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شیوع اینتگرون‌های کلاس ۱ و ۲ در جدایه‌های اشریشیا کلی یوروپاتوژنیک از بیماران دیابتی در کرمانشاه، ایران

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چکیده

زمینه و هدف: اشریشیا کلی یکی از پاتوژن‌های غالب است که باعث ۸۰ تا ۹۰ درصد عفونت‌های ادراری اکتسابی از جامعه و ۳۰ تا ۵۰ درصد عفونت‌های ادراری اکتسابی از بیمارستان می‌شود. جدایه‌های مقاوم به چند دارو که توسط ژن‌های مقاومت مرتبط کدگذاری می‌شوند، روی اینتگرون‌ها قرار دارند.

مواد و روش‌ها: در این مطالعه مقطعی، تعداد کل ۹۰ جدایه اشریشیا کلی از نمونه‌های ادرار بیماران دیابتی در کرمانشاه، ایران، طی یک دوره شش ماهه (مهر تا اسفند ۱۳۹۴) به دست آمد. نمونه‌های ادرار با روش‌های استاندارد باکتری‌شناسی آزمایش شدند و آزمایش حساسیت آنتی‌بیوتیکی برای همه جدایه‌ها با روش انتشار دیسک در آگار کربی بائر انجام شد. تشخیص اینتگرون‌های کلاس ۱ و کلاس ۲ با روش PCR در جدایه‌های MDR انجام شد.

یافته‌ها: شایع‌ترین فنوتیپ‌های مقاوم به ترتیب سفالوتین (۸۸,۸۸٪)، نالیدیکسیک اسید (۸۳,۳۳٪)، کوتریموکسازول (۷۰٪)، سیپروفلوکساسین (۵۸,۸۸٪)، تتراسایکلین (۵۶,۶۶٪)، نورفلوکساسین (۵۵,۵۵٪)، سفتازیدیم (۴۰٪)، جنتامایسین (۳۲,۲۳٪)، کلرامفنیکل (۲۶,۶۶٪)، نیتروفورانسیون (۲۱,۱۱٪)، آمیکاسین (۱۷,۷۷٪) و ایمیپنم (۲,۲۲٪) بودند. در این مطالعه، در مجموع ۹۰ جدایه در ۷۶ نمونه، MDRگزارش شدند. اینتگرون کلاس I در ۱۶ جدایه (۲۱,۰۵٪) و اینتگرون کلاس ۲ در ۸ جدایه (۱۰,۵۲٪) یافت شد. هیچ سویه‌ای حاوی هر دو اینتگرون کلاس ۱ و ۲ یافت نشد. در این مطالعه،

نتیجه‌گیری: بین وجود ژن intI1 و مقاومت به نورفلوکساسین و سفالوتین رابطه معنی‌داری وجود داشت. این نشان می‌دهد که ژن‌های مقاومت آنتی‌بیوتیکی در داخل اینتگرون قرار دارند.

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Introduction:

Urinary Tract Infections (UTIs) represent one of the most common diseases encountered in the medical practice today, accounting for 150 million UTIs per annum worldwide (1). Indicated that 40%-50% of women had UTIs throughout their lives. Also, a previous report of WHO showed that the UTIs were the common causes of febrile illness in 3–8% of girls and 1% of boys. *Escherichia coli* is the primary etiological agent, accounting for 75–90% cases of UTI (2). Although it is one of the most easily treatable diseases, it has been reported to be the most common hospital-acquired infection, affecting mainly women, children and the elderly (3). The gram negative bacteria are the most common pathogens and among them, *E coli* is responsible of for the acute UTI. The other gram negative bacteria (*Proteus, Pseudomonas* and *Klebsiella*) are probably related to chronic infections (4). Urinary tract infections comprise a wide range of disorders including pyelonephritis (infection of the kidney) and cystitis (infection of the bladder), which are defined by the presence of microorganisms in the urinary tract (5). Antimicrobial resistance and, in particular, multidrug resistance (MDR) are turning in to a big problem worldwide. MDR encoded bylinked resistance genes occurs on integrons, which are potentially mobile genetic elements involved in the transfer of MDR (6). Integrons are genetic units containing elements for site specific recombination, capture and mobilization of gene cassettes. To date, five distinct integron classes have been found. More than 60 different antibiotic resistance genes have been identified within gene cassettes,alone or in combination (7). Integrons were first described by Hall and Collis. They are genetic elements that contain the specific determinants of the component of a site-specific recombination system that recognizes and captures mobile gene

