Original Article

High Frequency of Class 2 and 3 Integrons Related to Drug-Resistance in Clinical Isolates of *Diarrheagenic E. coli* in Iran

Zahra Mohammadalipour[1], Mohammad Kargar[1*], Abbas Doosti[2]

[1] Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran.
2 Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Received: 2016-04-21; Accepted: 2016-11-27

Abstract

Background: Integrons are mobile genetic elements able to obtain the antibiotic resistance gene cassettes. The prevalence of integrons in the *Enterobacteriaceae* family has been varied and played an important role in the development of the drug resistant bacteria. The present study aimed to investigate the contribution of class 2 and 3 integrons in drug resistant *Diarrheagenic Escherichia coli* strains.

Materials and Methods: The 164 *Diarrheagenic E. coli* collected from feces samples of children in the Yasuj –Iran and all isolates were identified by standard biochemical tests. The antimicrobial susceptibility for 14 antibiotics, which are used conventionally was determined by disk diffusion. The presence of class 2 and 3 integrons in all isolates was investigated by PCR.

Results: Of 164 *E. coli* isolates from children, 80.49% carried class 2 integron and the length of the amplicons ranged from 800 bp to 2 kb. Class 3 integrons were identified among 24 *E. coli* isolates. All the *E.coli* isolates were susceptible to imipenem and the greatest resistance was correspondent to nalidixic acid. A significant correlation was revealed between Class 2 integron and resistance to kanamycin, amikacin, gentamicin, ceftazidime, chloramphenicol and cephalexin. The presence of class 3 integron was significantly associated with resistance to ampicillin, gentamicin, streptomycin, kanamycin, tetracycline and trimetoprime-sulfametoxazol.

Conclusion: The results indicated that integrons are widespread in *Diarrheagenic E. coli* and its carriage contributed significantly to the emergence of resistance among *Diarrheagenic E. coli*. However, factors leading to the wide spread of integrons are still to be determined.

Keywords: *Diarrheagenic E. coli*, disseminate antibiotic resistance, class 2 integron, class 3 integron.

***Corresponding Author:** Dr Mohammad Kargar, Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran. Tel: (+98) 917 314 9203, Email: mkargar@jia.ac.ir

Please cite this article as: Mohammadalipour Z, Kargar M, Doosi A. High Frequency of Class 2 and 3 Integrons Related to Drug-Resistance in Clinical Isolates of *Diarrheagenic E. coli* in Iran. Novel Biomed. 2017;5(1):30-6.

Introduction

Diarrheagenic Escherichia coli affect a large proportion of the population, particularly children[1], six major classes of diarrheagenic *E. coli* (DEC) are recognized as being associated with diarrheal illness. They are enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffuse adhering

E. coli (DAEC)[2], this bacteria are a major target of antimicrobial therapy. Increasing drug resistance among bacteria is therefore of great concern in global public health[3]. Resistance to antibiotics is widespread in bacterial isolates, particularly in developing countries[4]. The emergence of antibiotic resistance is a global health concern. Surveillance of antibiotic resistance genes in the community is necessary for developing strategies to minimize the spread of antimicrobial resistance[5].

The evolution of antimicrobial resistance in bacteria is a complex process involving a variety of different mechanisms. Some bacteria show a high natural resistance to specific antibiotics, while susceptible bacteria may achieve resistance by modification in their genome by mutations or the transfer of resistance genes located on mobile DNA elements such as integrons [6-10].

Integrons are a well-organized gene expression system that can capture, integrate one or more gene cassettes and convert them into functionally expressed genes. Integrons can transfer among different bacterial species easily because of their association with mobilizing elements such as transposons, plasmids or phages. Therefore, integrons are particularly adapted to transfer and disseminate antibiotic resistance as a result of their mobility and ability to quickly acquire diverse resistance genes[11].

Integrons are widely dispersed among gram-negative isolates[12]. Integron distributions in *E. coli* have been recently studied in many countries. The prevalence of integrons ranges from 22 to 59% in clinical *Escherichia coli*[13].

