

Molecular epidemiology of antibiotic-resistant *Escherichia coli* among clinical samples isolated in Azerbaijan, Iran

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ABSTRACT

Background and Objectives: The immediate emergence of resistant bacteria poses an increasingly growing problem to human society and the increasing prevalence of antibiotic resistance in *Escherichia coli* strains is one of the most important health problems. This study aimed to review the molecular epidemiology of drug resistance among clinical isolates of *E. coli* in north-west portion of Iran Azerbaijan.

Materials and Methods: A complete of 219 clinical isolates of *E. coli* had been collected from the various clinical samples. The disk diffusion and agar dilution assays were used to determine antimicrobial susceptibility. The presence of antibiotics resistance genes was carried out by the PCR method.

Results: The highest susceptibility was shown to imipenem (3%) and fosfomycin (3%), and the most antibiotic resistance was presented to ampicillin (99%). The highest frequent ESBL gene among isolates was *bla*_{CTXM-15} in 70% followed by *bla*_{CMY-2} in 67%, and *bla*_{TEM-1} in 46%. The most common fluoroquinolone (FQ) resistance genes were *oqx*B (34%), followed by *oqx*A (25%), and *qnr*B (18%). The frequency of tetracycline resistance genes (*tet*A, *tet*B, *tet*C, and *tet*D) were detected in 24.8%, 31.6%, 1.8%, and 4.2%, respectively. The highest frequent genes to fosfomycin were *fos*A 10%, *fos*A3 30%, *fos*C 40%, and *fos*X 20%. The dominant founded aminoglycosides resistant genes were *arm*A (12.96%) and *npm*A (4.93%).

Conclusion: The prevalence of antibiotics resistance in the tested *E. coli* isolates was high in Azerbaijan, Iran and these findings showed that *E. coli* is one of the major drug-resistant pathogens.

Keywords: *Escherichia coli*; Antibiotic resistance; Epidemiology

INTRODUCTION

Escherichia coli (*E. coli*) is a facultative, anaerobic Gram-negative rods bacterium, that is the most common commensal inhabitant of the gastrointestinal tracts of people and warm-blooded animals (1).

E. coli causes many human infections such as sepsis, gastroenteritis, neonatal meningitis, wound infections, pneumonia, peritonitis, and especially urinary tract infections and kidney failure in children (2). Since 2015, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* have accounted for 70% of Gram-negative hospi-

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tal infections (3). During the last two decades, there has been an evident increase in infections due to antibiotic-resistant *E. coli* that could have altered the outcome in patients. Multidrug-resistant (MDR) *E. coli* and especially extended-spectrum β -lactamase (ESBL)-producing *E. coli* are of big worries caused by their increased incidence and their resistance to a broad range of β -lactams and different groups of antimicrobial agents (4, 5). This expanding resistance limits treatment choices and may influence the prognosis of *E. coli* infections. Many *E. coli* strains established multi, extensively or pan-drug resistance (MDR, XDR, and PDR) that posing a great challenge to infection treatment (6, 7).

The antimicrobial resistance mechanisms are possibly placed on the bacterial chromosome and develop naturally in each member of a species (intrinsic) or develop other bacteria, generally through a plasmid (acquired) (8). Gram-negative bacteria extensively use some mechanisms for resistance and are very competent in the horizontal transfer of resistance elements (9). This bacterium has to transport an aggregate of antibiotic genes with chromosomal and plasmid origins. Acquiring antibiotic resistance genes with plasmid and other transposable agents and mutations after antibiotic pressure has created super-resistant strains of high-risk MDR and XDR *E. coli* isolates in clinical environment and conceivably is extra serious in low- and middle-income countries (10). Antibiotic resistance reasons long disease, surplus mortality, and bigger expense for patients and healthcare systems (11). Regardless of increased alarms and many efforts to involve it, antibiotic resistance has been expanding (12).

