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High Frequency of Class 1 Integrons in *Escherichia coli* Isolated From Patients With Urinary Tract Infections in Yasuj, Iran

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Abstract

Background: Most urinary tract infections (UTI) are caused by *Escherichia coli*. Integrons have an important role in distributing antibiotic resistance genes among bacteria.
Objectives: The aim of this study was to investigate the presence of class 1, 2 and 3 integrons and their association with antibiotic resistance in *E. coli* isolated from patient with UTI in Yasuj, Iran.
Patients and Methods: In this cross-sectional study a total of 200 *E. coli* were collected from 1820 patients diagnosed with UTI that had been referred to two clinical laboratories between February 2013 and November 2014 in Yasuj city, southwest of Iran. Susceptibility of isolates to 11 different antibiotics was determined by the disk agar diffusion method. multiplex-polymerase chain reaction (PCR) was used for detection of class 1, 2 and 3 integrons. The data were analyzed using the SPSS software (version 16) and the chi-square test. A P value of < 0.05 was considered statistically significant.
Results: The highest rate of resistance was observed toward cephalothin (99%) and amoxicillin (76%) while only two (1%) isolates showed resistance to imipenem. Overall, 79% of isolates were multi drug resistant (MDR). Class 1 and 2 integrons were detected in 104 (52%) and 5 (2.5%) isolates respectively, while none of the isolates were positive for class 3 integrons. A significant association was observed between the presence of integrons and resistance to co-trimoxazole, nalidixic acid, ciprofloxacin, amoxicillin, ceftazidime and tetracycline (P < 0.05).
Conclusions: High MDR isolates of *E. coli* were observed in this study. The significant association between class 1 integrons and resistance to ciprofloxacin, nalidixic acid, co-trimoxazole, amoxicillin, ceftazidime and tetracycline showed that class 1 integrons have an important role in resistance to these antibiotics in this region.

Keywords: Integrons, Urinary Tract Infections, Multidrug Resistant, *Escherichia coli*

1. Background

Urinary tract infection (UTI) is one of the most prevalent infections in human beings (1). Community acquired UTI and nosocomial infection are two types of urinary tract infections (2). Most of the cases of UTI are commonly caused by gram negative bacteria; *Escherichia coli* is responsible for 80% of infections (3) followed by *Klebsiella pneumonia*, *Acinetobacter* spp and *Enterobacter* spp (4). The Uropathogenic *Escherichia coli* (UPEC) strains are the most important causes of UTIs (5, 6). However, the gram-positive bacteria, *Staphylococcus saprophyticus*, can cause UTI in young females (7). Increasing resistance to antibiotics by UTI-causing organisms is a serious public health problem (8, 9). High levels of resistance to antibiotic agents that are frequently used to treat UTI infections have been reported in UTI-causing *E. coli* (10, 11). Horizontal gene transfer (HGT) has an important role in spreading resistance genes among bacteria through mobile genetic elements such as, plasmids, transposons and integrons (12). Integrons are genetic elements that are capable of integration of resistance gene cassettes in their structure and subsequently denote resistance phenotype to their bacterial host (13). Integrons can be located within transposons or conjugated plasmids and transfer along with them, facilitating the dissemination of antibiotic resistance genes between bacteria (14). Structurally, integrons consist of two 5' conserved segments (5'-CS) and 3' conserved segments (3'-CS) that flank the central variable region or gene cassettes, which encode antibiotic resistance traits (15). The 5'-CS of integrons encode the integrase

(*IntI*) gene, the recombination site (*attI*) and the common promoter (Pc), while the 3'-CS harbors the *qacE Δ1* gene and *sul1* gene that mediate resistance to certain detergents and sulphonamides, respectively (16). In addition to encoding resistance genes by gene cassettes, they include a recombination site (*attC*). Recombination between the *attl* and *attC* sites leads to insertion of the gene cassette downstream of a resident promoter, which mediated by the *intI* gene (17). Based on the homology of the *intI* gene, four classes (1-4) of integrons have been identified (18); class 1 integrons are the most prevalent followed by class 2 integrons (19). More than 194 gene cassettes encode antibiotic resistance to aminoglycoside, β-lactams, chloramphenicol, quinolones and trimethoprim (20). One or more gene cassette can be incorporated in integrons, however usually less than five cassettes with numerous combinations are carried by isolated bacteria (20-22). Hence, the presence of integrons among bacteria is associated with multiple drug resistance (MDR), especially in enteric bacteria such as *E. coli* (23). The MDR enterobacteriaceae serve as a major public health problem and a strong association between MDR and the presence of integrons has been shown in Enterobacteriaceae, independent of species or strain origin (24). Many studies have investigated the presence of integrons in *E. coli* isolated from patient with UTI, and they reported a significant association between antimicrobial resistance and existence of integron (23-28). Pattern of resistance to antibiotics change continuously in UTI-causing organisms, therefore appropriate information about local and national antimicrobial resistance will be needed for empirical therapy of UTI (28).