A common feature of all integrons is an integrase enzyme that can integrate gene cassettes. At least six classes of integron identified are well-known by their respective integrase genes[14]. Accordingly, three types of integrons, Class 1, 2 and 3 integrons, are known to be associated with antibiotic resistance[15].

These elements contain an *intI* gene encoding a site-specific recombinase association to the integrase family and a recombination site *attI*. A gene cassette contain an open reading frame (ORF) and, at the 3′-end, a recombination site *attC*. Integration of cassettes occurs by a site-specific recombination mechanism catalyzed by the integrase[14].

Class 2 integrons include a gene *intI2*. The *intI2* gene is located 5′ to the first gene cassette and its gene product, has 46% homology with *IntI1*[14,16]. Class 2 integrons are typically located in a unique site near the left end of the non-replicative transposon Tn_7. Class 2 integrons have been isolated from *E. coli, Shigella sonnei, Salmonella enterica* serovar Typhimurium, *Proteus vulgaris, Proteus mirabilis, Providencia stuartii, Acinetobacter baumannii, Vibrio cholerae* and *Klebsiella* spp[16,17].

Class 3 integrons appear to be much less common in the spread of drug resistance. Class 3 integrons have been isolated from *Serratia marcescens, Klebsiella pneumonia, Delftia* spp., *E. coli, Acinetobacter* spp., *Pseudomonas aeruginosa, Pseudomonas putida, Citrobacter freundii, Alcaligenes xylosoxidans* and *Salmonella* spp.[3,18].

Only some studies have analyzed the frequency of occurrence of integrons, and the association between integron carriage and antimicrobial susceptibility in Iran. However, there is not enough information available on prevalence of class 3 integron and their association with drug resistance. To the best of our awareness, this study was to evaluate the prevalence of class 2 integron and describes the distribution of class 3 integrons among diarrheagenic *E. coli* strains isolated from children less than 5 years in Iran and then to investigate associations between drug resistance and the existence of integrons.

Methods

Bacterial and clinical specimens: This descriptive cross – sectional study included 164 cases of *Diarrheagenic E. coli* strains obtained from four medical centers of Southwest Iran, in a period of five months from January to June 2012. Samples were feces of children under 5 suffering from diarrhea caused by *E. coli*. Bacteria were isolated and identified by standard bacteriological methods. Subsequently identified bacteria were transferred to tryptic soy broth containing 20% glycerol, and stored at -70°C as stock.

Antibiotic susceptibility test: Antimicrobial susceptibility of all isolates was determined using the standard Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guidelines. Antibiotics used were included imipenem (10µg), Ceftazidime (30µg), Cefixime (5µg) (ROSCO,

Denmark), gentamicin (10µg), amikacin (30µg), ampicillin (10µg), chloramphenicol (30µg), tetracycline (30µg), streptomycin (10µg), nalidixicacid (30µg), trimethoprim–sulfamethoxazole (25µg), cephalexin (30µg), and kanamycin (30µg) (padtanteb, Iran). *E. coli* ATCC 25922 was used as the control strain.

PCR amplification: Template DNA for PCR was prepared by boiling method and stored at -20°C[19]. In order to identify the isolates carrying class 2 integron, the conserved region and variable regions of integron were amplified using *IntI2-F/IntI2-R* and *attI-F/orfX-R* pair primers (obtained from Cinna Gene Co.), respectively. Class 3 integron was detected using PCR with *IntI3-F/IntI3-R* primers designed for conserved regions of integron encoded integrase gene *IntI3*. Primer Sequences and PCR conditions are shown in Table I.

The polymerase chain reaction was performed in 25µl volumes. A tube containing PCR mixture without DNA template was used as a negative control. Amplicons were analyzed by conventional 1% agarose gel electrophoresis in 1× TBE buffer and stained with ethidium bromide.

DNA analysis: Chi-square test and Fisher's exact test were performed to calculate the relationship between antibiotic resistance and the presence of class 2 and 3 integron, using *SPSS* software version 15. The significance level was considered P< 0.05.

