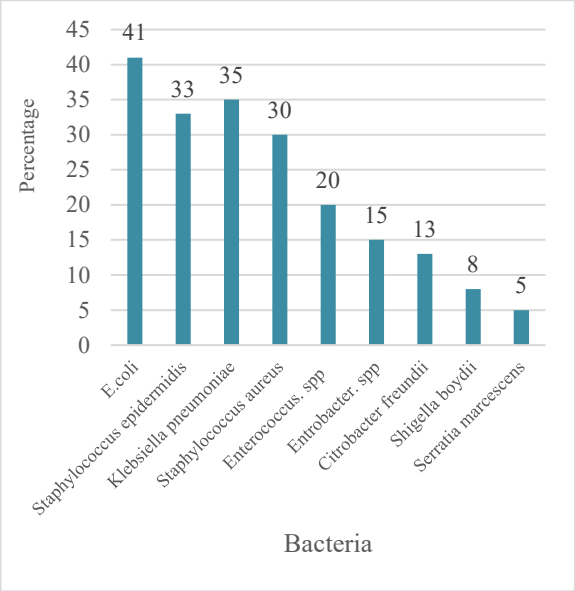

Figure 1. Frequency of isolated bacteria

All gram-negative isolates with results of oxidase negative, catalase-positive, lactose positive, H_2S negative, gas positive, indole positive, methyl-red positive, Voges-Proskauer negative, and citrate negative was identified as *E. coli*. All *E. coli* isolates were confirmed using *16S rRNA* gene-specific primers in the PCR (Figure 2).

The antibiotic susceptibility of *E. coli* isolates was evaluated by the disk diffusion method. The Kolmogorov-Smirnov test among antibiotics-resistance *E. coli* isolates showed a significant resistant to Cefotaxime ($P>0.001$) (Figure 3).

Table 3 represents high resistance to Cefotaxime (74.60 %) among the isolates. *E. coli* isolates showed the highest sensitivity to Gentamicin (58.74 %).

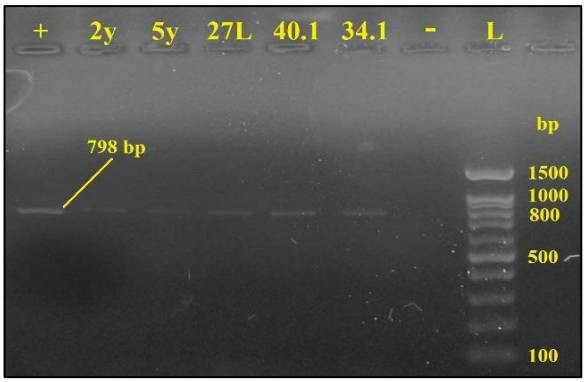

Figure 2. Gel electrophoresis of *16S rRNA* gene for some *E. coli* isolates with 100bp ladder (L: Ladder, +: positive control, -: negative control)

Table 3. Antibiotic susceptibility of *E. coli* isolates

Type of antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)	*p-Value*
Tetracycline	38(60.32)	10(15.87)	15(23.81)	
Cefixime	41(65.08)	6(9.52)	16(25.40)	
Ampicillin	(63.49) 40	7(11.11)	16(25.40)	
Ciprofloxacin	39(61.90)	5(9.93)	18(28.17)	*P*> 0.001
Ceftazidime	45(71.43)	5(7.94)	13(20.63)	
Ceftazidime clavulanate	44(69.83)	4(6.36)	15(23.81)	
Gentamicin	14(22.22)	12(19.04)	37(58.74)	
Cefotaxime	47(74.60)	4(6.36)	12(19.04)	