2. Objectives

Considering the importance of regional and local information about antibiotic resistance pattern among *E. coli* isolated from patients with UTIs, and the association between integrons and MDR among gram negative bacteria, the aim of this study was to determine the pattern of antibiotic resistance to commonly used antimicrobial agents, and to investigate the presence of class 1, 2 and 3 integrons and their association with antibiotic resistance in *E. coli* isolated from patient with UTIs in Yasuj, Iran.

3. Materials and Methods

3.1. Sample Collection and Antimicrobial Susceptibility Test

In this cross-sectional study, midstream urine samples of 1820 patients diagnosed with UTIs were collected in sterile universal containers from February 2013 to November 2014.

All patients had attended two clinical laboratories in Yasuj city, southwest Iran. Exclusion criteria were having an indwelling urinary catheter, being pregnant, having genitourinary abnormalities and antibiotics therapy within the previous two weeks. Urine samples were inoculated on MacConkey agar and blood agar plates using calibrated loops (0.001 mL) and incubated at 37°C for 18 to 24 hours. The number of colony forming units (cfu) was counted. A growth of $> 10^5$ colony forming units/mL of one type of organism was considered as significant bacteriuria. The isolates were identified to the species level using conventional biochemical tests, such as indole production, methyl red, Voges-Proskauer, Simmons' citrate, hydrogen sulfide, and urea hydrolysis (29). Finally 200 isolates of *E. coli* were identified. All of the isolates were stored at -20°C in trypticase soy broth (Mast Group Ltd. UK) containing 15% glycerol (29).

Disks containing antibiotics (Himedia, India) were used to determine the susceptibility of *E. coli* isolates to the following antibiotics, according to the clinical and laboratory standards institute (CLSI) guidelines (30): amoxicillin (25 µg), gentamycine (10 µg), amikacin (30 µg), co-trimoxazole (25 µg), tetracycline (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), imipenem (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), ceftazidime (30 µg). *Escherichia coli* ATCC 25922 was used as a quality control strain. This study was approved by the ethical committee of Yasuj University of Medical Sciences (ethical code number: 20.11.91-91011020).

3.2. Multiplex-Polymerase Chain Reaction for Detection of Class 1, 2 and 3 Integrons

Genomic DNA was extracted according to the method of Yu et al. (23) with some modifications. Briefly, three to five colonies of overnight culture of *E. coli* on brain heart infusion agar was suspended in 300 µL of sterile distilled water and boiled at 95°C for ten minutes then cell debris was removed by centrifugation for tenminutes at 13000 rpm, and 200 µL of the supernatant was stored at -20° C for DNA amplification.

The *intI1*, *intI2* and *intI3* genes were amplified by a multiplex-polymerase chain reaction (PCR) method using primers described by Goldstein et al. (8) (Table 1). The PCR amplification was performed in a total volume of 25 µL containing 12.5 µL of master mix (Cinnagen, Iran), 25 pmol of each primer and 3 µL of DNA template. The PCR conditions are presented in Table 2. Expected amplified products, 280 bp for integron class 1, 233 bp for integron class 2 and 600 bp for integron class 3 were separated by electrophoresis on 1% agarose gel containing 0.5 µg/mL ethidium bromide and photographed under UV illumination.

3.3. Statistical Analysis

The SPSS software (version 16, Chicago, IL, USA) was used for statistical analysis. The association between presence of integrons and antibiotic resistance was determined by χ^2 or Fisher's exact test. A P value of < 0.05 was considered statistically significant.