Results

A total of 164 *Diarrheagenic E. coli* isolates was collected from fecal specimens obtained in four medical centers of Southwest Iran. Of the 164 isolates tested, 35.7% (n=58) had been isolated from girls patients and 64.63% (n＝106) had been isolated from boys patients and According to the World Health Organization's, assortment samples were divided in to 9 groups, where the majority of the patients were in the age group of 6-8 months.

The antibiotic resistance results were surveyed and Percentage of resistance to antibiotics determined as follows: imipenem 0%, ceftazidime 28.05%, cefixime 63.41%, gentamicin 19.51%, amikacin 14.63%, ampicillin 59.76%, chloramphenicol 6.10%, tetracycline 28.05%, streptomycin 46.34%, nalidixic acid 75.61%, trimethoprim–sulfamethoxazole 34.15%, cephalexin 26.83% and kanamycin 35.37%. Only 1.22% of strains were fully susceptible to all tested antibiotics. The remaining strains were resistant to one or more antibiotics

Table 1: Primer sequences and PCR conditions.

Primer	Oligonucleotide sequence (5' to 3')	PCR conditions
IntI2-F	CAC GGA TAT GCG ACA AAA AGG T	1 cycle of 12 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 62°C, 1 min at 72°C; 1 cycle of 8 min at 72°C
IntI2-R	GTA GCA AAC GAG TGA CGA AAT G	
attI2-F	GAC GGC ATG CAC GAT TTG TA	1 cycle of 12 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 58°C, 3.5 min at 72°C; 1 cycle of 10 min at 72°C
orfX-R	GAT GCC ATC GCA AGT ACG AG	
IntI3-F	5'-AGT GGG TGG CGA ATG AGT G -3'	1 cycle of 12 min at 94°C; 32 cycles of 1 min at 94°C, 1 min at 60°C, 1 min at 72°C; 1 cycle of 8 min at 72°C
IntI3-R	5'- TGT TCT TGT ATC GGC AGG TG -3'	

From total 164 *E. coli* isolates, 132 (80.49%) and 24 (14.63%) isolates identified being positive for class 2 and class 3 integrons, respectively (Figure I). The integron cassette region be amplified by PCR in 56 (42.42%) of the class 2 integron-containing isolates and the length of the amplicons ranged from 800 to 2000 bp (Figure II and Table II). The relationship between antibiotic resistance and class 2 integron is shown in Table III.

The incidence of class 3 integron among diarrheagenic *Eschericha coli* isolates from stool collected in this study is shown in Table IV. Drug resistance to ampicillin, gentamicin, streptomycin, kanamycin, tetracycline, and trimetoprime-sulfametoxazol with the

Table 2: Sizes of variable regions of integron class 2 cassettes in *intI2* positive isolates.

Pattern of integron 2 cassettes bands (pb)	No. of isolates (%)
800	14 (10.61)
1300	2 (1.51)
1500	6 (4.54)
2000	34 (25.76)
Without PCR product	76 (57.58)
Total no. of *intI2* positive isolates	132 (100)

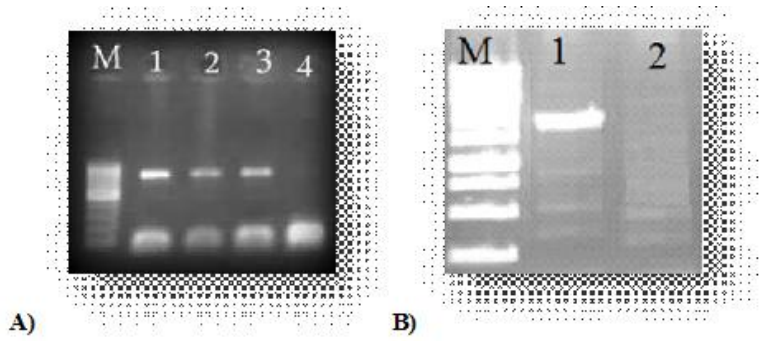

Figure 1. A) PCR amplicons of class 2 integrons. M: size marker 100 bp, line 1-3: *IntI2* (788 bp), line 4: negative control B) PCR amplicons of class 3 integrons. M: size marker 100 bp, line 1: *IntI3* (600 bp), line 2: negative control.

presence of class 3 integron was statistically significant (Table IV). Also, Class 3 integron distribution analysis by age group showed a significant correlation between the age group of 6-8 months and the class 3 integron (p= 0) (Figure III).

