Carriage of Class 1 and Class 2 Integron in Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Burn Patients in Tehran Hospitals, Iran

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ABSTRACT

Objective: *To investigate the antimicrobial susceptibility patterns of* Pseudomonas aeruginosa *clinical isolates and their associations with the existence of integrons.*
Methods: *During a 12-month study, 140 clinically significant* Pseudomonas aeruginosa *isolates were collected from patients hospitalized in the burn ward of different hospitals in Tehran.* Pseudomonas aeruginosa *isolates were identified using standard laboratory procedures. Antimicrobial susceptibility testing was performed for 13 antimicrobial agents according to the standard Kirby-Bauer disk diffusion method and Clinical and Laboratory Standards Institute (CLSI) guidelines. The frequency of Class 1, 2 and 3 integrons was detected using a polymerase chain reaction (PCR) method.*
Results: *The resistance rates of* Pseudomonas aeruginosa *isolates to 13 antimicrobial agents were between 34.7% and 90.8%. Ceftriaxone and imipenem had good activity against the isolates. Of 140 tested isolates, 91 (65%) were multidrug resistant. The most predominant resistance profile among the isolates included resistance to 10 (12.14%), 9 (12.14%) and 8 (12.14%) antibiotics. Class 1 and 2 integrons were detected in 57.2% (56/98) and 30.6% (30/98) of tested* Pseudomonas aeruginosa *isolates, respectively. Of 98 (70%) integron positive isolates, only 12 (12.2%) isolates were positive for both classes of integrons. Resistance of the isolates to cefotaxime, aztreonam, imipenem, tobramycin, ticarcillin, ciprofloxacin and cloxacillin was observed to be significantly associated with the existence of integrons.*
Conclusion: *These data confirmed high prevalence of Class 1 integrons among* Pseudomonas aeruginosa *isolates from burn patients in this study. Based on these results, integrons may play an important role in the possible transmission of resistance genes to the clinical* Pseudomonas aeruginosa *isolates.*

Keywords: *Pseudomonas aeruginosa*, integron, multidrug resistant

Transporte de Integrones de Clase 1 y Clase 2 en *Pseudomonas aeruginosa* Multifármaco-resistente Aislada de Pacientes con Quemaduras en Hospitales de Teherán, Irán

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RESUMEN

Objetivo: *Investigar los patrones de susceptibilidad antimicrobiana de aislados clínicos de* Pseudomonas aeruginosa *y sus asociaciones con la existencia de integrones.*
Métodos: *Durante un estudio de 12 meses, 140 aislados clínicamente significativos de* Pseudomonas aeruginosa *se obtuvieron de pacientes hospitalizados en la sala de quemados de diversos hospitales en Teherán. Los aislados de* Pseudomonas aeruginosa *fueron identificados utilizando procedimientos estándar de laboratorio. Se realizaron pruebas de susceptibilidad antimicrobiana a 13 agentes antimicrobianos siguiendo el método estándar de difusión de disco Kirby-Bauer y las pautas del denominado Clinical and Laboratory Standards Institute (CLSI). La frecuencia de los integrones de clase 1, 2 y 3 fue detectada usando el método de reacción en cadena de la polimerasa (RCP).*

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Resultados: *Las tasas de resistencia de los aislados de* Pseudomonas aeruginosa *a los 13 agentes antimicrobianos estuvieron entre 34.7% y 90.8%. La ceftriaxona y el imipenem mostraron buena actividad contra los aislados. De los 140 aislados analizados, 91 (65%) resultaron multifármaco-resistentes. El perfil de resistencia más predominante entre los aislados incluyó la resistencia a 10 (12.14%), 9 (12.14%) y 8 (12.14%) antibióticos. Los integrones de clase 1 y 2 fueron detectados en 57.2% (56/98) y 30.6% (30/98) de los aislados de* Pseudomonas aeruginosa *analizados, respectivamente. De 98 (70%) aislados positivos de integrón, sólo 12 aislados (12.2%) fueron positivos para ambos integrones. Se observó que la resistencia de los aislados a la cefotaxima, el aztreonam, el imipenem, la tobramicina, la ticarcilina, la ciprofloxacina y la cloxacilina, se hallaba significativamente asociada con la presencia de integrones.*
Conclusión: *Estos datos confirmaron la alta prevalencia de los integrones de clase 1 entre los aislados de* Pseudomonas aeruginosa *en los pacientes quemados de este estudio. De acuerdo con estos resultados, los integrones pueden desempeñar un papel importante en la posible transmisión de genes de resistencia a los aislados clínicos de* Pseudomonas aeruginosa.