4. Results

Among the 200 UTI *E. coli* strains, 144 (72%) strains were isolated from females and 56 (28%) strains were isolated from males. High frequencies of resistance were observed toward cephalothin (99%) and amoxicillin (76%) while only two (1%) isolates showed resistance to imipenem. The *E. coli* isolates were also resistant to co-trimoxazole (62%), tetracycline (50%), nalidixicacid (48.5%), ceftazidime (40.5%), ciprofloxacin (29%), gentamicin (15.5%), chloramphenicol (13%) and amikacin (3%). Of the 200 tested *E. coli* isolates, 158 (79%) were MDR and were resistant to three or more antibiotics. None of the isolates were fully susceptible or resistant to all of the tested antibiotics.

Sixty-six antibiotic resistance patterns were observed in *E. coli* isolates, yet 30 resistance phenotypes were presented by only one isolate. Resistance to cephalothin was the most common phenotype, which was detected in 27 isolates. The second resistance phenotype was towards 13 isolates, including resistance to cephalothin, amoxicillin, co-trimoxazole and tetracycline.

Class 1 and 2 integrons were detected in 104 (52%) and 5 (2.5%) isolates, respectively, while none of isolates were positive for class 3 integrons. Two isolates had both class 1 and 2 integrons. Ninety-three of the isolates (46.5%) did not contain any classes of integrons. Among the 158 MDR *E. coli*, 101 (63.9%) harbored class 1 or class 2 integrons yet only 5 (11.9%) of the 42 non-MDR isolates had integrons.

The carriage of class 1 integron was found to be significantly higher in co-trimoxazole ($P < 0.001$), nalidixic acid ($P < 0.001$), ciprofloxacin ($P < 0.015$), amoxicillin ($P < 0.001$), ceftazidime ($P < 0.003$) and tetracycline ($P < 0.012$) resistant isolates (Table 3).There was no significant association between resistance to chloramphenicol, amikacin and gentamicin with the presence of class 1 integrons.

Table 1. Sequences of Primers Used for Detection of *intI-1*, *intI-2* and *intI-3* Genes in *Escherichia coli* Isolates

Gene Type/Primer	Sequence (5' → 3')	Product Size, bp	References
intI-1		280	(8)
F:	CCTCCCGCACGATGATC		
R:	TCCACGCATCGTCAGGC		
intI-2		233	(8)
F:	TTATTGCTGGGATTAGGC		
R:	ACGGCTACCCTCTGTTATC		
intI-3		600	(8)
F:	AGTGGGTGGCGAATGAGTG		
R:	TGTTCTTGTATCGGCAGGTG		

Abbreviations: F, forward; R, reverse.

Table 2. Multiplex Polymerase Chain Reaction Conditions for Detection of Class 1, 2 and 3 Integrons Among 200 *Escherichia coli* Isolates

	Temperature, °C	Time, s
First Denaturation	94	240
Extension Final	72	600
Cycles: 30		
Denaturation	94	50
Annealing	55	30
Extension	72	60

Table 3. The Correlation Between Presence of Integrons and Antibiotic Resistance Among 200 *Escherichia coli* Isolated From Patients With Urinary Tract Infections

Antibiotics	Integron Class 1 Positive[a]	Integron Class 2 Positive[a]	Total Resistance	Association With Class 1 Integrons (P Value)
Co-trimoxazole	88 (44)	4 (2)	124	< 0.001[b]
Chloramphenicol	19 (9.5)	0	26	< 0.44
Tetracycline	55 (27.5)	4 (2)	100	< 0.012[b]
Amikacin	2 (1)	0	6	< 0.28
Gentamicin	21 (10.5)	2 (1)	31	< 0.09
Ciprofloxacin	38 (19)	3 (1.5)	58	< 0.015[b]
Nalidixic acid	63 (31.5)	4 (2)	97	< 0.001[b]
Amoxicillin	93 (46.5)	5 (2.5)	152	< 0.001[b]
Ceftazidime	52 (26)	5 (2.5)	81	< 0.003[b]
Cephalothin	104 (52)	5 (2.5)	198	NA[c]
Imipenem	0	0	2	NA[d]

Abbreviation: NA, not available.
[a]Values are expressed as No. (%).
[b]P values that indicate significance.
[c]Only two sensitive isolates.
[d]Only 2 resistant isolates.