cassettes (8,9). Integron system is a dynamic force in the evolution of multidrug resistance (MDR) and helps bacteria to acquirenovel combinations of resistance genes. Integrons are horizontally transferable genetic elements which play animportant role in dissemination and accumulation of resistance genes in bacteria (10). Most integrons encoding antibiotic resistance have been found in gram-negative bacteria (e.g., *Pseudomonas* species, *Acinetobacter* species, *Vibrio* species, and various enterobacteriaceae species including *Escherichia coli, Klebsiella pneumoniea*, and *Enterobacter cloacae*). However, integrons coding for antibiotic resistance have also been described in *Corynebacterium* species, *mycobacteria*, and *Enterococcus faecalis (7*). There are four different classes of integrons in bacteria carrying genes for antimicrobial resistance, each with a distinct integrase gene. Nearly all known gene cassettes from class 1, 2 and 3 integrons encode resistance to antibiotics or disinfectants. Class 1 integrons are the most prevalent and well characterized. Class 4 is a distinctive class of integrons located in the *Vibrio cholera* genome and not known to be associated with antibiotic resistance (11-13). Several studies have examined integron distributions in multi-drug resistant *Escherichia coli* strains around the world (14). However, no publicized information is available on the detection of integrons in MDR *Escherichia coli* isolated from urinary tract infections in Kermanshah, Iran. The aim of this study was to determine the prevalence of multi-drug resistant *Escherichia coli* isolated from urinary tract infections in Kermanshah Iran and investigate the associations between multi-drug resistance and the existence of integrons.

Material and Methods:

Bacteria isolation

A total number of 90 *Escherichia coli isolates* were obtained from urine samples of patients at Medical diagnostic laboratories in

Kermanshah, Iran during a six-month period (October to March, 2015). The bacterial isolates were identified according to the cultural and biochemical properties. Urine samples were inoculated on blood and Mac Conkey agar plates and incubated at 37°C for 18-24h. *Escherichia coli* isolates were distinguished by post growth on solid medium. It produced a large, smooth, pink (lactose fermented) on MacConkey. Furthermore, the biochemical tests were performed for the identification of *Escherichia coli* isolates from other isolates. These tests included Indole test, Methyl red test, Voges proskaur test, Simmon`s citrate test, triple sugar iron test, urease test, and Motility test (15).

Antmicrobial Susceptibility Testing:

Antibioticsusceptibility tests were done for all isolates by Kirby-Bauer agar disk diffusion method as recommended by CLSI on Muler-Hinton agar medium (Merck,Germany). The antibiotic disks used in this study were: Gentamicin (GM 10µg), Amikacin (AN 30µg), Nalidixic Acid (NA 30µg), Ceftazidim (CAZ 30 µg), Norfeloxacine (NOR), Choloramphenicol (C 30µg), Cephalotin (CF 30 µg), Imipenem (IPM 10 µg), Ciprofloxacin (CP 5 µg), Co-trimoxazole (SXT 25 µg), Tetracycline (TE 30 µg) and Nitrofurantoin (FM 30 µg) (16,17).

DNA extraction and PCR ampilification

Template DNA for PCR was prepared by the boiling method. Briefly, bacteria were harvested from 1.5 ml of an overnight Luria Bertani broth culture (Merck, Germany). It was suspended in sterile distilled water and incubated at 95°C for 10 min. Following the centrifugation of the lysate, the supernatant was stored at -20°Cand used as the template DNA stock.

PCR Amplification:

Detection of class 1 and class 2 integrons was performed by PCR. The primers used for

the detection of *int1*and *int2* genes by PCR method are presented in Table (1) (18).

PCR amplification was carried out in a 25 µl reaction mixture containing 2 µl of DNA template, 50 pm of each oligonucleotide primer, 0.2 mM of deoxynucleoside triphosphates sets, 1.5 mM of MgCl2, 2.5 µl of 10X PCR buffer (100 mMTris-HCl, pH 8.3 and 500 mM KCl) and 2.5 U of Taq polymerase. It was performed as follows: the initial denaturation at 94°C for 12 min was followed by 30 cycles consisting of 94°C for 1 min, annealing at 60°C for 30 sec, elongation at 72 for 2 minutes,and the final extension was conducted at 72°C for 10 min (18).