Palabras claves: *Pseudomonas aeruginosa*, integrón, resistencia a múltiples fármacos

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INTRODUCTION

Pseudomonas aeruginosa, as an opportunistic nosocomial pathogen, is the major cause of infection in immunocompromised and burn patients (1). *Pseudomonas aeruginosa* is responsible for a broad spectrum of infections that can range from urinary or wound infections to bacteraemia, endocarditis and death (2). Overall, infections associated with *Pseudomonas aeruginosa* in the immunocompromised and burn patients have a higher mortality rate than any other Gram-negative bacterial infection (1, 2). Infections resulting from this bacterium require antimicrobial therapy, but due to decreased susceptibility and increased resistance to many antimicrobial agents, infections caused by this micro-organism are often difficult to treat. The prevalence of resistance to applied antibiotics in treatment of infections caused by *Pseudomonas aeruginosa* is steadily increasing, and despite reduced use, the resistance rate to them has remained at a high level (3). During the past several decades, *Pseudomonas aeruginosa* has exhibited a remarkable ability to develop multidrug resistance (MDR) rapidly. Widespread multidrug resistant *Pseudomonas aeruginosa* strains isolated from burn patients has become a severe and worldwide concern in the healthcare setting (4, 5). Increase of resistance not only leads to increased economic burden, but also can directly threaten the life of the patient (6). Although the mechanisms of resistance among bacteria are very different and complex, it has been documented that bacterial resistance is caused by chromosomal gene mutations or acquisition of extra-chromosomal pieces of DNA that can be spread through the world's bacterial populations by horizontal transfer mechanism, mediated by mobile genetic elements (7). The mobile genetic elements facilitate horizontal transfer of resistance genes and lead to dissemination of antibiotic resistance genes. However, recently, it has been found that apart from plasmids and transposons, the dissemination of MDR among Gram-negative bacteria, especially in *Pseudomonas aeruginosa*, is linked to integrons (6–8).

Integrons are genetic elements which consist of 5′and 3′-conserved segments with gene cassettes containing antibiotic resistance genes (9). Integrons contribute to the rapid transmission of MDR among clinical isolates of bacteria. The genes carried on integrons usually encode multiple resistances to antimicrobial agents. To date, several classes of integrons have been described that are recognized by their distinct integrase genes (9, 10). Class 1 integrons are the most common and widely distributed among Gram-negative bacteria isolated from clinical samples and often are associated with lateral transfer of antibacterial resistance genes (11).

Class 2 integrons are less common than Class 1 and have been frequently reported in Gram-negative bacteria. Other classes of integrons were reported rarely (9, 10, 12). Several studies have reported the existence of integrons and their role in the development of MDR in isolates of *Pseudomonas aeruginosa* from different world regions. It has been suggested that multidrug resistant *Pseudomonas aeruginosa* can serve as reservoirs for resistance genes and can spread them to other micro-organisms (9, 13).

This study investigated the frequency of integrons as a marker of antibiotic resistance and MDR in *Pseudomonas aeruginosa* isolates from burns patients and their associations with different classes of integrons.

MATERIAL AND METHODS

Bacterial strains

A total of 140 clinical isolates of *Pseudomonas aeruginosa* was collected from patients hospitalized in the burn units of different hospitals in Tehran from November 2012 to October 2013. Duplicate isolates from the same patients were excluded. All *Pseudomonas aeruginosa* isolates were recovered from routine clinical samples and obtained from the depth of the lesion by sterile swab. All samples were transported to the laboratory and were processed immediately. Isolates were identified as *Pseudomonas aeruginosa* using standard microbiological

procedures such as Gram stain, oxidase test, catalase test, arginine dihydrolase, ornithine decarboxylase, growth at 42 ˚C, growth on cetrimide agar medium (Liofilchem, Italy), O/F (oxidation-fermentation) test and pigment production (14). Samples confirmed as *Pseudomonas aeruginosa* isolates were stored in tryptic soy broth (TSB; Merck, Germany) containing 20% glycerol at -70 ˚C for further studies.

Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method according to Clinical Laboratory and Standards Institute (CLSI) criteria (15). The following antimicrobial agents were used in this study: cefotaxime (CTX), amikacin (Ak), gentamicin (GEN), aztreonam (AT), imipenem (IPM), tobramycin (Tob), piperacillin (PIP), ticarcillin (TIC), norfloxacin (NX), ciprofloxacin (CP), ceftriaxone (CRT), cloxacillin (COX) and carbenicillin (CB). All antibiotic disks used in this research, with the exception of piperacillin and carbenicillin (Rosco Diagnostica, Denmark) were supplied by Himedia Co, India. *Pseudomonas aeruginosa* ATCC 27853 was used as the control strain for antimicrobial susceptibility tests.

DNA extraction and PCR assay

Preparation of DNA templates for polymerase chain reaction (PCR) was performed according to Enne *et al* (16). Detection of integron classes was carried out using PCR with degenerate primers designed to hybridize to conserved regions of integron encoded integrase genes intI1, intI2 and intI3. Polymerase chain reaction was used to amplify integrase gene and the primers were designed as follows: forward primer: 5′-TGCGGGT YAARGATBTKGATTT -3′ and reverse primer: 5′- CARCACATGC GTRTARAT - 3′ where B = C or G or T, K = G or T, R = A or G and Y = C or T (17). The amplicons (expected size 491 bp) were separated on 1% agarose gel (Invitrogen, Carlsbad, CA, USA) prepared in TAE buffer and visualized using ultraviolet light (UVItec, Cambridge, UK) after staining with ethidium bromide.

DNA purification and PCR-RFLP analysis

To determine type of integrons, positive PCR products were digested with restriction enzymes *Rsa I* and *Hinf I* (Takara Bio Inc., Otsu, Japan). The products were separated in a 1.5% agarose gel. The size and number of generated fragments are shown in Table 1 and Fig. 1. Amplified DNA fragments corresponding to the variable regions of each distinct Class 1 and Class 2 restriction fragment length polymorphism (RFLP) type integrons were sequenced using an ABI Prism 377 automated sequencer (Applied Biosystems, Perkin-Elmer, Foster City, CA). Nucleotide sequences were compared to the published sequences in the GenBank and the European Molecular Biology Laboratory (EMBL) databases using the BLASTN local alignment search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

Statistical analysis was performed using SPSS software for Windows, version 17.0 (SPSS Inc, Chicago, IL). Chi-squared and Fisher's exact tests were applied to our analysis. A *p*-value less than 0.05 was considered statically significant.

Table 1: Restriction fragment length polymorphism classification of integrase polymerase chain reaction products

PCR product	Enzyme	No of fragment	Fragment size (s)(bp)
Int I1	*Rsa I*	1	**491**
	Hinf I	1	**491**
Int I2	*Rsa I*	2	**334, 157**
	Hinf I	2	**300, 191**
Int I3	*Rsa I*	3	**97-104-290**
	Hinf I	**2**	**119-372**

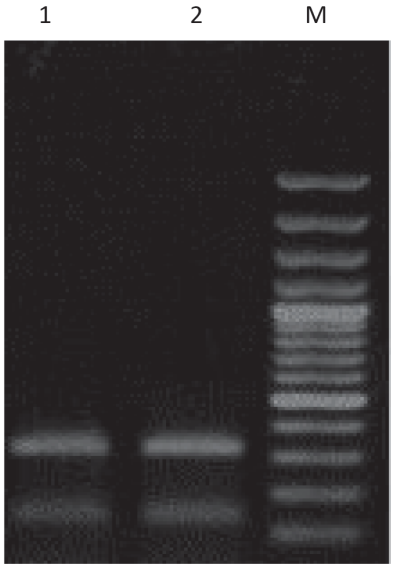

Fig. 1: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of integrase gene products. Lane 1: *RsaI* digested of amplified products represent Class 2 integrons. Lane 2: control positive, Lane M: molecular marker (ladder 100 bp).