5. Discussion

Consistent with previous reports, in this study class 1 integrons were the most prevalent compared to the other tested integrons. In accordance with our study, Chang et al. from Taiwan and Li et al. from China detected class 1 integrons in 64% and 66.5% of *E. coli* isolated from different clinical specimens and blood stream infections, respectively (31, 32). Many reports have shown the prevalence rate of integrons to be between 6.25% and 54.6% of clinical isolates (33-38). There have been various studies from different cities of Iran: Fallah et al. from Tehran detected class1 integrons in 50.3% of isolates (37) while Rezaee et al. from Tabriz, and Japoni et al. and Farshad et al. from Shiraz identified this class of integrons in 22.05%, 33.34% and 6.25% of *E. coli*, respectively (33-35). Farshad et al. detected integrons in only 6.25% of *E. coli* isolated from children with UTI and suggested that antibiotic resistance cassettes may be carried on transposable elements or other plasmids rather than integrons. Class 2 integrons were detected in 2.5% of isolates in this study, which is lower than that reported by other studies, such as Fallah et al. (12.5%), Farshad et al. (10.41%), Rezaee et al. (5.08%), Cao et al. (5.88%) and (6.7%) Japoni et al. (33-35, 37, 39). Similar to previous studies we couldn't find class 3 integrons in the present study (31-39). Antibiotic resistance encoding gene cassettes for fluoroquinolones, β-lactams, aminoglycosides, trimethoprim and chloramphenicol have been detected in integrons (20). A significant association between presence of class 1 integrons and resistance to co-trimoxazole, nalidixic acid, ciprofloxacin, amoxicillin, ceftazidime and tetracycline was shown in this study. In accordance with our study, a significant association between presence of class 1 integrons and resistance tociprofloxacin, nalidixic acid and co-trimoxazole was reported by Japoni et al, to co-trimoxazole, nalidixic acid and ceftazidime by Fallah et al. and to nalidixic acid and tetracycline by Mathai et al. (27, 35, 37). While in many studies resistance to other antibiotics such as gentamicin, amikacin, cephalothin (35) and chloramphenicol (34) were correlated with class 1 integrons. In spite of many resistance gene cassettes including aadA, aadB, aadA7, aadA4 and aacA1, which confer resistance to aminoglycosides like amikacin and gentamicin, in our study this association was not significant. This may be due to the low resistance rate to amikacin (2.5%), or other resistance mechanisms (mutation in the rRNA gene, efflux and permeability mechanisms) to aminoglycosides rather than integrons by bacteria in this study. Sincemost of the isolates (99%) were resistant to cephalothin and only 1% were resistant to imipenem, no significant association was found between presence of integrons and resistance to these antibiotics, similar to many previous studies (33, 34). In this study, 79% of isolates were MDR, which is approximately similar to other reports from Iran that showed 77% and 84.2% MDR in isolated *E. coli* (33-37), while in the USA and Slovenia this was 7.1% and 42%, respectively (39, 40). Empirical therapy for MDR isolates is a major concern; hence a periodical local study will be needed to assess the antibiotic susceptibility pattern. In the present study a high resistance rate was seen towards cephalothin (99%), which is higher than other studies that reported 77.8%, 60% and 40% (34, 35, 37). Extreme sensitivity of *E. coli* to imipenem was observed in this study. Previous studies by Tariq et al. Japoni et al. Farshad et al. and Rezaee et al. showed similar results (33-35, 41) while, Fallah et al. reported that 27.1% of their isolates were sensitive to imipenem (37). Although this antibiotic may be used as a drug of choice for treatment of UTI caused by *E. coli*, yet it is only recommended for complicated urinary tract infections. Resistance to ciprofloxacin and nalidixic acid was 29% and 48.5% in this study, which was lower than the studies of Fallah et al. and Rezaee et al. (34, 37), yet higher than other studies (35, 42, 43). These two antibiotics are among the drugs of choice for treatment of UTI, hence increased use of these antibiotics without knowledge about the pattern of antibiogram may gradually lead to a rise in antibiotic resistance. Sixty-two percent of the isolates were resistant to co-trimoxazole, which was similar to the study of Fallah et al. (67.7%) but higher than the study of Japoni et al. and lower than Rezaee et al. and Farshad et al. (33-35, 37).