Table 1: Oligonucleotide primers used in the PCR assay

Primer	**Oligonucleotide sequence (5’ - 3’)**
Int 1-F	GGTCAAGGATCTGGATTTCG
Int 1-R	ACATGCGTGTAAATCATCGTC
Int 2-F	CACGGATATGCGACAAAAAGGT
Int 2-R	GTAGCAAACGAGTGACGAAATG

Results:

Of the total collected samples, 90 *Escherichia coli* strains were detected based on culture characterization, biochemical and morphological features. In this study, 75% (n = 60) were female and 25% (n = 20) were male. The rate of resistance to 12 antibiotics was Nalidixic acid (83.33%), Tetracycline (56.66%), Norfeloxacin (55.55%), Ciprofloxacin (58.55%), Cephalothin (88.88%), Ceftazidim (40%), Chloramphenicol (26.26%), Co-trimoxazole (70%), Gentamicin (32.22%) Amikacin (17.77%), Nitrofurantoin (21.11%) and Imipenem (2.22%) (Table2). The highest and lowest levels of resistance were to

Cephalothin and Imipenem, respectively. Resistance to 3 antibiotics was shown in 15 isolates (19.73%), 4 antibiotics in 15 isolates (19.73%), 5 antibiotics in 15 isolates (19.73%), 6 antibiotics in 8 isolates (10.52%), 7 antibiotics in 8 isolates (10.52%) and 9 antibiotics in 7 isolates (9.21%).

In this study, a total of 76 MDR isolates in 24 samples (31.57%) integrons were found. The results that show in figure 1. Class I integron was found in 16 isolates (21.05%) and class 2 integron was in 8 isolates (10.52%). No strain was found to contain both class 1 and 2 integrons. The association of resistance to antibiotics and integrons is show in Table 2 and Table 3.

Table 2: prevalence of antibiotic resistant in MDR *Escherichia coli* isolates from urinary tract infections and associations between multi-drug resistance and the existence of integrons

Antibiotic	**Total resistance (%)**
Nalidixic acid (NA)	75 (83.33%)
Ciprofloxacin (CP)	53 (58.88%)
Ceftazidim (CAZ)	36(40%)
Norfloxacin (NOR)	50 (55.55%)
Co-trimoxazole (SXT)	63(70%)
Gentamicin (GM)	29 (32.23%)
Cephalothin (CF)	80 (88.88%)
Amikacin (AN)	16 (17.77%)
Nitrofurantoin (FM)	19 (21.11%)
Chloramphenicol (C)	24 (26.66%)
Imipenem (IPM)	2 (2.22%)
Tetracycline (TE)	51(56.66%)

Table 3: Prevalence of *Int* 1 and *Int* 2 in MDR *Escherichia coli* isolates from urinary tract infections

Resistance	**Number of Isolates (%)**	**No. of *intI*+ isolates**	**No. of *int II*+ isolates**
Three Anti microbial	**8 (10.52)**		
NA/ CF/TE		0	0
NA/CF/SXT		1	0
NA/CF/**FM**		0	0
NA/CF/SXT		0	0
NA/CF/GM		0	0
NA/CF/SXT		0	0
NA/CF/**CAZ**		0	0
NA/CF/SXT		0	0
		0	0
Four Anti microbial	**15(19.73%)**		
NA/CF/ **SXT**/**TE**		0	0
CF/SXT/**CP**/**NOR**		0	0
NA/CF/ **SXT**/**C**		0	0
NA/CF/**SXT**/ TE		1	0
NA/CF/ **SXT**/**FM**		0	0
CF/SXT/**CP**/**NOR**		0	0
NA/CF/ **NOR**/**CP**		0	0
NA/CF/**SXT**/ C		0	0
SXT/ TE/ CAZ/C		0	1
NOR/ TE/ **SXT**/C		0	0
NA/CF/SXT/**CP**		0	0
NA/CF/ **SXT**/**CP**		0	0
NA/CF/ SXT/ TE		0	0
NA/CF/SXT/**CP**		0	0
NA/ CF/ SXT/**CP**		0	0