RESULTS

During the 12-month study period, a total of 140 *Pseudomonas aeruginosa* isolates were recovered from hospitalized burn patients. Of the 140 isolates included in the study, 83 isolates (59.3%) had been isolated from male and 57 isolates (40.7%) had been isolated from female. The median age was 48.9 years with an age range from 10 months to 76 years old. The result of the antimicrobial susceptibility testing of 140 *Pseudomonas aeruginosa* clinical isolates by the disk diffusion method to 13 antibiotics tested revealed resistance to cefotaxime was in 120 isolates (85.7%), cloxacillin in 117 (83.5%), carbenicillin in 116 (82.8%), piperacillin in 105 (75%), tobramycin in 102 (72.8%), ciprofloxacin in 102 (72.8%), amikacin in 99 (70.7%), aztreonam in 81 (57.9%), ticarcillin in 77 (55%), gentamicin in 74 (52.8%), norfloxacin in 67 (47.9%), imipenem in 66 (47.1%) and ceftriaxone in 55 (39.3%). The rates of resist-

ance to the majority of antibiotics tested varied between 50% and 86%. *In vitro* susceptibility of the *Pseudomonas aeruginosa* isolates to 13 antibiotics tested are shown in Fig. 2.

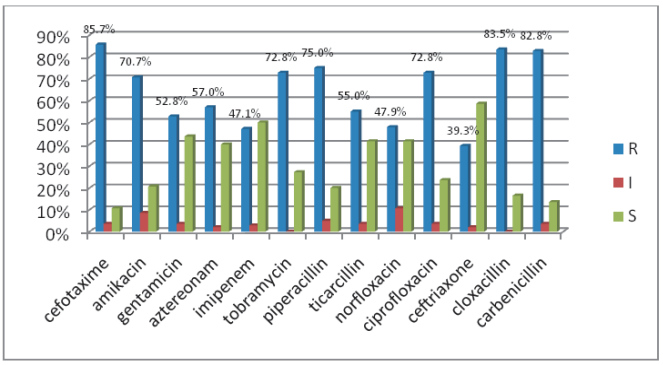

Fig. 2: Antibiotic resistance pattern of 140 *Pseudomonas aeruginosa* isolated from burn patients.
R: resistant; I: intermediate; S: sensitive

None of the strains tested was sensitive to all antimicrobial agents. Multidrug resistance was defined as resistance to six or more antibiotics and this correlated strongly with the presence of integrons (18). Of 140 isolates, 91 (65%) were multidrug resistant. The resistance profile among our isolates included resistance to 11 (7.85%), 10 (12.14%), 9 (12.14%), 8 (12.14%), 7 (11.42%), 6 (9.28%), 5 (9.28%), 4 (11.42%) and 3 (7.14%) antibiotics (Table 2). The existence of integrons was confirmed in 98 (70%) of the isolates PCR (Fig. 3). The PCR-RFLP results of integrase gene revealed a dominant presence of Class 1 integron in 66 (67.3%) isolates, in the event that only 32 isolates (32.7%) had the Class 2 integrase gene (intI2). The existence of Class 3 integron was not confirmed in any of the multidrug resistant strains. Co-existence of Class 1 and 2 integron was detected in 12 isolates (12.2%). The relationship between the existence of integrons and drug resistance to cefotaxime, aztreonam, imipenem, tobramycin, ticarcillin, ciprofloxacin and cloxacillin was statistically significant [$p < 0.05$] (Table 3).

Table 2: Different pattern of multidrug resistance in *Pseudomonas aeruginosa* strains isolated from burn patients