Discussion

According to previous studies, regardless of the antibiotic consumption pattern, antibiotic resistant gene can be passed among bacterial populations by movable elements such as the integron[20]. In this study, the highest antibiotic resistance rates in *Escherichia coli* isolates corresponds to nalidixic acid (75.61%) and the lowest resistance to imipenem (0%). Similar results were observed in Razaghi *et al*[21] study, but on the contrary, Phongpaichit *et al*[22] reported a 27% resistance to nalidixic acid. In addition Japoni *et al*[23]. published resistance rates of 57.5% to Tetracycline, 48% to Cotrimoxazole, 8.5% to Amikacin, 36% to Nalidixic acid, 18% to Gentamicin and 18% to chloramphenicol, in southern Iran. The difference in the percentage of resistance in different parts of the world is due to differences in the prevalence of antibiotic consumption in each country.

According to Japoni's surveys by PCR-RFLP technique, class 2 integrone were present in 6.7% of *E. coli* strains[23], it is while amongst total collected isolates of *E. coli* strains in this study, 80.49% were

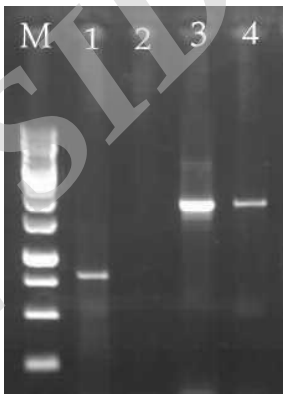

Figure 2. PCR amplicons of class 2 integrons gene cassette. M: size marker 1 kb, line 1: gene cassette (800 bp), line 2: negative control, line 3, 4: gene cassette (2000 bp).

positive for class 2 integron. In addition class 2 integron prevalence in the present study, was much higher than bakhshi's[24] research, in which, reported an occurrence of 14.2%, class 2 integron in *Enteropathogenic* strains of *Diarrheagenic E. coli* in children under 5 years in Iran. In addition, in 42.42% of the samples, variable gene cassettes of 800 to 2000 bp sequences were observed. Similar results were also obtained by bakhshi *et al*[24,25]. Confirming previous studies, resistant to cephalexin, gentamicin, chloramphenicol, ceftazidime, kanamycin, and amikacin was associated with class 2 integron[10,16]. In respect to the results from antibiotic resistance gene cassette bearing isolates in this study (Table III), it can be remarked that resistance to Gentamicin,

Table 3: The association between antibiotic resistance and integron existence in isolates.

Antibiotics	No. (%) of resistant isolates without genes cassettes	No. (%) of resistant isolates with gene cassettes	Resistant isolates No. (%) of total	Association of resistance with gene cassettes
Ampicillin	62 (37.80)	36 (21.95)	98 (59.76)	$P = 0.394$
Gentamicin	10 (6.09)	22 (13.41)	32 (19.54)	$P = 0$[d]
Amikacin	8 (4.88)	16 (9.76)	24 (14.63)	$P = 0.000$[d]
Chloramphenicol	2 (1.22)	8 (4.88)	10 (6.10)	$P = 0.002$[c]
Tetracycline	26 (15.85)	20 (12.20)	46 (28.05)	$P = 0.116$
Streptomycin	44 (26.83)	32 (19.51)	76 (46.34)	$P = 0.056$
Nalidixicacid	82 (50)	42 (25.61)	124 (75.61)	$P = 0.896$
Trimethoprim–sulfamethoxazole	32 (19.51)	24 (14.63)	56 (34.15)	$P = 0.090$
Cephalexin	22 (13.41)	22 (13.41)	44 (26.83)	$P = 0.010$[c]
Kanamycin	32 (19.51)	26 (15.85)	58 (35.37)	$P = 0.033$[b]
Ceftazidime	38 (23.17)	8 (4.88)	46 (28.05)	$P = 0.005$[c]
Imipenem	0 (0)	0 (0)	0 (0)	-[a]
Cefixime	72 (43.90)	32 (19.51)	104 (63.41)	$P = 0.230$

a. No statistics are computed
b. At the 5%
c. At the 1%

Table 4: Antibiotic sensitivity of *E. coli* isolated and correlation with class 3 integron.