Five Anti microbial	**12 (15.78%)**		
NA/CF/SXT/**CP**/**NOR**		**0**	0
NA/CF/**SXT**/NOR/**CP**		**0**	0
NA/CF/**SXT**/NOR/ TE		**1**	0
NA/CF/SXT/**CP**/**NOR**		**0**	0
NA/CF/**SXT**/CAZ/ TE		**0**	1
NA/CF / SXT/**CAZ**/ TE		**0**	0
NA/CF/ **SXT**/**CP**/ TE		**0**	0
NA/CF/**SXT**/**CAZ**/ TE		**0**	0
NA /CF /**SXT**/**CP**/ TE		**0**	0
NA/CF/**SXT**/**CAZ**/ TE		**0**	0
NA/CF/**SXT**/**CP**/ TE		**1**	0
NA/CF/**SXT**/ TE/ **NOR**		**0**	0

Six Anti microbial	**8 (10.52%)**		
NA/CF/ **CP**/SXT/ **NOR**/ TE		**0**	0
NA/CF/**CP**/**SXT**/**NOR**/ **CAZ**		**1**	0
NA/ CF/ NOR/ **CP**/ SXT/ TE		**0**	0
NA / CF/ **SXT** / **NOR**/ **CP**/ **CAZ**		**0**	0
NA/ CF/ **CP** / NOR/ SXT/ TE		**0**	1
NA/CF/**SXT**/NOR/**CP**/ TE		**0**	0
NA/ CF/**SXT**/**NOR**/**CP**/**CAZ**		**0**	0
NA /CF /**SXT**/ **NOR**/**CP**/ TE			

Seven Anti microbial	**4 (5.26%)**		
NA/CF/ NOR/ **CP**/SXT/ TE/ CAZ		0	0
NA /CF /SXT/**NOR**/**CP**/ GM/ SXT		0	0
NA/CF/ NOR/ **CP**/SXT/ TE/ CAZ	1	1	0
NA /CF /SXT/**NOR**/**CP**/ GM/ SXT		0	1
Eight Anti microbial	**9 (11.82%)**		
NA/CF/**CP**/NOR/ SXT/ **GM** / TE/ C		0	0
NA/CP/**CP**/NOR/SXT/ TE/ CAZ/ FM		0	0
NA/CP /**CP**/NOR/SXT/TE/ **GM**/ C		1	0
NA /NOR/**CP**/SXT/ TE/ FM/ **GM** / C		0	1
NA/ CF/**CP**/NOR/ SXT/ TE/ CAZ/ C		0	0
NA/CF /**NOR**/**CP**/SXT/ TE/ CAZ/**GM**		0	0
NA/CF/**NOR**/SXT/**CP**/ CAZ/**GM**/ C		1	0
NA /CF /NOR/SXT/ **CP**/ TE/ **GM**/ C NA/CF/**SXT** /NOR/CAZ/TE/**GM**/ C		0	0
		0	0
Nine Anti microbial	4 **(5.26%)**		
NA/CF/ **SXT**/**NOR**/**CP**/CAZ TE/**GM**/ **FM**		**0**	**0**
NA/ CF/**SXT** /NOR/**CP**/ CAZ/GM/ TE/ C		**0**	**1**
NA/ CF/**SXT** /NOR/**CP**/CAZ/ TE/**GM**/ C		**1**	**0**
NA/ CF/SXT/ **CP**/ TE/CAZ/NOR/**GM**/ **FM**		**0**	**0**