Antibiotic resistance profiles	**Number of antibiotic resistant isolates**	**Antibiotics no.**	**Total number of resistant isolates (%)**
CTX-COX-CB-PIP-Tob-CIP-AK-AT-TIC-GEN-NX	8	11	11 (7.85)
CTX-COX-CB-PIP-Tob-CIP-AK-AT-TIC-GEN-CRT	3		
CTX-COX-CB-PIP-Tob-CIP-AK-AT-TIC-IMP	5	10	17 (12.14)
CTX-COX-CB-PIP-Tob-CIP-AK-AT-TIC-NX	5		
CTX-COX-CB-PIP-Tob-CIP-AK-AT-GEN-NX	4		
CTX-COX-CB-PIP-Tob-CIP-AK-NX-IMP-CRT	3		
CTX-COX-CB-PIP-Tob-CIP-AT-IMP-CRT	5	9	17 (12.14)
CTX-COX-CB-PIP-Tob-AK-TIC-GEN-IMP	4		
CTX-COX-CB-PIP-Tob-TIC-GEN-NX-IMP	5		
CTX-PIP-Tob-AK-AT-TIC-GEN-IMP-CRT	3		
CTX-COX-CB-PIP-Tob-CIP-AK-AT	6	8	17 (12.14)
CTX-COX-CB-PIP-Tob-CIP-AK-TIC	5		
CTX-COX-CB-PIP-Tob-TIC-GEN-NX	4		
CTX-COX-CB-TIC-AK-NX-IMP-CRT	2		
CTX-COX-PIP-TOB-CIP-AK-AT	3	7	16 (11.42)
CTX-COX-Tob-CIP-AT-TICGEN	4		
CTX-COX-CIP-AK-AT-GEN-NX	2		
CTX-COX-AT-TICNX-IMP-CRT	3		
CTX-AT-TIC-GEN-NX-IMP-CRT	4		
CTX-COX-CB-PIP-Tob-CIP	3	6	13 (9.28)
CTX-CB-PIP-AT-TIC-IMP	2		
CTX-Tob-CIP-AK-GEN-NX	3		
CTX-AK-TIC-GEN-NX-IMP	1		
COX-CB-PIP-Tob-CIP-AK	2		
COX-CB-AK-AT-GEN-NX	2		
CTX-COX-CB-Tob-CIP	1	5	13 (9.28)
CTX-PIP-CIP-AK-AT	3		
CTX-CB-CIP-AT-GEN	3		
CIP-AK-GEN-NX-IMP	5		

Table 2 (cont'd): Different pattern of multidrug resistance in *Pseudomonas aeruginosa* strains isolated from burn patients

Antibiotic resistance profiles	Number of antibiotic resistant isolates	Antibiotics no	Total number of resistant isolates (%)
CTX-COX-CB-PIP	3	4	16 (11.42)
CTX-COX-PIP-Tob	4		
CTX-PIP-AK-AT	5		
CB-PIP-Tob-CIP	3		
CB-AK-AT-TIC	3		
CTX-COX-CB	1	3	10 (7.14)
CTX-CB-PIP	2		
CTX-PIP-Tob	1		
CTX-CIP-AT	3		
CTX-AT-TIC	1		
CTX-PIP-CRT	**2**		

CTX: cefotaxime; Ak: amikacin; GEN: gentamicin; AT: aztreonam; IPM: imipenem; Tob: tobramycin; PIP: piperacillin; TIC: ticarcillin; NX: norfloxacin; CP: ciprofloxacin; CRT: ceftriaxone; COX: cloxacillin; CB: carbenicillin

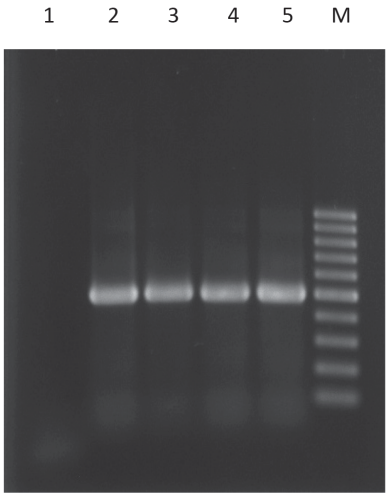

Fig. 3: Lane 1: negative control; Lane 2: control positive; Lane 3: polymerase chain reaction products of conserved region of integrase; Lane 4: *Rsa I* digested of amplified products represent Class 2 integrons; Lane 5: *Hinf I* digested of amplified products represent Class 2 integrons; Lane M: molecular marker (ladder 100 bp).

DISCUSSION

Beta-lactam antibiotics, aminoglycosides and fluoroquinolones are the most important antimicrobial agents used for the treatment of *Pseudomonas aeruginosa* infections (4). Nowadays in our treatment protocols, these antibiotics are commonly prescribed for treatment of burn patients, but with this infection, several studies revealed that this organism rapidly develops resistance to these antibiotics (6, 19).

Resistance to beta-lactam antibiotics is of considerable concern to the health system; they are important drugs of choice for treatment of *Pseudomonas aeruginosa* infections. The findings of our study demonstrated a notably high resistance rate to beta-lactam antibiotics that is in agreement with the results of previous studies (6, 19).