Antibiotic	Total resistant n (%)	Positive integron n (%)	P value
Ampicillin	98 (59.75)	24 (14.63)	**0**[*]
Nalidixic acid	124 (75.61)	14 (8.54)	0.061
Sulfamethoxazole-trimethoprim	56 (34.14)	14 (8.54)	**0.013**[*]
Gentamicin	32 (19.51)	12 (7.32)	**0**[*]
Cefalexin	44 (26.83)	10 (6.10)	0.127
Tetracycline	46 (28.05)	14 (8.54)	**0.001**[*]
Streptomycin	76 (46.34)	18 (10.98)	**0.005**[*]
Kanamycin	58 (35.36)	16 (9.76)	**0.001**[*]
Chloramphenicol	10 (6.10)	2 (1.22)	0.642
Amikacin	24 (14.64)	6 (3.66)	0.127
Ceftazidime	46 (28.05)	4 (2.44)	0.224
	0 (0)	0 (0)	-[a]
Cefixime	104 (63.41)	14 (8.54)	0.741

[*]**:** significant values
a) No statistics are computed

Chloramphenicol, Kanamycin and Amikacin could be due to the existence of resistance genes in class 2 integron, while the significant correlation between the presence of integron and resistance to Cephalexin and Ceftazidime could be explained by genetic association of integrones with tranposones or conjugative plasmids, since isolates resistant to these two antibiotics contained less gene cassettes.

In some studies, variable region primers are used to identify the class 2 integron, however, our PCR results using variable region specific primers showed that in some strains despite having the integrase gene, no gene cassette could be detected.

Accordingly, the differences in PCR results of integron identification in the samples could be explained by the fact that the class 2 integrase gene (*intI2*) contains an early stop codon resulting in truncation form of the enzyme. The resultant integrase is therefore unable to excise existing cassettes or insert new ones[26].

The first class 3 integron was discovered in the *Serratia marcescens* isolated from patient with UTI in Japan in 1993 and the second report in *Klebsiella pneumoniae*[18].

The results in Australia[26], Korea[10], France[13], Spain[27], China[28], Iran[23], Taiwan[29], Malaysia[30] and Pakistan[7] showed that there is no class 3 integron in *E. coli* samples. Unlike other studies, present investigation

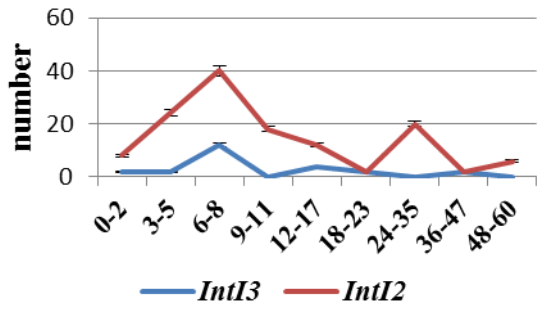

Figure 3. Comparing the distribution of integron in Population According to age group.

revealed 24/164 (14.63%) of *E. coli* isolates harbored class 3 integrons.

Previous studies showed that Class 3 integron, possess resistance genes for chloramphenicol, kanamycin and ESBLs. Also Poirel *et al.*[31] reported that class 3 integrase, sharing 60% amino acid identity with the class 1 integrase and carried the gene coding for resistance to beta-lactams. In this study, a significant relationship was found between class 3 integron and resistance to kanamycin, streptomycin, tetracycline, gentamicin, ampicillin and Trimethoprim-sulfamethoxazole. Furthermore, using statistical analysis, a significant relationship was identified between class 3 integron and the age group of 6-8 months, while most of the studies did not specify any age range for the prevalence of integrons and showed that the high incidence of integrons is largely dependent on several factors such as hospitalization and the antibiotic pattern of usage.