Ten Anti microbial	8 **(10.52%)**		
NA /CF /**SXT**/ **NOR** /**CP**/TE/ **AN**/ CAZ/**GM**/ C		**1**	**0**
NA/CF/ **SXT/NOR/CP /CAZ/TE/ FM/ AN/GM**		**0**	**0**
NA/CF/**SXT/NOR/CP /CAZ/ TE/** C/ **AN/GM**		**0**	**0**
NA/CF/**SXT/NOR/CP/CAZ/TE/ FM/ AN/GM**		**0**	**0**
NA/CF /**SXT/ NOR/CP/FM**/C/ **TE/CAZ/AN**		**1**	**0**
NA/CF/**SXT/NOR /CP /FM/TE/CAZ/ AN/GM**		**0**	**1**
NA/CF/**SXT/NOR/ CP/GM/ AN/TE/ CAZ/ FM**		**0**	**0**
NA/CF/ **SXT/NOR/CP/CAZ/AN/FM/TE/GM**		**0**	**0**
Eleven Anti microbial	6 (7.89%)		
NA/CF/**SXT/NOR/CP**/C/ **AN**/ TE/ CAZ/ FM/**GM**		**1**	**0**
NA/CF/SXT/**NOR** / **CP**/AN/FM/ TE/ CAZ/**GM**/ C		**0**	**0**
NA/CF/ **SXT/NOR/ CP/GM/ AN/TE/ CAZ/ FM/C**		**0**	**1**
NA/CF/ **SXT/NOR/CP /CAZ/AN/FM/TE/GM**/C		**0**	**0**
NA/CF/ **SXT/NOR/ CP/GM/ AN/TE/ CAZ/ FM/C**		**1**	**0**
NA/CF/ **SXT/NOR/CP /CAZ/AN/FM/TE/GM**/C		**0**	**0**
Tewlve	**2 (2.63%)**		
NA/ **CF**/ SXT/ NOR/ CP /GM/ AN/TE/ CAZ/IPM/ FM/ C		**1** **1**	
NA / **CF**/ SXT/NOR/CP/ CAZ /AN/ FM/TE/IPM/ GM/**C**			

NA: NalidixicAcid, NOR: Norfloxacine, TE:Tetracycline, CF:Cephtazidim, SXT: Cotrimoxazole, CRO: Ciprofloxacine, GM: Gentamycine, CP: Ciprofloxacin, AM: Amikaycine, C: Choloramphenicol, FM: Nitroforanteine, IMP: Imipenem.

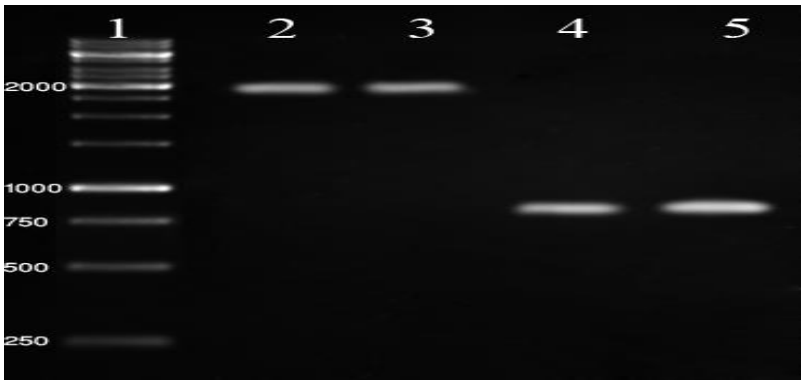

Fig 1: Gel electrophoresis of *Int* 1 and *int* 2 genes; Lane 1: 1 kb Ferments marker, Lanes 2 and 3: 1900 fragment base pair of *int* 1 gene, Lanes 4 and 5: 789 fragment base pair of *int* 2 gene

Discussion:

Multi drug resistant bacteria are now a problem in patients hospitalized throughout the world. The prevalence of MDR among clinical isolates varies greatly worldwide and in geographic areas, and rapidly changes over time (18,19). Knowledge of antibiotic susceptibility, prevalence of MDR, and the presence of integrons in clinical isolates can facilitate the selection of appropriate treatment agents and also help control nosocomial infections. In the present study, we investigated the antimicrobial susceptibility patterns and the presence of class 1 and class 2 integrons in the clinical isolates of *Escherichia coli* from Kermanshah, Iran. Integrons are genetic elements located on the bacterial chromosome or a plasmid that often carry genetic determinants for antimicrobial drug resistance (20, 21). The results of the present study showed a high level of antimicrobial resistance among *Escherichia coli* isolates. A total of 90 *Escherichia coli* isolates were analyzed in this study, up to 50% of resistance was observed against different antimicrobial agents, including Nitrofurantoin, Co-trimoxazole, Ceftazidime, Cephalothin, Gentamicin, Tetracycline, Nalidixic acid, Chloramphenicol, and Amikacin. This study revealed that a major prevalence of resistant