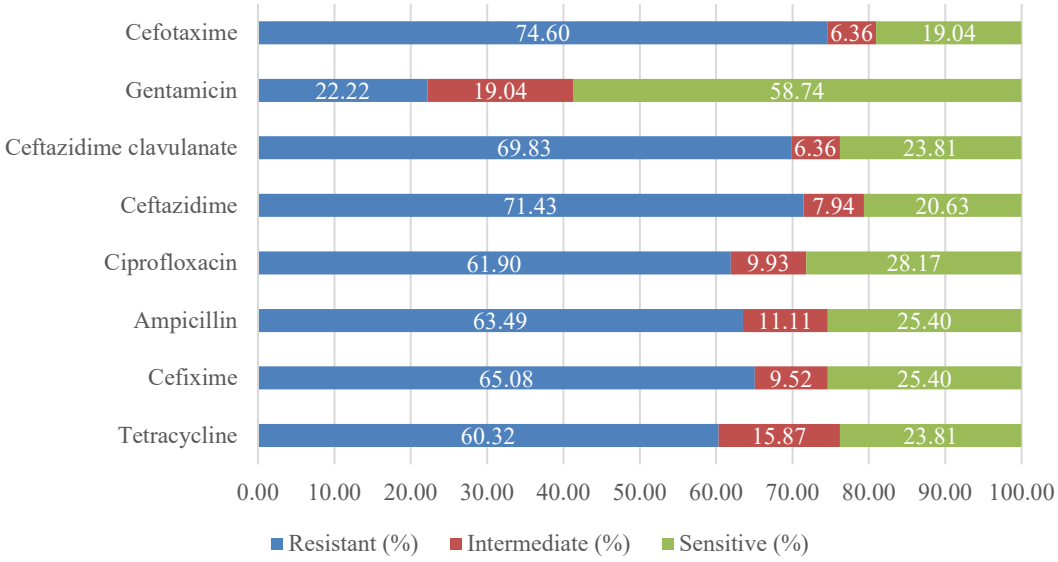

Figure 3. Antibiotics susceptibility patterns of *E. coli* isolates (*P*> 0.001)

Detecting of bla_{TEM-1}, bla_{OXA-10}, and bla_{OXA-48} genes among 20 beta-lactam-resistant isolates by PCR indicated that 6 isolates (30%) had bla_{TEM-1} gene (Figure 4); 4 (20%) had bla_{OXA-10} gene (Figure 5) and 5 isolates (25%) had bla_{OXA-48} gene (Figure 6).

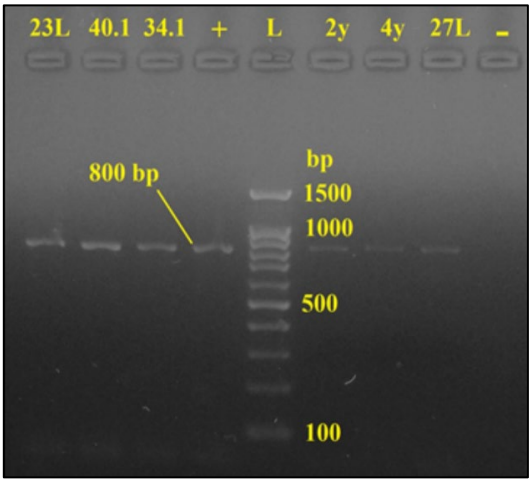

Figure 4. Electrophoresis gel of bla_{TEM-1} gene for some resistant *E. coli* isolates to beta-lactam antibiotic with 100bp ladder (L: Ladder, +: positive control, -: negative control)

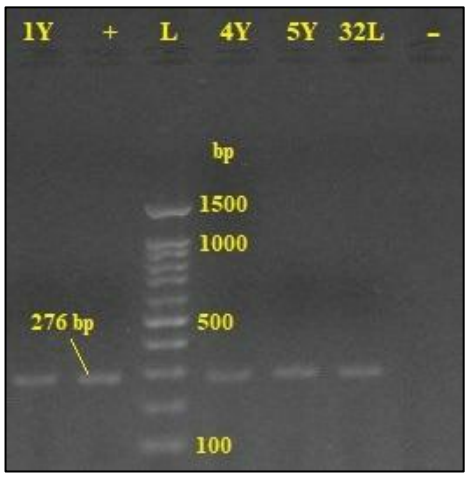

Figure 5. Electrophoresis gel of bla_{OXA-10} for some resistant *E. coli* isolates to beta lactam antibiotic with 100bp ladder (L: Ladder, +: positive control, -: negative control)

To detect the bla_{TEM-1} gene, a strain from the Microbiology Laboratory of Mashhad Azad University collection containing the desired gene was used as a positive control **(19)**.

To detect the bla_{OXA-10} *and* bla_{OXA-48} *genes*, a strain from the Microbiology Laboratory of Mashhad Azad University collection containing the desired gene was used as a positive contrast **(16)**.

Among 38 tetracycline-resistant isolates, (7.89%) carried the *tetA* (Figure 7) and 2 (5.25%) carried the *tetR* gene (Figure 8). The *tetB* gene was not detected in any tetracycline-resistant isolates.

The strains carrying *tetA* and *tetR* genes from microbial collection of the Microbiology Laboratory of Mashhad Azad University were used as positive controls **(20)**.