Nosocomial infections are obtained throughout hospital treatment or in a hospital environment. One such infecting agent, *E. coli*, harbors several virulence genes that allow it to be made pathogenic, inducing damage to the host. Antibiotic resistance among *E. coli* isolates are unexpectedly raising around the world and this bacterium is considered to be a characteristic index of antimicrobial resistance of Gram-negative bacteria (13). Despite numerous studies about antibiotic resistance and susceptibility profiles in Iran, the complete epidemiology of *E. coli* resistance genes is now not clear still in a study to examine different antibiotics resistance genes simultaneously. According to the increasing use of antibiotics and the consequent increase in antibiotic resistance and the different susceptibility of *E. coli*

isolated in each region, it is necessary to investigate the antibiotic resistance of bacteria. Therefore, this study desired to assay the molecular epidemiology of drug resistance in clinical isolates of *E. coli* in the north-west portion of Iran Azerbaijan.

MATERIALS AND METHODS

Patients and bacterial isolates. This study was performed from July 2019 until June 2020 at the hospitals of Tabriz, in Iran. In this study, 219 clinical *E. coli* isolates were collected from different wards of the hospitals including, infectious, surgery, burn, emergency, internal wards and ICUs. They were isolated from urine, blood, tracheal tubes, ulcers and sputum. All specimens were cultured on sheep blood agar and MacConkey agar. *E. coli* isolates were detected utilizing conventional biochemical tests (14). This study was approved by the research ethics committee (IR.TBZMED.REC 1399.570) at Tabriz University of Medical Sciences, Tabriz, Iran.

Disk diffusion assay. Antibiotic susceptibility testing was achieved using Kirby-Bauer method, according to the CLSI guidelines (15), with a panel of following antibiotics: ciprofloxacin (5 μ g); nalidixic acid (30 μ g); ofloxacin (5 μ g); levofloxacin (5 μ g); moxifloxacin (5 μ g); gatifloxacin (5 μ g); streptomycin (10 μ g); trimethoprim-sulfamethoxazole (1.25/23.75 μ g); tetracycline (30 μ g); tobramycin (10 μ g); gentamicin (10 μ g); amikacin (30 μ g); kanamycin (30 μ g); cefoxitin (30 μ g); ceftriaxone (30 μ g); ceftazidime (30 μ g); imipenem (10 μ g); ertapenem (10 μ g); meropenem (10 μ g); cefepime (30 μ g); ampicillin (10 μ g); amoxicillin (10 μ g); gentamicin (10 μ g); ciprofloxacin (5 μ g); piperacillin/tazobactam (100/10); and cefixime (5 μ g). For quality control *E. coli* ATCC (American type culture collection) 25922 was used.

MIC determination. The minimum inhibitory concentration (MIC) of nalidixic acid, ciprofloxacin, levofloxacin, tetracycline, trimethoprim-sulfamethoxazole, trimethoprim, sulfamethoxazole, gentamicin, tobramycin, amikacin, kanamycin, cefoxitin, imipenem, ampicillin, amoxicillin, and fosfomycin was determined on Mueller-Hinton agar by agar dilution method according to the CLSI guideline. In ambient air, the plates were incubated at 35°C for 16 to 20 hours.

The MIC of every antimicrobial agent was deter-

mined as the lowest concentration that inhibited the apparent growth of the organism. The results were explained according to the CLSI guidelines. For quality control, *E. coli* ATCC (American type culture collection) 25922 was used (15).

Detection of ESBL by phenotypic tests. The ceftazidime (30 µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 g discs) were used with an increase of equal or more than 5 mm in the zone diameter of inhibition of the combination discs to the only ceftazidime disc was measured to be an ESBL producer. The results were explained according to the CLSI guidelines.

Determination of carbapenemases by the phenotypic tests. The ability of carbapenem-resistant isolates to produce carbapenemases was investigated by the Carba NP test and modified Hodge Test (MHT) technique (16, 17).

PCR amplification and evaluation of the antimicrobial resistance genes. Among the collection, 219 isolates, which showed a phenotype of resistance to one or more antimicrobial agents, were investigated for antimicrobial resistance genes. The genomic DNA for PCR was extracted by the cetyltrimethylammonium bromide (CTAB) method as described previously (18). PCR assays were achieved by specific primers for the resistance genes of various classes such as ESBLs, carbapenemases; fluoroquinolones, tetracycline, trimethoprim/sulfamethoxazole, aminoglycosides, and fosfomycin. PCR amplification for detecting antimicrobial resistance genes was conducted by the previous reports (19-25). PCR products were analyzed by electrophoresis in 1% agarose gel, and then staining with 0.5 µg/mL safe stain. The gel was visualized under ultraviolet (UV) light.