5.1. Conclusions

A large number of MDR isolates of *E. coli* were observed in this study, among which 62.6% had class 1 integrons.The significant association between class 1 integrons and resistance to ciprofloxacin, nalidixic acid, co-trimoxazole, amoxicillin, ceftazidime and tetracycline suggests that class 1 integrons have an important role in resistance to these antibiotics. We suggest that further research should be done on the association between antibiotic resistance and presence of integrons, integrase genes and gene cassettes.

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Footnotes

Authors' Contribution: Study concept and design: Seyed Sajjad Khoramrooz; acquisition of data: Seyed Ali Asghar Malek Hosseini; analysis and interpretation of data: Mehdi Mirzaii and Seyed Abdolmajid Khosarvani; drafting of the manuscript: Seyed Sajjad Khoramrooz, Mohammad Emaneini and Farzaneh Gharibpour; critical revision of the manuscript for important intellectual content: Mohammad Emanenini, Seyed Sajjad Khoramrooz and Farzaneh Gharibpour; statistical analysis: Mohammad Zoladl; administrative, technical and material support: Mahboubeh Yazdanpanah, Seyed Ali Asghar Malek Hosseini and Najmeh Parhizgari; study supervision: Seyed Sajjad Khoramrooz and Asghar Sharifi.

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References

1. Su J, Shi L, Yang L, Xiao Z, Li X, Yamasaki S. Analysis of integrons in clinical isolates of Escherichia coli in China during the last six years. *FEMS Microbiol Lett.* 2006;**254**(1):75–80. doi: 10.1111/j.1574-6968.2005.00025.x. [PubMed: 16451182]
2. Gales AC, Sader HS, Jones RN. Urinary tract infection trends in Latin American hospitals: report from the SENTRY antimicrobial surveillance program (1997-2000). *Diagn Microbiol Infect Dis.* 2002;**44**(3):289–99. [PubMed: 12493177]
3. Perfetto EM, Keating K, Merchant S, Nichols BR. Acute uncomplicated UTI and E. coli resistance: implications for first-line empirical antibiotic therapy. *J Manag Care Pharm.* 2004;**10**(1):17–25. [PubMed: 14720102]
4. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. *Ann Clin Microbiol Antimicrob.* 2007;**6**:4. doi: 10.1186/1476-0711-6-4. [PubMed: 17378940]
5. Dormanesh B, Safarpoor Dehkordi F, Hosseini S, Momtaz H, Mirnejad R, Hoseini MJ, et al. Virulence factors and o-serogroups profiles of uropathogenic Escherichia coli isolated from Iranian pediatric patients. *Iran Red Crescent Med J.* 2014;**16**(2):e14627. doi: 10.5812/ircmj.14627. [PubMed: 24719745]
6. Navidinia M, Najar Peerayeh S, Fallah F, Bakhshi B. Phylogenetic Groups and Pathogenicity Island Markers in Escherichia coli Isolated From Children. *Jundishapur J Microbiol.* 2013;**6**(10):e8362. doi: 10.5812/jjm.8362.
7. Minardi D, d'Anzeo G, Cantoro D, Conti A, Muzzonigro G. Urinary tract infections in women: etiology and treatment options. *Int J Gen Med.* 2011;**4**:333–43. doi: 10.2147/IJGM.S11767. [PubMed: 21674026]
8. Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, et al. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. *Antimicrob Agents Chemother.* 2001;**45**(3):723–6. doi: 10.1128/AAC.45.3.723-726.2001. [PubMed: 11181350]
9. Heidary M, Momtaz H, Madani M. Characterization of Diarrheagenic Antimicrobial Resistant Escherichia coli Isolated From Pediatric Patients in Tehran, Iran. *Iran Red Crescent Med J.* 2014;**16**(4):e12329. doi: 10.5812/ircmj.12329. [PubMed: 24910786]
10. Teichmann A, Agra HN, Nunes Lde S, da Rocha MP, Renner JD, Possuelo LG, et al. Antibiotic resistance and detection of the sul2 gene in urinary isolates of Escherichia coli in patients from Brazil. *J Infect Dev Ctries.* 2014;**8**(1):39–43. doi: 10.3855/jidc.3380. [PubMed: 24423710]
11. Asadi S, Kargar M, Solhjoo K, Najafi A, Ghorbani-Dalini S. The Association of Virulence Determinants of Uropathogenic Escherichia coli With Antibiotic Resistance. *Jundishapur J Microbiol.* 2014;**7**(5):e9936. doi: 10.5812/jjm.9936. [PubMed: 25147722]
12. van Essen-Zandbergen A, Smith H, Veldman K, Mevius D. Occurrence and characteristics of class 1, 2 and 3 integrons in Escherichia coli, Salmonella and Campylobacter spp. in the Netherlands. *J Antimicrob Chemother.* 2007;**59**(4):746–50. doi: 10.1093/jac/dkl549. [PubMed: 17307772]
13. Stokes HW, Gillings MR. Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. *FEMS Microbiol Rev.* 2011;**35**(5):790–819. doi: 10.1111/j.1574-6976.2011.00273.x. [PubMed: 21517914]
14. Leverstein-Van Hall MA, Paauw A, Box AT, Blok HE, Verhoef J, Fluit AC. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. *J Clin Microbiol.* 2002;**40**(8):3038–40. [PubMed: 12149373]