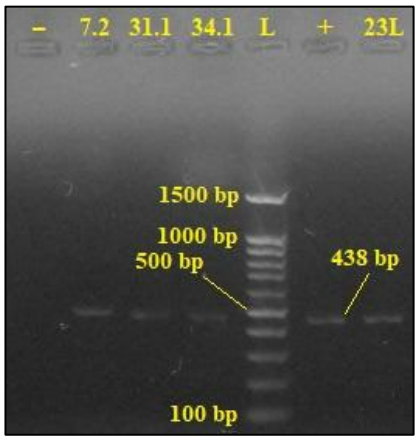

Figure 6. Electrophoresis gel of bla_{OXA-48} gene for some resistant *E. coli* isolates to tetracycline with 100bp ladder (L: Ladder, +: positive control, -: negative control)

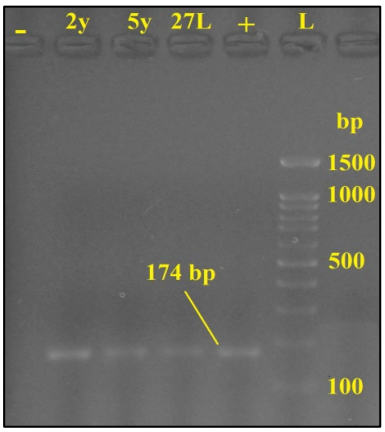

Figure 7. Electrophoresis gel of *tetA* gene for some resistant *E. coli* isolates to tetracycline with 100bp ladder (L: Ladder, +: positive control, -: negative control)

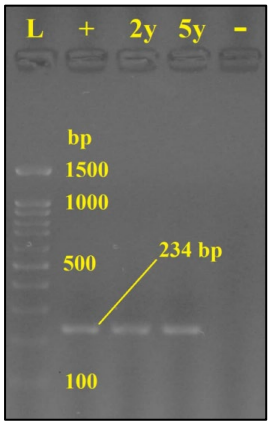

Figure 8. Electrophoresis gel of *tetR* gene for some resistant *E. coli* isolates to tetracycline with 100bp ladder (L: Ladder, +: positive control, -: negative control)

Gene Sequencing

PCR products of bla_{OXA-10}, bla_{OXA-48}, bla_{TEM-1}, *tetA*, *tetB,* and *tetR* genes were sent for sequencing. The data were obtained from the Genetics Laboratory of Razavi Hospital in Mashhad and was BLAST on the NCBI website. The results were shown in Table 4.

Table 4. Accession Number of similar sequences in the NCBI database and their degree of similarity

Gene name	Similar sequence Accession Number in the NCBI database	Alignment Explanation
bla_{OXA-10}	HM240865	Nucleotide C was deleted in row 56.
bla_{OXA-48}	MN654467	Alignment 100 %
bla_{TEM-1}	-	
tet-A	KX117210	Alignment 100 %
tet-B	-	
tet-R	KX117210	There is nucleotide A instead of nucleotide G in row 546.

4. Discussion

The spread of beta-lactamases among gram-negative bacteria has been reported as the most serious threat to infectious diseases. The increasing use of antibiotics has led to inherent and acquired resistance among these gram-negative microbes **(21)**. *OXA* enzymes are a class of beta-lactamase enzymes that hydrolyze Oxacillin antibiotics. These enzymes are widely distributed among various strains of *Enterobacteriaceae* and *Acinetobacter*. These enzymes can also hydrolyze Carbapenem antibiotics. Given that these antibiotics are among the most beneficial ones for treating infections, resistance to these antibiotics is a significant risk **(22, 23)**.

In this research, from 200 samples collected, 41 isolates (20.5%) were identified as *E. coli*. Antibiogram results showed that they had the highest resistance to Cefotaxime with 74.60% and the most heightened sensitivity to gentamicin with 58.74%. Regarding the detection of beta-lactam resistance genes, among 20 isolates, six isolates (30%) carried the bla_{TEM-1} gene, four isolates (20%) carried bla_{OXA-10} gene and five isolates (25%) carried the bla_{OXA-48} gene.

Also, research on the frequency of Tetracycline-resistance genes, among 38 isolates, three isolates (7.89%) had the *tetA* gene, and two isolates (5.26%) had the *tet*R gene. None of the isolates had the *tetB* gene. From the results of tracking *tet* genes in our work, it is inferred that, fortunately, the prevalence of these genes in the population study is low.