Statistical analysis. The results of study were analyzed utilizing expressive statistics in SPSS software for Windows (version 21 SPSS Inc., Chicago, IL, USA). According to the study results, $p \leq 0.05$ was considered statistically significant.

RESULTS

Patients and bacterial isolates. The mean age of patients was 46 ± 31 years (range, 1-89 years), and

included 78 (36%) males and 141 (64%) females. The *E. coli* isolates (n=219) were identified in biochemical and microbiological tests. These isolates had been cultured from urine (n=157), bloodstream (n=29), wound (n=16), respiratory (n=4), and peritonea (n=5); in various wards of the hospitals internal 144 (66%), surgery 37 (17%), intensive care unit (ICU) 24 (11%), and pediatrics 14 (6%) were collected.

Antibiotic susceptibility patterns. The antibiotics susceptibility testing was evaluated by the disk diffusion agar method (DDA) according to the CLSI guidelines. The least resistance was for imipenem (3%) and fosfomycin (3%) and the most resistance was for ampicillin (99%). Due to the disk diffusion agar (DDA), the prevalence of resistance of *E. coli* to antimicrobial agents was as follows: sulfamethoxazole (87%), trimethoprim (78%), nalidixic acid (73%), trimethoprim-sulfamethoxazole (70%), ciprofloxacin (68%), moxifloxacin (66%), gatifloxacin (63%), tetracycline (60%), levofloxacin (58%), streptomycin (54%), gentamicin (51%), tobramycin (51%), kanamycin (49%), ceftriaxone (45%), cefotaxime (44%), doxycycline (44%), ceftazidime 174 (35%), pirazinamide (31%), cefotaxin (13%), amikacin (8%), and imipenem 7 (3%) (Fig. 1).

The most resistance to antibiotics was observed in the internal ward (n=86, 60%) followed by, surgery (n=21, 57%), ICU (n=9, 38%), and pediatrics (n=9, 30%). There was a significant association among resistance to antibacterial agents and the various wards of the hospital ($P \leq 0.05$). There were no significant relationships between age, gender, antimicrobial resistance ($P > 0.05$). Of the MDR isolates with phenotypic confirmatory, 42.6% were ESBLs-producer. The carbapenem-resistant isolates by the modified Hodge test and Carba NP test were 4.8%. The MIC method results were consonant with the DDA method and they were not so different (Fig. 2). Considering the agar dilution assay, 49, 93, and 62 of the isolates had been incredibly resistant; $\text{MIC} \geq 64$ µg/mL, $\text{MIC} \geq 512$ µg/mL, and $\text{MIC} \geq 128$ µg/mL to ciprofloxacin, nalidixic acid and levofloxacin, respectively.

Molecular epidemiology. The most predominant resistance genes were *sul1* (69.5%) followed by beta-lactamases resistance genes *bla*_{TEM-1} (46%) and fluoroquinolone resistance genes *OqxB* (34%). The distribution of other antibiotics resistance genes among *E. coli* isolates is shown in Table 1.

