

Multidrug resistance and integron carriage in clinical isolates of *Pseudomonas aeruginosa* in Tehran, Iran

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Background/aim: *Pseudomonas aeruginosa* is the cause of 10% of hospital-acquired infections. The organisms are often multidrug-resistant, mediated mostly by antibiotic-resistant integrons. The aim of this research was to study integron carriage and its association with multidrug resistance in burn and nonburn clinical isolates of *P. aeruginosa*.

Materials and methods: A total of 112 *P. aeruginosa* clinical isolates were collected from the Motahari and Shohadaye Tajrish hospitals in Tehran between July and December 2011. Antibiotic susceptibility to 13 antibiotics was determined by disk diffusion. Detection of integron classes 1 and 2 and amplifications of internal variable regions (IVRs) of class 1 integrons were carried out by PCR and specific primers.

Results: Among the 112 isolates, 77 were from burn patients and 35 were nonburn isolates. Multidrug resistance and class 1 integron carriage were both significantly higher in the burn isolates compared to the nonburn strains (97.4% vs. 22.8% and 82.3% vs. 17.7%, respectively). Class 2 integron (2.7%) was only present in the burn isolates. Amplification of IVRs of class 1 integrons revealed 3 different fragment arrays.

Conclusion: The significant association between multidrug resistance and integron carriage among *P. aeruginosa* burn isolates suggests a dissemination of resistance determinants by horizontal gene transfer.

Key words: *Pseudomonas aeruginosa*, integron, multidrug resistance

1. Introduction

Pseudomonas aeruginosa is the cause of 10% of all hospital-acquired infections, particularly in immune-compromised hosts suffering from respiratory diseases, cancer, and burns; in children; and in young adults with cystic fibrosis (1–3). *P. aeruginosa* possesses a number of resistance mechanisms and frequently displays multidrug resistance, which can complicate treatment of infections caused by the organism (4,5). Multidrug-resistant (MDR) *P. aeruginosa* has been shown to be responsible for outbreaks in burn units in Iran (6–8).

Integrons are genetic elements that recognize and capture mobile gene cassettes, including the antimicrobial drug resistance determinants (9,10). Three classes of antibiotic resistant integrons (classes 1–3) have been historically associated with MDR phenotypes. These are often located on plasmids, which can facilitate their horizontal transfer among bacteria (9,11). A high prevalence of integron carriage among clinical isolates of *P. aeruginosa* has been reported worldwide (12–14).

In fact, some studies have shown strong associations between MDR isolates of *P. aeruginosa* and class 1 integrons (15,16). Little information is available on the distribution of different classes of integrons and their relation to multidrug resistance in clinical isolates of *P. aeruginosa* in Iran. We investigated the frequency of class 1 and 2 integron carriage as well as the diversity of internal variable regions (IVRs) of the class 1 integron in MDR burn and nonburn isolates of *P. aeruginosa*.

2. Materials and methods

2.1. Clinical isolates

A total of 112 *P. aeruginosa* clinical isolates were collected from Motahari and Shohadaye Tajrish hospitals in Tehran between July and December 2011. Among these, 58% were from male patients and 42% were from females. Most of the isolates ($n = 77$, 68.7%) were recovered from burn patients. The majority of the burn isolates were from wound infections (88.3%), followed by blood (6.5%) and subclavian catheter infections (5.2%). Among the

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35 nonburn isolates, 51.4% were recovered from urine, 28.6% from blood, 17.2% from the trachea, and 2.8% from abdominal fluids. All isolates were maintained at -20°C in brain-heart infusion broth (Oxoid, UK) containing 10% dimethyl sulfoxide (v/v) before use. *P. aeruginosa* ATCC 27853 was used as the control for antibiotic susceptibility tests and *Klebsiella pneumoniae* ATCC 1029, harboring a class 1 integron (kindly provided by Dr Rajabnia, Babol University of Medical Sciences, Iran), was used as the positive control for the PCR studies.