In a study in Bojnourd, out of 50 urine samples, 34 *E. coli* samples had resistance genes, and among them, 12 isolates (24%) had bla_{TEM-1} beta-lactamase. The highest frequency of antibiotic resistance was observed for Ampicillin (84%), Co-trimoxazole (60%),

and the highest frequency of antibiotic susceptibility was observed, Nitrofurantoin (94%) and Gentamicin (78%) **(24)**.

The higher frequency of the *blaTEM-1* gene and the difference in antibiotic-resistance in this research compared to Amiri *et al*. could be due to differences in the geographical areas studied.

In another similar study by Mousavi *et al*. in Robat Karim (Iran), from 253 urine samples collected from patients, 100 samples were identified as *E. coli* (55%). Among them, 60 isolates were positive for ESBL production, of which 48 isolates with the *TEM* gene were reported **(25)**.

Similar to the present work, ESBL-producing strains have a high frequency, indicating a high prevalence of beta-lactamase genes in the community; however, the frequency of *TEM* gene is much higher than in this study, which may be due to differences in the type of sample.

Studies in Iran, Tunisia, Sudan, Brazil, South Africa, and India have reported widespread prevalence of beta-lactamases by *E. coli* carrying the *TEM* gene, with an average of 53% indicating the high prevalence of this gene in developing countries **(26-30)**.

Examination by Kamrani Hemmat *et al*., in several hospitals in different cities in Iran (Tehran, Borojerd, and Sanandaj), Out of 150 isolates of *E. coli*, 88 isolates (27.86%) had *tetA* gene, and 83 isolates (37.81%) had the gene *tetB* were identified. In 74 isolates (54.72%), both genes had Tetracycline resistance, and in five isolates (90.4%), they lacked resistant genes **(3)**.

A study by Kadai *et al*., in Zabol City, out of 100 isolates of *E. coli*, 57 isolates were tetracycline resistant, of which 32 isolates (14.56%) were resistant to *tetA* gene, and 28 isolates (12.49%) were carriers of the *tetB* gene. Both *tetA* and *tetB* genes were present in 15 isolates **(10)**.

A comparison of the results of these studies with the present study shows that the prevalence of *tet* genes in hospitalized patients and clinical specimens is much higher than in the samples obtained in this study. Therefore, the presence of tetracycline resistance genes is the main cause of tetracycline resistance in clinical samples not in environmental samples.

In a study by Gurung *et al*. in Nepal, 141 *E. coli* isolates were identified from 154 gram-negative isolates. Ninety-seven isolates carried beta-lactamase enzymes, of which 57 isolates (33.3%) had the *OXA48* gene **(31)**.

The results of a research by Gurung *et al*. are similar to the present study, and the prevalence of the bla_{OXA-48} gene is the same. In the academic work of Hashemi *et al*., the prevalence of bla_{OXA-10} and bla_{OXA-48} genes among clinical isolates of *Pseudomonas aeruginosa* resistant to Imipenem was 26.3% and 23.6%, respectively **(16)**. The results of these studies were almost identical.

However, a comparison of the results of the two recent studies mentioned with the current study, it cannot be concluded that the prevalence of *OXA* genes is the same in clinical and environmental samples. Given that there is no information on the health status of people who have used the health services; it is possible that the isolates carrying the *OXA* genes belonged to people who had disease.

bla_{TEM-1}, bla_{OXA-10}, and bla_{OXA-48} genes are very common in beta-lactamase-producing (ESBL) samples in this study, the outbreak of the present genes was more frequent, and according to studies conducted in Iran and European-American countries, it shows that the prevalence of resistance to beta-lactam antibiotics is increasing. To prevent the overuse of antibiotics, doctors and health professionals are expected to be informed about the drugs through preventive training programs.

5. Conclusion

The results of this research showed that *E. coli* carrying antibiotic resistance genes are found in toilets and the resistance gene sequence was very similar to NCBI database. Since these bacteria can play a role in the transmission and spread of antibiotic resistance, it is recommended to reduce the possibility of transmission and spread of pathogenic bacteria as well as the growth of antibiotic-resistant strains, by observing personal hygiene and daily disinfection of toilets.

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Conflict of Interest

The authors declared no conflict of interest.