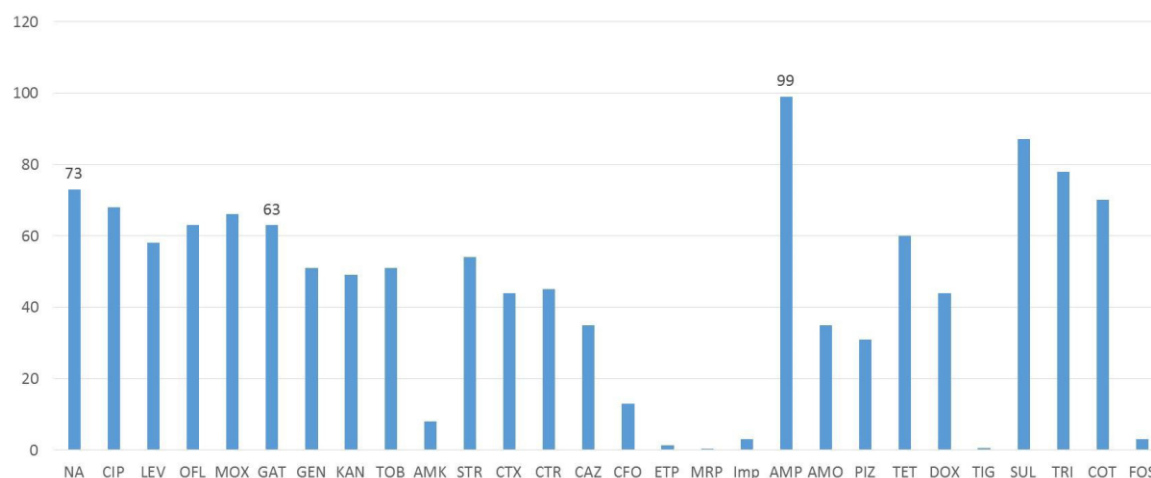


Fig. 1. Antibiotic resistance patterns of *E. coli* isolates according to the disk diffusion assay. NA; Nalidixic Acid, CIP; Ciprofloxacin, OFL; Ofloxacin, MOX; Moxifloxacin, GAT; Gatiocyclin, GEN; Gentamicin, KAN; Kanamycin, TOB; Tobramycin, AM; Amikacin, STRP; Streptomycin, CTX; Cefotaxime, CTR; Ceftriaxone, CAZ; Ceftazidime, CFO; Cefoxitin, ETP; Ertapenem, MRP; Meropenem, IMP; Imipenem, AMP; Ampicillin, AMO; Amoxicillin, PIZ; Pyrazinamide, TET; Tetracycline, DOX; Doxycycline, TIG; Tigecycline, SUL; Sulfafurazole, TRI, Trimethoprim, COT, Trimethoprim/sulfamethoxazole, FOS, Fosfomycin.

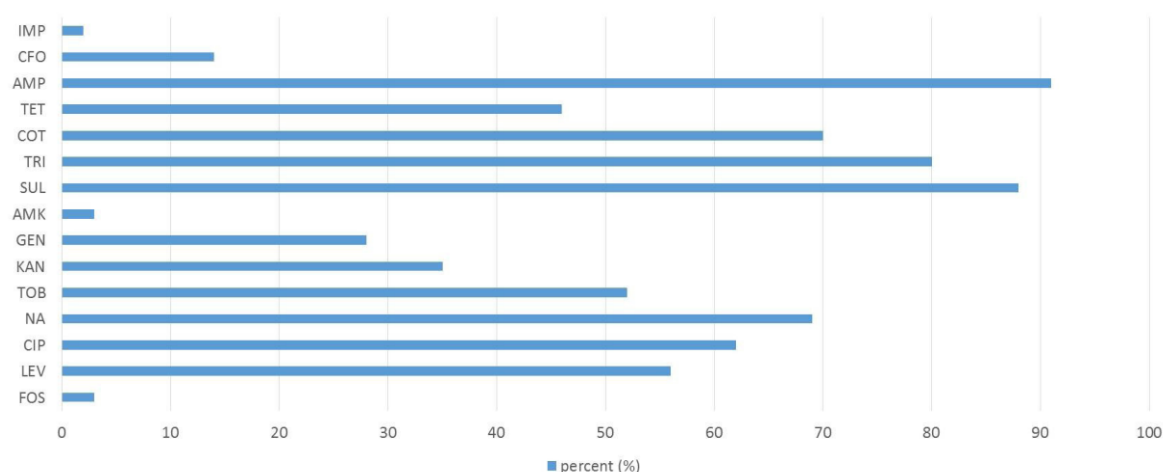


Fig. 2. Antibiotic resistance patterns of *E. coli* isolates according to the Agar dilution assay. IMP; Imipenem, AMP; Ampicillin, COT, Trimethoprim/sulfamethoxazole, SUL; Sulfafurazole, GEN; Gentamicin, , TOB; Tobramycin, CIP; Ciprofloxacin, FOS, Fosfomycin.