Conclusion

The high prevalence of class 2 integron and antibiotic resistance genes in this study indicates a serious threat to the treatment of infectious diseases, in the future. Therefore, in order to control bacterial resistance and prevent the spread of integrons, necessary measures must be adopted. Moreover, considering the mentioned conclusions it can be suggested that integrase gene mutations should be studied among variable region amplification negative strains in future researches, and, further investigation of integron prevalence and its association with resistance to other antibiotics in specific age groups, is essential. Integrons appeared to contain one of the common features of multidrug-resistant *E. coli* isolates in Iran. The appearance of the class 3 integron and its relationship to drug resistance in this study, represents a serious threat to the spread of antimicrobial resistance which caused health problems. Therefore, the molecular surveillance, sequencing and prevent the effluence of a class 3 integron in other parts of the country is recommended.

Acknowledgment

The authors express the appreciation to deputy of research and all personnel of the Biotechnology Research Center, Islamic Azad University, Shahrekord branch, for their support during this study.

References

1. Kargar M, Homayoon M. Outbreaks of infection sources and antibiotic resistance in EHEC strains among children under 5 years old in Marvdasht. Medical Sciences Journal of Islamic Azad University Tehran Medical Branch. 2010;19:268-73 [In Persian]

2. Nweze E.I, Virulence Properties of Diarrheagenic *E. coli* and Etiology of Diarrhea in Infants, Young Children and Other Age Groups in Southeast, Nigeria, American-Eurasian Journal of Scientific Research, 2009; 4 (3): 173-179

3. 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, polish journal of microbiology, 2008; Vol. 57, No. 3, 193-198

4. Frisca F, Lay B.W, Waturangi D.E. Identification of class 1 integron of escherichia coli from street foods in jakarta. *Microbiology Indonesia* 2010; 1 (1)

5. Dawes F.E, Kuzevski A, Bettelheim K.A, Hornitzky M.A, Djordjevic S.P, Walker M.J. Distribution of class 1 integrons with is26-mediated deletions in their 3′-conserved segments in escherichia coli of human and animal origin. *PloS one* 2010; 5 (9), e12754

6. Barlow R.S, Pemberton J.M, Desmarchelier P.M, Gobius K.S. Isolation and characterization of integron-containing bacteria without antibiotic selection. *Antimicrobial agents and chemotherapy* 2004; 48 (3), 838-842

7. Normark B.H, Normark S. Evolution and spread of antibiotic resistance. *Journal of internal medicine*, 2002; 252 (2), 91-106.

8. Rowe-Magnus D.A, Guerout A.-M, Ploncard P, Dychinco B, Davies J, Mazel D. The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons. *Proceedings of the National Academy of Sciences* 2001. 98 (2), 652-657.

9. Rowe-Magnus D.A, Guerout A.M, Mazel D. Bacterial resistance evolution by recruitment of super-integron gene cassettes. *Molecular microbiology* 2002. 43 (6), 1657-1669.

10. Yu H.S, Lee J.C, Kang H.Y, Ro D.W, Chung J.Y, Jeong Y.S, 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. *Journal of clinical microbiology* 2003; 41 (12), 5429-5433.

11. Su H.C, Ying G.G, Tao R, Zhang R.Q, Zhao J.L, Liu Y.S. Class 1 and 2 integrons, sul resistance genes and antibiotic resistance in Escherichia coli isolated from dongjiang river, south china. *Environmental Pollution* 2012. 169, 42-49.