Reference

1. Belaynehe KM, Shin SW, Yoo HS. Interrelationship between tetracycline resistance determinants, phylogenetic group affiliation and carriage of class 1 integrons in commensal Escherichia coli isolates from cattle farms. BMC Vet Res. 2018;14(1):1-11. [PMCID] [DOI:10.1186/s12917-018-1661-3] [PMID]
2. Blum SE, Leitner G. Genotyping and virulence factors assessment of bovine mastitis Escherichia coli. Vet Microbiol. 2013;163(3-4): 305-12. [DOI:10.1016/j.vetmic.2012.12.037] [PMID]
3. Kamrani Hemat N, Mirzaee M, Najar-peerayeh S. Prevalence of tetracycline resistance genes (tetA, tetB) and antibiotic resistance pattern in uropathogenic Escherichia coli isolates. New Cellular Mol Biotechnol j. 2017;7(25):9-18.
4. Olowe O, Idris O, Taiwo S. Prevalence of tet genes mediating tetracycline resistance in Escherichia coli clinical isolates in Osun State, Nigeria. Eur J Immunol Microbiol Immunol. 2013;3(2):135-40. [PMID] [PMCID] [DOI:10.1556/EuJMI.3.2013.2.7]
5. Taghinejad J, Barati B, Sadeghi A. A study of the drug resistance pattern of Group B Streptococcus isolated from urinary samples in the city of Salmas during the year 2015. New Cellular Mol Biotechnol j. 2018;8(30):79-84.
6. Ullmann A. Escherichia coli and the Emergence of Molecular Biology. EcoSal Plus. 2011;4(2). [DOI:10.1128/ecosalplus.1.1.2] [PMID]
7. Ruppé E. Épidémiologie des bêta-lactamases à spectre élargi: l'avènement des CTX-M. Antibiotiques. 2010;12(1):3-16. [DOI:10.1016/j.antib.2010.01.003]
8. Roshdi Maleki M, Taghinejad J, Momen Amir H. Drug susceptibility of E. coli strains isolated from patients with UTI by disc diffusion agar method in Salmas city of Iran. Health MED.1146.
9. Akya A, Gheisari H, Mohammadi G, Khodadoost M. Study of the pattern of plasmid and antibiotic resistance of Escherichia coli isolated from outpatients with urinary tract infection. Sci J Kurd Univ Med Sci. 2015;20(1):89-96.
10. Kadaei V, Rashki Ghalenoo Z. Identification and determination of tetracycline resistance genes in uropathogenic Escherichia coli isolates in Sistan. Vet Microbiol. 2019;2(1):1-9.
11. Tuckman M, Petersen PJ, Howe AY, Orlowski M, Mullen S, Chan K, et al. Occurrence of tetracycline resistance genes among Escherichia coli isolates from the phase 3 clinical trials for tigecycline. Antimicrob Agents Chemother. 2007;51(9):3205-11. [DOI:10.1128/AAC.00625-07] [PMID] [PMCID]
12. Forbes BA, Sahm DF, Weissfeld AS. Diagnostic microbiology: Mosby St Louis; 2007.
13. Satlin MJ, Lewis JS, Weinstein MP, Patel J, Humphries RM, Kahlmeter G, et al. Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing position statements on polymyxin B and colistin clinical breakpoints. Clin Infect Dis. 2020; 71(9):e523-e9. [DOI:10.1093/cid/ciaa121] [PMID]
14. Li X, Jensen BB, Højberg O, Noel SJ, Canibe N. Development of a species-specific TaqMan-MGB real-time PCR assay to quantify Olsenella scatoligenes in pigs offered a chicory root-based diet. AMB Express. 2018;8(1):1-13. [PMCID] [DOI:10.1186/s13568-018-0627-y] [PMID]
15. Moldagulova N, Ayupova A, Sembayeva D, Duambekov M, Khassenova E, Nagyzbekkyzy E, et al. Data on the isolation and identification of thermotolerant microorganisms from cow manure promising for organic waste processing. Data in brief. 2020;31:105761. [PMID] [PMCID] [DOI:10.1016/j.dib.2020.105761]
16. Hashemi AB, Nakhaei Moghaddam M, Forghanifard MM, Yousefi E. Detection of blaOXA-10 and blaOXA-48 Genes in Pseudomonas aeruginosa Clinical Isolates by Multiplex PCR. J Med Microbiol Infect Dis. 2021; 9(3):142-7. [DOI:10.52547/JoMMID.9.3.142]
17. Xu G, An W, Wang H, Zhang X. Prevalence and characteristics of extended-spectrum β-lactamase genes in Escherichia coli isolated from piglets with post-weaning diarrhea in Heilongjiang province, China. Front Microbiol. 2015;6:1103. [DOI:10.3389/fmicb.2015.01103] [PMID] [PMCID]
18. Møller TS, Overgaard M, Nielsen SS, Bortolaia V, Sommer MO, Guardabassi L, et al. Relation between tetR and tetA expression in tetracycline resistant Escherichia coli. BMC Microbiol. 2016; 16(1):1-8. [DOI:10.1186/s12866-016-0649-z] [PMID] [PMCID]
19. Moghadam M N, MM F. Antibiotic susceptibility and multi-drug resistance of escherichia coli isolates producing CTX-M and TEM type beta-lactamases in Mashhad, Iran, in 2010. 2012.
20. Naji-Hamad M, Nakhaei-Moghaddam M. Detection of Tetracycline-Resistant Genes by