DISCUSSION

The results of current study exhibits that among the *E. coli* isolates, elevated resistance rates were related to ampicillin (99%), sulfamethoxazole (87%), and trimethoprim (78%). The rate of resistance of *E. coli* to trimethoprim-sulfamethoxazole account has risen over the three years, and it was nearly 100 percent (26). In Kerman city a province in Iran, Mansouri

et al. reported resistance rates to trimethoprim/sulfamethoxazole (93.4%) and amoxicillin (91.4%) among *E. coli* isolates (27). In another study from Iran, Arabi et al. reported that 60.4% of *E. coli* isolates from urine samples were resistance to trimethoprim/sulfamethoxazole (co-trimoxazole) (28). Shehata, et al. showed that *E. coli* from humans were resistant to ampicillin (72.7%).

The lowest resistance prevalence was found against

Table 1. Distribution of antibiotic resistance genes in the current study.

Antibiotics	Genes conferring resistance (%)
Fluoroquinolone	<i>OqxA</i> (25%), <i>OqxB</i> (34%), <i>qepA</i> (7%), <i>qnrA</i> (5%), <i>qnrB</i> (18%), <i>qnrC</i> (6.5%), <i>qnrD</i> (15%), <i>qnrS</i> (17.6%)
Ciprofloxacin, ofloxacin, gatifloxacin, levofloxacin, and moxifloxacin	
Tetracycline	<i>tetA</i> (24.8%), <i>tetB</i> (31.6%), <i>tetC</i> (1.8%), <i>tetD</i> (4.2%)
Tetracycline, doxycycline, and tigecycline	
Co-trimoxazole	<i>dfr</i> (33.3%), <i>Sul1</i> (69.5%), <i>Sul2</i> (40%), <i>Sul3</i> (4.5%)
Aminoglycosides	<i>ArmA</i> (12.96%), <i>npmA</i> (4.93%), <i>rmtA</i> (1.23%), <i>rmtB</i> (0.61%), <i>rmtCy</i> (1.23%)
Gentamicin, amikacin, kanamycin, and tobramycin	
Beta-lactamases	<i>bla_{TEM-1}</i> (46%), <i>bla_{TEM-16}</i> (41%), <i>bla_{TEM-12}</i> (0.9%), <i>bla_{TEM-24}</i> (0%), <i>bla_{SHV-1}</i> (19%), <i>bla_{SHV-5}</i> (10%), <i>bla_{SHV-11}</i> (24%), <i>bla_{SHV-12}</i> (29%), <i>bla_{SHV-28}</i> (0.9%), <i>bla_{SHV-2a}</i> (0%), <i>bla_{SHV-27}</i> (10%), <i>bla_{SHV-110}</i> (0.8%), <i>bla_{CTXM-15}</i> (70%), <i>bla_{CTXM-3}</i> (8%), <i>bla_{CTXM-1}</i> (0%), <i>bla_{CTXM-55}</i> (4%), <i>bla_{CTXM-27}</i> (5%), <i>bla_{CTXM-14}</i> (5%), <i>bla_{CTXM-61}</i> (1%), <i>bla_{CTXM-9}</i> (1%), <i>bla_{CTXM-24}</i> (0%), <i>bla_{MBL}</i> (0.5%), <i>bla_{NDM}</i> (0.3%), <i>bla_{AmpC}</i> (8%), <i>bla_{CMY-2}</i> (67%), porin loss (3%)
Cefoxitin, ceftazidime, cefotaxime, ertapenem, imipenem, meropenem, ampicillin, ceftriaxone, amoxicillin-clavulanate, piperacillin-tazobactam, and aztreonam	
Fosfomycin	<i>fosA</i> (10), <i>fosA3</i> (30), <i>fosC</i> (40), <i>fosX</i> (20)

imipenem (1%) and amikacin (8%). In another Iranian study, various resistance levels have been reported. Similar to our study Pouladfar et al. in Shiraz, reported two imipenem-resistant urinary *E. coli* (1.9%) (29). Also, in another study, Soleimani et al. showed that resistance to amikacin among isolates were 3.62% (30). On the other hand in contrast to our study, another report from Pakistan showed 91% and 59% of resistance to amikacin and tobramycin, respectively (30, 31). Differences in the frequency of resistance to these antibiotics are due to the differences in geographic areas, the program of infections control, and the pattern of antibiotics utilization. In our study, the rate of resistance to ciprofloxacin was 63% that in another study from Iran was 51% (32).