Escherichia coli isolates UTI samples. *Escherichia coli* isolate showed a high degree of resistance to Cephalothin (88.88%), Nalidixic acid (83.33%), Co-trimoxazole (70%), Ciprofloxacin (55.55%), Tetracycline (53.33%) and ceftazidime (32.89%). In other parts of the world, several studies have identified entrobacteriacea family isolates, showing high rates of resistance (up to 50%) to different antimicrobial agents, including Ceftazidime, Amikacin, Gentamicin, Co-trimoxazole and Ciprofloxacin. In our study, 76 (84.44%) isolates showed MDR. The rate of occurrence of MDR *Escherichia coli* isolates observed in our study was similar to that reported by this study (22-24). In this study, MDR *Escherichia coli* isolates with resistance to three or more different antibiotics were common. Seventy-Six samples (84.44%) had MDR, similar to the rate of multi-drug resistance reported in *E. coli isolates* by Ahangarzadeh Rezaee *et al*., 2011 (14).

Escherichia coli isolates in our study were extremely resistant (88.88%) to Cephalothin, similar to Ahangarzadeh Rezaee *et al* (84%) in *K. pneumoniae* from Northwest Iran (21). A high level of antimicrobial resistance (83.33%) against Nalidixic Acid was also observed in this study as compared to Muhammad *et al* (84.16%), *E. coli* from Punjab in Pakistan (10) and Ahangarzadeh Rezaee *et al* (81.3%) in *K. pneumoniae* in Northwest of Iran. In this study, resistance rate to quinolones (Nalidixic Acid, Ciprofloxacin and Norfloxacin) were 83.33%, 84.1% and 55.55%, respectively. Ahangarzadeh Rezaee *et al*. conducted a similar study in Northwest of Iran and reported 60.7%, 43.3% and 59.7% resistance rate to these antibiotics. In another study in Tabriz, Ahangarzadeh Rezaee *et al* compared the prevalence of multi-drug resistant *Klebsiella pneumonia* in paediatric and adult patients with UTI in the educational health centers of Tabriz. The resistance rate

to Nalidixic Acid and Ciprofloxacin was 58.33% and 63.05% respectively, comparable with the results of this study (25). A low level resistance rate to Imipenem was found in this study (2.22%), similar to Soltan Dalal (2%) in Emam Khomeini hospital and Ahangarzadeh Rezaee *et al* (0.8%) in multi drug resistance *E. coli* isolates in Northern west of Iran (14, 26).

Mobilized integrons are substantial contributors to the spread of antibiotic resistance genes. The three classes of integron mostly contributing to the problem of multidrug resistance are classes 1, 2, and 3 (27-29), where they are determined based on sequence differences in the respective *IntI* proteins (30). Of the three, class 1 integrons are the most abundant and found in a diverse range of other mobile elements (31,32), such as transposons and plasmids. The antimicrobial resistant gene located on an integron-like structure is being increasingly reported worldwide (18,33).

The prevalence rate of integrons in Europe and Asia from clinical *E. coli* isolates has been reported to be 22% to 59% (17). In the 76 MDR isolates, the class 1 integrase gene (*intI1*) showed a dominant presence. A positive test result for integrons was found in 24 (31.57%) of 76 MDR isolates screened, including 16 isolates (21.05%) of class 1 and 8 isolates (10.52%) of class 2. No strain was found to contain both class 1 and 2 integrons. In the present study, class 1 integrone was more prevalent than class 2, similar to Aarati et al (2006), Rao et al (2006), Ahangarzadeh Rezaee et al. (2012) (20-22). Falakian *et* al (20011) reported the high prevalence of class1 integron (49.1%) among *Escherichia coli* isolates of patients with urinary tract infection in Shahrekord. Reports from Asian countries have also noted a high prevalence of class I integrons in gram-negative clinical isolates (33). Hak Sun *et a* (2004) reported 54% and 5% for *intI* and *int2* respectively

among *Escherichia coli* isolates from urine specimens collected in Korea (34). Reports from different countries have described a high prevalence of class 1 and class 2 integrons in gram-negative clinical isolates. These data suggest that integrons are relatively common, especially in enterobacteriaceae, and they contribute to the spread of antimicrobial drug resistance in healthcare settings (14).