Multiplex Polymerase Chain Reaction in Clinical Isolates of Klebsiella Pneumonia. J Isfahan Med Sch. 2021;39(647):815-20.

21. Chaudhary M, Payasi A. Prevalence, genotyping of Escherichia coli and Pseudomonas aeruginosa clinical isolates for Oxacillinase resistance and mapping susceptibility behaviour. J Microb Biochem Technol. 2014;6(2):63-7. [DOI:10.4172/1948-5948.1000123]

22. Bokaian M, Shahraki S, Raeisi J, Mohammadzadeh Rostami F. Prevalence of OXA-2 and OXA-10 Type ESBL among Acinetobacter Strains Isolated from Patients of Zahedan (South Eastern Iran). Med J Mashhad Univ Med Sci. 2016;59(1):26-34.

23. Hujer Kristine M, Hujer Andrea M, Hulten Edward A, Bajaksouzian S, Adams Jennifer M, Donskey Curtis J, et al. Analysis of Antibiotic Resistance Genes in Multidrug-Resistant Acinetobacter sp. Isolates from Military and Civilian Patients Treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother. 2006;50(12):4114-23. [DOI:10.1128/AAC.00778-06] [PMID] [PMCID]

24. Amiri M, Farzin H, Jamshidian-Mojaver M. Phenotypic and Genotypic Study of Antibiotic Resistance among Escherichia coli Isolates from Human Urinary Infection Cases in Bojnord Province. Avicenna J Clin Med. 2019;26(3):173-80. [DOI:10.29252/ajcm.26.3.173]

25. Mousavi P, Zaker Bostan Abad S, Mirnejad R. Detection of TEM beta-lactamase Gene in Escherichia Coli Isolated from Urinary Tract Infection of referred to the clinic boarding Robat Karim to the molecular method. N Cell Mol Biotechnol J. 2018;8(30):85-94.

26. Maurya AP, Dhar D, Basumatary MK, Paul D, Ingti B, Choudhury D, et al. Expansion of highly stable bla OXA-10 β-lactamase family within diverse host range among nosocomial isolates of Gram-negative bacilli within a tertiary referral hospital of Northeast India. BMC Res Notes. 2017;10 (1):1-6. [DOI:10.1186/s13104-017-2467-2] [PMID] [PMCID]

27. Adefisoye MA, Okoh AI. Identification and antimicrobial resistance prevalence of pathogenic Escherichia coli strains from treated wastewater effluents in Eastern Cape, South Africa. MicrobiologyOpen. 2016;5(1):143-51 . [DOI:10.1002/mbo3.319] [PMID] [PMCID]

28. Malik I, Elhag K, Gustaw K. Characterisation of extended-spectrum β-lactamases among multidrug resistant Enterobacteriaceae from Sudan. J Pure Appl Microbiol. 2019;13(1):8. [DOI:10.22207/JPAM.13.1.06]

29. Boroumand M, Naghmachi M, Ghatee MA. Detection of phylogenetic groups and drug resistance genes of Escherichia coli causing urinary tract infection in southwest Iran. Jundishapur J Microbiol. 2021;14(2). [DOI:10.5812/jjm.112547]

30. Badi S, Cremonesi P, Abbassi MS, Ibrahim C, Snoussi M, Bignoli G, et al. Antibiotic resistance phenotypes and virulence-associated genes in Escherichia coli isolated from animals and animal food products in Tunisia. FEMS Microbiol Lett. 2018;365(10):fny088. [DOI:10.1093/femsle/fny088] [PMID]

31. Gurung S, Kafle S, Dhungel B, Adhikari N, Shrestha UT, Adhikari B, et al. Detection of OXA-48 gene in carbapenem-resistant Escherichia coli and Klebsiella pneumoniae from urine samples. Infect Drug Resist. 2020;13:2311. [DOI:10.2147/IDR.S259967] [PMID] [PMCID]