The frequency of ESBL genes was investigated among the ESBL-positive clinical isolates of *E. coli* that the most frequent ESBL genes in the tested isolates were *bla_{CTXM-15}* in 70% followed by *bla_{CMY-2}* in 67% and *bla_{TEM-1}* in 46%. In our previous review study conducted in Iran, we reported that in most regions of Iran ESBL-producing prevalence is high and *E. coli* is the most ESBL-producing bacterium in this country, which in some regions up to 89.8% of the isolates were positive for ESBL (33). The prevalence of ESBL-producing isolates is high in all parts of Iran. Moreover the dominant ESBL-producing bacterium in Iran is *E. coli* and we understood that up to 89.8% of these isolates were ESBL positive (33).

A study showed that among different β -lactam resistance genes present in *E. coli* strains, *bla_{TEM}* was the most frequent gene (100%), followed by *bla_{CTXM-15}* (16%) and plasmid-mediated *ampC* (3%) (34). According to the results of a systematic review in Iran with an analysis of several articles, *bla_{TEM}* reported as the most common gene (51%) in Iran (37). In recent years, the public health challenge is that *CTXM-15* is an increasing number of found in *E. coli* isolates with the ability to produce ESBL. A study investigated the gene *CTXM-15* in various countries of Africa and reported that highest prevalent in ESBL-producing *Enterobacteriaceae* (26). Rezai et al. in Iran reported that among one hundred isolates of urine bacteria producing ESBL the abundance of *TEM* genes of *E. coli* was 49% (35).

The resistance rate to tetracycline by the DDA assay in the current study was 60%. The distinct frequency of tetracycline resistance was described in some countries. In contrast to our study, from Pakistan, the rate of tetracycline resistance was reported 93% in *E. coli* isolates (35). The prevalence of tetracycline resistance in some regions in Europe was found to be 66.9% ESBL-producing *E. coli* (36). Molecular recognition of *E. coli* tet genes in our region shows a rise in resistance genes that among *tet* genes the *tetB* gene was the highest prevalent tetracycline resistance determinant detected. In the current study, *tetA*, *tetB*, *tetC* and *tetD* among the detected tetracycline resistance genes, were detected in 24.8%, 31.6%, 1.8%,

and 4.2%, respectively. Another study from Tabriz, Iran, *tetA*, *tetB*, *tetC*, and *tetD* in among *Enterobacteriaceae* isolates were 14.4%, 18.4%, 2%, and 4.4%, respectively (37).

Fosfomycin is being reconsidered as a choice for treatment of MDR Enterobacterales infections including ESBL-producing isolates. *E. coli* appears to show low rate of resistance to fosfomycin. Resistance to this antibiotic in Gram-negative bacteria demonstrates the formation of fosfomycin-inactivating enzymes (*fos* genes). The *fosA3* gene is the main type in Enterobacterales and at the minimum 10 types of *fos* genes are known. The plasmid-mediated resistance in isolates inducing human infections had been shown only in Asian countries (38). In the present study, the resistance rate to fosfomycin was 3%. The resistance rate to fosfomycin in another study from Iran, among *E. coli* was 15% (39). In the current study, the most detected resistance genes to fosfomycin were *fosA* (10%), *fosA3* (30%), *fosC* (40%), *fosX* (20%), which is different from some other studies in Iran. In a study from Iran *fosC2* and *fosA3* genes were not detected in *E. coli* isolates (40). Another study from Tabriz in 2018, among *E. coli* isolates, the frequency of *fosA*, *fosA3*, *fosC*, and *fosX* reported as 0.59%, 1.18%, 1.77%, and 1.18%, respectively (41). Although fosfomycin has a good antibacterial function, the growing resistance genes in recent years in our region is warning because of the special locations on mobile genetic elements like the plasmids.