15. Recchia GD, Hall RM. Gene cassettes: a new class of mobile element. *Microbiology.* 1995;**141** (**Pt 12**):3015–27. doi: 10.1099/13500872-141-12-3015. [PubMed: 8574395]
16. Fluit AC, Schmitz FJ. Class 1 integrons, gene cassettes, mobility, and epidemiology. *Eur J Clin Microbiol Infect Dis.* 1999;**18**(11):761–70. [PubMed: 10614949]
17. Collis CM, Hall RM. Site-specific deletion and rearrangement of integron insert genes catalyzed by the integron DNA integrase. *J Bacteriol.* 1992;**174**(5):1574–85. [PubMed: 1311297]
18. Ahangarzadeh Rezaee M, Langarizadeh N, Aghazadeh M. First report of class 1 and class 2 integrons in multidrug-resistant Klebsiella pneumoniae isolates from northwest Iran. *Jpn J Infect Dis.* 2012;**65**(3):256–9. [PubMed: 22627310]
19. Levesque C, Piche L, Larose C, Roy PH. PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother.* 1995;**39**(1):185–91. [PubMed: 7695304]
20. Partridge SR, Tsafnat G, Coiera E, Iredell JR. Gene cassettes and cassette arrays in mobile resistance integrons. *FEMS Microbiol Rev.* 2009;**33**(4):757–84. doi: 10.1111/j.1574-6976.2009.00175.x. [PubMed: 19416365]
21. Hall RM, Collis CM. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Mol Microbiol.* 1995;**15**(4):593–600. [PubMed: 7783631]
22. Bennett PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *Br J Pharmacol.* 2008;**153 Suppl 1**:S347–57. doi: 10.1038/sj.bjp.0707607. [PubMed: 18193080]
23. Yu HS, Lee JC, Kang HY, Ro DW, Chung JY, Jeong YS, et al. Changes in gene cassettes of class 1 integrons among Escherichia coli isolates from urine specimens collected in Korea during the last two decades. *J Clin Microbiol.* 2003;**41**(12):5429–33. [PubMed: 14662921]
24. Leverstein-van Hall MA, Blok HE, Donders ART, Paauw A, Fluit AC, Verhoef J. Multidrug resistance among Enterobacteriaceae is strongly associated with the presence of integrons and is independent of species or isolate origin. *J Infect Dis.* 2003;**187**(2):251–9. doi: 10.1086/345880. [PubMed: 12552449]
25. Chang LL, Chang TM, Chang CY. Variable gene cassette patterns of class 1 integron-associated drug-resistant Escherichia coli in Taiwan. *Kaohsiung J Med Sci.* 2007;**23**(6):273–80. doi: 10.1016/s1607-551x(09)70409-7. [PubMed: 17525011]
26. Rao AN, Barlow M, Clark LA, Boring JR, Tenover FC, McGowan JE. Class 1 integrons in resistant Escherichia coli and Klebsiella spp., US hospitals. *Emerg Infect Dis.* 2006;**12**(6):1011–4. [PubMed: 16707065]
27. Mathai E, Grape M, Kronvall G. Integrons and multidrug resistance among Escherichia coli causing community-acquired urinary tract infection in southern India. *APMIS.* 2004;**112**(3):159–64. doi: 10.1111/j.1600-0463.2004.apm1120301.x. [PubMed: 15153157]
28. Hryniewicz K, Szczypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *J Antimicrob Chemother.* 2001;**47**(6):773–80. [PubMed: 11389109]
29. Mahon CR, Lehman DC, Manuselis G. *Textbook of diagnostic microbiology.* 4th ed. Maryland: Saunders/Elsevier; 2011.
30. Clinical Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement.* 2011. Available from: http://antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/M100S22E.pdf.
31. Chang CY, Chang LL, Chang YH, Lee TM, Chang SF. Characterisation of drug resistance gene cassettes associated with class 1 integrons in clinical isolates of Escherichia coli from Taiwan, ROC. *J Med Microbiol.* 2000;**49**(12):1097–102. doi: 10.1099/0022-1317-49-12-1097. [PubMed: 11129722]
32. Li LM, Wang MY, Yuan XY, Wang HJ, Li Q, Zhu YM. Characterization of integrons among Escherichia coli in a region at high incidence of ESBL-EC. *Pak J Med Sci.* 2014;**30**(1):177–80. doi: 10.12669/pjms.301.4079. [PubMed: 24639856]
33. Farshad S, Japoni A, Hosseini M. Low distribution of integrons among multidrug resistant E. coli strains isolated from children with community-acquired urinary tract infections in Shiraz, Iran. *Pol J Microbiol.* 2008;**57**(3):193–8. [PubMed: 19004239]
34. Rezaee MA, Sheikhalizadeh V, Hasani A. Detection of integrons among multi-drug resistant (MDR) Escherichia coli strains isolated from clinical specimens in northern west of Iran. *Braz J Microbiol.* 2011;**42**(4):1308–13. doi: 10.1590/S1517-838220110004000010. [PubMed: 24031757]
35. Japoni A, Gudarzi M, Farshad S, Basiri E, Ziyaeyan M, Alborzi A, et al. Assay for integrons and pattern of antibiotic resistance in clinical Escherichia coli strains by PCR-RFLP in Southern Iran. *Jpn J Infect Dis.* 2008;**61**(1):85–8. [PubMed: 18219144]