12. Heir E, Lindstedt B, Leegaard T, Gjernes E, Kapperud G. Prevalence and characterization of integrons in blood culture *Enterobacteriaceae* and gastrointestinal *Escherichia coli* in Norway and reporting of a novel class 1 integron-located lincosamide resistance gene, *Annals of Clinical Microbiology and Antimicrobials*, 2004; 3:12 doi:10.1186/1476-0711-3-12

13. Skurnik D, Menac'h A, Zurakowski D, Mazal D, Courvalin P, Denamur E, et al. Integron-Associated Antibiotic Resistance and Phylogenetic Grouping of *Escherichia coli* Isolates from Healthy Subjects Free of Recent Antibiotic Exposure, Antimicrobial agents and Chemotherapy. 2005, Vol. 49, No. 7, p. 3062-3065

14. Iyer A, Barbour E, Azhar E, El Salabi A.A, Hassan H.M.A, Qadri I. et al. Transposable elements in escherichia coli antimicrobial resistance. *Advances in Bioscience and Biotechnology* 2013; 4, 415.

15. Bass L, Liebert CA, Lee MD, Summers AO, White DG, Thayer SG, Maurer JJ. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian Escherichia coli. Antimicrob Agents Chemother 1999; Dec;43(12):2925e9.

16. Dawes F.E. Antibiotic resistance genes located in integrons isolated from escherichia coli recovered from humans and animals. 2009

17. Hansson K, Sundström L, Pelletier A, Roy P.H. Inti2 integron integrase in tn7. *Journal of bacteriology* 2002; 184 (6), 1712-1721

18. Yu G. Role of integrons in antimicrobial resistance: A review, *African Journal of Microbiology Research*, 2013,Vol. 7(15), pp. 1301-1310

19. Rezaee M.A, 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, 256-259.

20. Eslami G, Seyedjavadi S.S, Goudarzi H, Fallah F, Goudarzi M. Distribution of integrons among multidrug resistant e. Coli and klebsiella strains. *Pejouhesh* 2010; 34 (1), 61-65

21. Razaghi M, Alebouyeh M, Rajabi Bazl M, Zali M.R. Frequency of β-lactamase producing isolates of escherichia coli and their diversity in enzyme activities among the resistance isolates from patients with diarrhea and nosocomial infections in tehran, iran. *Koomesh* 2014. 15 (2), 197-205.

22. Phongpaichit S, Wuttananupan K, Samasanti W. Class 1 integrons and multidrug resistance among escherichia coli isolates from human stools. Southeast Asian J Trop Med Public Health. 2008 Mar;39(2):279-87

23. 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. *Japanese journal of infectious diseases* 2008; 61 (1), 85.

24. Bakhshi B, Fallahzad S, Pourshafie M.R. The occurrence of atypical enteropathogenic escherichia coli strains among children with diarrhea in iran. *Journal of Infection and Chemotherapy* 2013; 19 (4), 615-620.

25. Hernandes R.T, Elias W.P, Vieira M.A, Gomes T.A. An overview of atypical enteropathogenic escherichia coli. *FEMS microbiology letters* 2009; 297 (2), 137-149.

26. White P.A, Mciver C.J, Rawlinson W.D. Integrons and gene cassettes in theenterobacteriaceae. *Antimicrobial agents and chemotherapy* 2001; 45 (9), 2658-2661.

27. Machado E, Canton R, Baquero F, Galan J, Rollan A, Peixe L, et al. Integron Content of Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Strains over 12 Years in a Single Hospital in Madrid, Spain, Antimicrobial agents and Chemotherapy, 2005; Vol. 49, No. 5, p. 1823-1829

28. 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 254*, 2006, 75-80

29. Vinue L, Saenz Y, Somalo S, Escudero E, Moreno MA, Ruiz-Larrea F, Torres C. Prevalence and diversity of integrons and associated resistance genes in faecal *Escherichia coli* isolates of healthy humans in Spain, *Journal of Antimicrobial Chemotherapy* 2008; 62, 934–937

30. Ibrahim N, Wajidi M.F, Yusef M.Y, Tay S.T. The integron prevalence of extended-spectrum betalactamase producing *enterobacterial* isolates in a Malaysian teaching hospital, Tropical Biomedicine, 2011; 28(3): 668-671

31. Poirel L, Carattoli A, Bernabeu S, Brudere T, Frei R, Nordmann P. A novel IncQ plasmid type harbouring a class 3 integron from *Escherichia coli*, *Antimicrob Chemother* 2010; 65: 1594–1598