Resistance to FQs has been growing following the widespread utilization of FQs in the world (42). In the present study, a higher FQ resistance rate was recognized among *E. coli* isolates (63.75%). The frequency of resistance to FQs is different in other countries, e.g. India (89%), Egypt (41.3%) and Iran (60.4%) (43-45). Fluoroquinolones are broad-spectrum synthetic drugs given comprehensively in the treatment of bacterial infections. In *E. coli*, mutational alterations in the FQ target enzymes, namely, DNA topoisomerase II (DNA gyrase) and topoisomerase IV, are established to be the important mechanisms through which resistance shows. In present study, the high prevalent PMQR gene was *oqxB* (34%) followed by *oqxA* (25%), *qnrB* (18%), *qnrS* (17.6%), *qepA* (7%), *qnrC* (6.5%), *qnrA* (5%). Yuan et al. in China, reported that 6.6% of *E. coli* isolated harbored the *oqxAB* genes (46). The prevalence of *oqxAB* in Kim et al. (47), was lower than the current results. Our results

showed that *qnrA* and *qnrC* were low in *E. coli* isolates. In other study, the presence of *qnrA* and *qnrC* genes was not detected in any isolates (48). These dissimilarities in the prevalence of PMQR principles and possibly connected to differences in FQ usage, study period and, geographical area.

The modification of the ribosomal target, enzymatic modification, and diminished intracellular antibiotic accumulation by alterations of the outer membrane permeability, reduced inner membrane transport, or active efflux pumps are the mechanisms of resistance to aminoglycosides. In the current study, the resistance of *E. coli* isolates to gentamicin, tobramycin, kanamycin, and amikacin was 51%, 51%, 49% and 8%, respectively. Another study from Iran, among *E. coli* isolates, reported antibiotic susceptibility testing showed that 21%, 24.6%, 23.18% and 3.62% of the isolates were resistant to gentamicin, tobramycin, kanamycin and amikacin respectively (30). Our findings are different since resistance to aminoglycosides antibiotics in the isolates are remarkable. The results of present study revaluated that the high frequent aminoglycosides resistant genes were *armA* (12.96%), *npmA* (4.93%), *rmtA* (1.23%), *rmtB* (0.61%) and *rmtCy* (1.23%). The rate of *armA* gene in our study is near to another report from Iran (9.5%) (22), but higher than results reported from Chinese (0.9%) (49), Belgium (0.12%) (50) and France (1.3%) (51). A study reported *armA* 11.6%, *rmtB* 82.1% genes of MDR *E. coli*, and also no *npmA* was detected in this study (52).

Cotrimoxazole is created from a combination of two antibiotics containing trimethoprim and sulfamethoxazole, both of which disrupt the folic acid metabolism in bacteria. The extensive use of this drug, low cost and availability have led to increased resistance to cotrimoxazole (53). In the current study, resistance was high against co-trimoxazole (70%). Other studies by Azizi al. reported 48% resistance to co-trimoxazole in *E. coli* isolated from Iran (54). The results of the present study show that high resistant genes co-trimoxazole were *dfr* (33.3%), *sul1* (69.5%), *sul2* (40%), *sul3* (4.5%). The frequency of *sul* genes in a previous study in Iran on *E. coli* isolates was close to our study results (28) and *sul1* (81%), *sul2* (67%) and *sul3* (2.29%) was detected which is higher and differs from other studies conducted worldwide (55, 56). The *sul* genes in a previous study from Iran on *E. coli* isolates was near to our study results (28).

CONCLUSION

The clinical isolates of *E. coli* from various sources of infection were investigated for the presence of antibiotic susceptibility testing and antibiotics resistance genes. The resistance to ampicillin is high in our study, followed by co-trimoxazole. Resistance to antibiotics and presence of antibiotics genes among *E. coli* isolates in our region, like some other parts of the world, substantially has been increased. The outcome of this study could be used in a strategic policy to limit the prevalence of antibiotics resistance among *E. coli*.

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