36. Martinez-Freijo P, Fluit AC, Schmitz FJ, Grek VS, Verhoef J, Jones ME. Class I integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. *J Antimicrob Chemother.* 1998;**42**(6):689–96. [PubMed: 10052890]
37. Fallah F, Karimi A, Goudarzi M, Shiva F, Navidinia M, Jahromi MH, et al. Determination of integron frequency by a polymerase chain reaction-restriction fragment length polymorphism method in multidrug-resistant Escherichia coli, which causes urinary tract infections. *Microb Drug Resist.* 2012;**18**(6):546–9. doi: 10.1089/mdr.2012.0073. [PubMed: 22816551]
38. Al-Assil B, Mahfoud M, Hamzeh AR. First report on class 1 integrons and Trimethoprim-resistance genes from group in uropathogenic (UPEC) from the Aleppo area in Syria. *Mob Genet Elements.* 2013;**3**(3):e25204. doi: 10.4161/mge.25204. [PubMed: 23956949]
39. Cao X, Zhang Z, Shen H, Ning M, Chen J, Wei H, et al. Genotypic characteristics of multidrug-resistant Escherichia coli isolates associated with urinary tract infections. *APMIS.* 2014;**122**(11):1088–95. doi: 10.1111/apm.12260. [PubMed: 24698634]
40. Sahm DF, Thornsberry C, Mayfield DC, Jones ME, Karlowsky JA. Multidrug-resistant urinary tract isolates of Escherichia coli: prevalence and patient demographics in the United States in 2000. *Antimicrob Agents Chemother.* 2001;**45**(5):1402–6. doi: 10.1128/AAC.45.5.1402-1406.2001. [PubMed: 11302802]
41. Tariq N, Jaffery T, Ayub R, Alam AY, Javid MH, Shafique S. Frequency and antimicrobial susceptibility of aerobic bacterial vaginal isolates. *J Coll Physicians Surg Pak.* 2006;**16**(3):196–9. [PubMed: 16542599]
42. 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]
43. Diekema DJ, BootsMiller BJ, Vaughn TE, Woolson RF, Yankey JW, Ernst EJ, et al. Antimicrobial resistance trends and outbreak frequency in United States hospitals. *Clin Infect Dis.* 2004;**38**(1):78–85. doi: 10.1086/380457. [PubMed: 14679451]