The data from our investigation showed that 66 isolates (47.1%) were resistant to imipenem. Decreased susceptibility to imipenem in *Pseudomonas aeruginosa* isolates has been reported by several investigators (9, 11). In Brazil, the rate of resistance to carbapenems was reported in 43% of clinical strains of *Pseudomonas aeruginosa* (9). In another study that was

Table 3: Association between integrons and antibiotic resistance in 140 *Pseudomonas aeruginosa* strains isolated from burn patients

Antibiotics	Antibiotic susceptibility (n = 140)			Integron positive (n = 98)			Inetgron negative (n = 42)			*P*-value[a]
	R n (%)	I n (%)	S n (%)	R n (%)	I n (%)	S n (%)	R n (%)	I n (%)	S n (%)	
Beta-lactams										
Cephalosporins										
cefotaxime	120 (85.7)	5 (3.6)	15 (10.7)	88 (89.8)	3 (3.06)	7 (7.14)	32 (76.2)	2 (4.7)	8 (19.1)	**0.05**
ceftriaxone	55 (39.3)	3 (2.1)	82 (58.6)	34 (34.7)	3 (3.06)	61 (62.24)	21 (50)	0 (0)	21 (50)	0.24
Penicillin										
piperacillin	105 (75)	7 (5)	28 (20)	75 (76.5)	5 (5.1)	18 (18.4)	30 (71.4)	2 (4.8)	10 (23.8)	0.61
cloxacillin	117 (83.5)	0 (0)	23 (16.5)	89 (90.8)	0 (0)	9 (9.2)	28 (66.7)	0 (0)	14 (33.3)	**0.001**
carbenicillin	116 (82.8)	5 (3.6)	19 (13.6)	79 (80.6)	3 (3.06)	16 (16.34)	37 (88.1)	2 (4.8)	3 (7.1)	0.23
ticarcillin	77 (55)	5 (3.6)	58 (41.4)	45 (45.92)	3 (3.06)	50 (51.02)	32 (76.1)	2 (4.8)	8 (19.1)	**0.001**
Monobactam										
aztreonam	81 (57)	3 (2.1)	56 (40)	53 (54)	0 (0)	45 (46)	28 (66.7)	3 (7.1)	11 (26.2)	**0.046**
Carbapenem										
imipenem	66 (47.1)	(2.9)	70 (50)	57 (58.2)	2 (2)	39 (39.8)	9 (21.4)	2 (4.8)	31 (73.8)	**0.001**
Aminoglycosides										
amikacin	99 (70.7)	12 (8.6)	29 (20.7)	70 (71.4)	7 (7.2)	21 (21.4)	29 (69)	5 (11.9)	8 (19.1)	0.92
gentamicin	74 (52.8)	5 (3.6)	61 (43.6)	50 (51)	4 (4.1)	44 (44.9)	24 (57.1)	1 (2.4)	17 (40.5)	0.76
tobramycin	102 (72.8)	0 (0)	38 (27.2)	78 (79.6)	(0)	20 (20.4)	24 (57.1)	0 (0)	18 (42.9)	**0.011**
Fluoroquinolones										
norfloxacin	67 (47.9)	15 (10.7)	58 (41.4)	5 (46)	15 (15.3)	38 (38.7)	22 (52.4)	0 (0)	20 (47.6)	0.43
ciprofloxacin	102 (72.8)	5 (3.6)	33 (23.6)	68 (69.4)	2 (2.04)	28 (28.56)	34 (90.9)	3 (7.1)	5 (12)	**0.05**

R: resistant; I: intermediate; S: sensitive
[a]Statistically significant values are in bold

done on 54 *Pseudomonas aeruginosa* isolates from the intensive care unit (ICU) by Moradian *et al*, the prevalence of imipenem-resistant imipenem was 53.7% (20). The high resistance rates observed in this study against beta-lactam antibiotics may be explained by the production of plasmid encoded extended spectrum beta-lactamase (ESBL) enzymes that are mostly located in integron structures.

Aminoglycosides as a major antipseudomonal component are often administered with other antimicrobials, most notably beta-lactams, for treatment of *Pseudomonas aeruginosa* infections (2, 4). In the present study, there was a remarkable increase in resistance to aminoglycoside groups: amikacin (70.7%), gentamicin (52.8%) and tobramycin (72.8%). In contrast to our findings, other studies have reported low resistance rates to tobramycin and amikacin (9, 11, 20). Decreased susceptibility to aminoglycosides, including tobramycin and amikacin in *Pseudomonas aeruginosa* isolated from burn wound infections has been reported previously (21).