In this study, we observed a significant relationship between the presence of an integron and the phenotypic resistance to some antimicrobial agents tested. All data were analyzed using SPSS software, version 19.0. The significance of differences between the resistance patterns of the isolates was determined using the chisquare test and Fisher exact test. There was a positive association between the presence of the *intI1* gene and resistance to Norfloxacin (P-value= 0.039) and Cephalotin (P-value=0.002). This indicated that the antibiotic resistance genes were located within the integron. Low prevalence of integron in this study indicated that the resistance gene cassettes were located in different elements such as transposons and prophage.

Islami *et al.,* 2010 found a significant correlation between integron and resistance to Cephalothin, Gentamicin, Norfeloxacin and Nalidixic acid (36). In another study by Ahangarzadeh Rezaee *et al* (2012), were found a positive association between the presence of the *intI1* gene and resistance to Gentamicin, Ttracycline, Ceftazidime, Cephalothin, Chloramphenicol, and |Nalidixic Acid has been reported. In this study, a positive association was also observed between the presence of the *intI2* gene and resistance to tetracycline (21).

In a study conducted by Falakian *et al*, the prevalence of class 1 integron was reported to be 49.01%. This study found a significant correlation between class 1 integrons and resistance to Gentamicin, Ceftriaxon,

Aztreonam, Ciprofloxacin and Co-trimoxazole, comparable with similar studies in south and northern west of Iran (17).

Most *E. coli* isolates were sensitive to imipenem, but only 2.2% were resistant to imipenem. Franklin *et al* (2002) reported that imipenem was the most active agent against Gram-negative isolates, there by confirming the results of this study (33).

Salem *et al*., 2003 reported that there was a correlation between UTIs with gender. In this study, 75% (n = 60) were female and 25% (n = 20) were male. It has been previously found that UTIs is always a very common phenomenon among the women (35). Women are mostly at the risk of developing UTIs and half of them develop a UTI during their lifetime (37). Men and women of elderly group have been found to be very much prone to UTI. In conclusion, the differential association of class 1 and class 2 integrons with resistance in *E. coli* isolates suggested that integrons could facilitate the spread of antimicrobial resistance. This is the first report on the association of multidrug resistance in the presence of class 1 and class 2 integrons in *E. coli* isolates from urinary tract infection in Kermanshah, Iran.

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Prevalence of class 1 and 2 integrons in *uropathogenic Escherishia coli* isolates from diabetic patients in Kermanshah, Iran

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Abstract:

Background and Objective: *Escherichia coli* is one of the most predominant pathogens causing 80-90% of community acquired UTIs and 30-50% of nosocomial acquired UTIs. Multi drug resistance isolates encoded by linked resistance genes occurs on integrons.

Materials & Methods: In this cross sectional study, a total number of 90 *E. coli* isolates were obtained from urine samples of diabetic patients in Kermanshah, Iran, during a six-month period (October to March, 2015). Urine samples were tested by standard bacteriological methods and antibiotic susceptibility tests were done for all isolates by Kirby Bauer agar disk diffusion. Detection of class 1 and class 2 integrons were performed by PCR in MDR isolates.

Findings: The most common resistant phenotypes were found to be Cefalothin (88.88%), Nalidixic acid (83.33%), Co-trimoxazole (70%), Ciprofloxacin (58.88%), tetracycline (56.66%), Norfloxacin (55.55%), Ceftazidim (40%), Gentamycine (32.23%), Choloramphenicol (26.66%), Nitrofurantion (21.11%), Amikacin (17.77%) and Imipenem (2.22%). In this study, a total of 90 isolates in 76 samples were reported MDR. Class I integron was found in16 isolates (21.05%) and class 2 integron was found in 8 isolates (10.52%). No strain was found to contain both class 1 and 2 integrons. In this study,

Conclusion: There was a significant relationship between the presence of the *intI1* gene and resistance to Norfloxacin and Cephalotin. This indicated that the antibiotic resistance genes were located within the integron.

Key Words: *Escherishia coli*, Integrons, Multi Drug Resistance, Urinary Tract Infectio