In the present study, the resistance rates of isolates to ciprofloxacin and norfloxacin, in spite of limitation in the use of them, were relatively high. This is in accordance with Poonsuk *et al* (19) and may be explained by the extensive use of these drugs. Overall, the main mechanisms of resistance that have been described in *Pseudomonas aeruginosa* clinical isolates for the three main classes (beta-lactams, aminoglycosides and fluoroquinolones) are permeability alterations, antibiotic-inactivating enzymes, target modifications (mutations in topoisomerase, ribosomal methylation) and aminoglycoside-modifying enzymes (2, 4). The difference in our findings compared to the previous studies is due to differences in the type of samples, the study duration, increased antibiotic usage for treatment of burn infection, ability of strains to acquire resistance genes by transposable elements such as plasmids, transposons and especially integrons, integron detection method, and epidemiological conditions.

The increasing frequency of multidrug resistant *Pseudomonas aeruginosa* has become an emerging challenge especially in the treatment of burn patients (2, 4). The results of the present study showed a high level of MDR among *Pseudomonas aeruginosa*; MDR among our isolates was 65%

which is higher than the rate of MDR reported in Turkey [20.9%] (22) and Iran [42.3%] (21) and lower than China [90.1%] (6) and Brazil [71%] (9).

The multiple resistance mechanism of *Pseudomonas aeruginosa* is extremely complicated but it could be attributable to transposable elements (transposons, plasmids and also integrons) which can transfer resistance genes among bacteria (23). The studies conducted by Paauw *et al* and Fallah *et al* demonstrated that acquisition of resistance genes is not random and the transfer of integron-carrying elements plays a dominant role in the development of MDR (23, 24).

In the present study, we investigated 140 *Pseudomonas aeruginosa* isolated from burn patients and found that from 140 isolates carrying integrons, 67.3% and 32.7% contained Class 1 and 2 integrons, respectively. As previously noted, the presence of Class 1 integron, in line with the results of other studies (11, 18, 25), was more than that of Class 2 integron. Class 2 integrons were detected in a limited number of isolates (32.7%). According to several studies, the Class 2 integron positive rates had been increased among *Pseudomonas aeruginosa* isolates. Xu *et al* showed that the Class 2 integron-positive rates had been rising during the five-year study period from 8.0% to 60.8% (26).

In the present study, a clear difference in the distribution and frequency of Class 1 integrons among multidrug resistant *Pseudomonas aeruginosa* clinical strains could be correlated with *Pseudomonas aeruginosa* strains isolated from burn wounds and antibiotic selective pressure in expression of integron-associated resistance gene cassettes.

We also found a significant relationship between integrons and resistance to cefotaxime, aztreonam, imipenem, tobramycin, ticarcillin, ciprofloxacin and cloxacillin. However, a statistically significant association was not observed between resistance to nearly half of the antibiotics that were tested including amikacin, gentamicin, piperacillin, norfloxacin, ceftriaxone and carbenicillin and the presence of integrons. This reflects the fact that other vehicles of acquisition and spreading of resistance genes (plasmids and conjugative transposons) and some other mechanisms (biofilm formation, up-regulation of multidrug efflux pumps, target modifications and changes of outer membrane permeability) could also be effective.

In addition, the detection of integrons with high-level resistance among *Pseudomonas aeruginosa* strains confirms the propensity of *Pseudomonas aeruginosa* to carry and transfer multidrug resistant genes between other pathogens. In our study, some isolates also had multiple resistance pattern, but they did not carry any integrons. These resistance may have resulted from chromosomal mutation, plasmid acquisition, or the presence of other integrons types.

CONCLUSION

According to our findings, cefotaxime, cloxacillin and carbenicillin are not effective drugs for treatment of *Pseudomonas aeruginosa* infections, especially in Iranian burn patients. High frequency of integrons among our isolates showed that they played an important role in multidrug resistant *Pseudomonas aeruginosa* and facilitate the spread of antimicrobial drug resistance. Continuous surveillance for *Pseudomonas aeruginosa* multidrug-resistant strains and integrons is necessary to prevent the further spread of resistant isolates. Further studies are needed to determine the types of gene cassettes in these integrons.

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