2.2. Antibacterial susceptibility

Antibacterial susceptibility profiles of the isolates were determined by disk diffusion according to the recommendations of the Clinical and Laboratory Standards Institute (17). The antibiotic disks (MAST Diagnostics, Merseyside, UK) were imipenem (10 μg), meropenem (30 μg), aztreonam (30 μg), cefepime (30 μg), ceftazidime (30 μg), amikacin (30 μg), ciprofloxacin (5 μg), piperacillin (100 μg), piperacillin/tazobactam (110 μg), carbenicillin (100 μg), ticarcillin (75 μg), cotrimoxazole (25 μg), and tobramycin (10 μg). All tests were repeated at least 3 times and the averages of the inhibition zones are reported.

2.3. Screening for integrons

DNA extraction was performed using a simple boiling method and the DNA extracts were stored at -20°C until use (18). PCR screening was carried out in reaction mixtures (25 μL) containing 1 μL of DNA extract, 1.5 mM MgCl_2 , 1 U of Taq DNA polymerase, 10 mM dNTPs (Cinnagen, Iran), and 10 pM of each primer (Alpha DNA, Canada), as shown in Table 1. The conditions used for amplification of the class 1 integrons were initial denaturation at 95°C for 5 min followed by 30 amplification cycles consisting of 1 min at 94°C , 1 min at 54°C , and 1 min at 72°C , followed by an extension period of 10 min at 72°C . For the class 2 integron, following the initial 5 min at 95°C , 30 amplification cycles were performed for 30 s at 94°C , 30 s at 52°C , and 2 min at 72°C , with a final extension period of 7 min at 72°C . Amplification of the IVRs of the class 1

integron was carried out for 4 min at 94°C followed by 35 amplification cycles of 1 min at 94°C , 1 min at 55°C , and 2 min at 72°C , followed by 10 min at 72°C . All amplifications were carried out in a Peqlab thermal cycler (Primus 96, South Korea). The PCR products were run on a 1% agarose gel and visualized using an image analysis system (UVItec, St John's Innovation Centre, UK). Class 2 integrons were confirmed by sequencing (Source Bioscience, UK) and the sequences were analyzed using BioEdit version 7.0.5.2.

2.4. Statistical analyses

Statistical analyses were performed using nonparametric analysis and the two-tailed Mann-Whitney U test in SPSS 19 for comparison of antibiotic resistance profiles, multidrug resistance, and integron carriage between burn and nonburn isolates.

3. Results

The antibiotic susceptibility results are shown Table 2. Overall, 74.1% of the isolates were MDR (resistant to ≥ 3 classes of antibiotics). As observed, resistance to all antibiotics was significantly higher in the burn isolates, except for cotrimoxazole. In fact, 97.4% of the burn isolates were MDR compared to 22.8% in the nonburn group ($P = 0.00$).

PCR amplification of the two classes of integron genes showed that 79 isolates (70.5%) carried class 1 integrons, 3 isolates (2.7%) harbored class 2 integrons, and 1 isolate (0.9%) had both classes of integron genes. The nucleotide sequence of the class 2 integron of this study was deposited in GenBank under accession number KC544959.

Integron carriage was significantly higher in burn isolates than nonburn strains. In fact, 82.3% of the burn isolates harbored the class 1 integron compared to 17.7% of the nonburn isolates ($P = 0.00$). The class 2 integron was only detected in 3 of the burn isolates and in none of the nonburn strains. The results also showed that integron carriage correlated with a drug resistance phenotype and was significantly higher among the MDR isolates than the non-MDR strains (92.4% vs. 7.6%) ($P = 0.00$).

Table 1. Primers used for amplification of class 1 and class 2 integrase genes and variable regions of the class 1 integron in *P. aeruginosa* clinical isolates.

Target	Primer	Sequence	Amplicon	Reference	GenBank number
Int 1	Forward	5'-ACGAGCGCAAGGTTTCGGT-3'	565	13	AF550415
	Reverse	5'-GAAAGGTCTGGTCATACATG-3'			
Int 2	Forward	5'-GTGCAACGCATTTTGCAGG-3'	403	13	AP002527
	Reverse	5'-CAACGGAGTCATGCAGATG-3'			
CS-5	Forward	5'-GGCATAACAAGCAGCAAGC-3'	Variable	28	U12338
CS-3	Reverse	5'-AAGCAGACTTGACCTGAT-3'			

Table 2. Comparison of the antibiotic susceptibility between burn and nonburn isolates of *P. aeruginosa*.

Antibiotic	All isolates (% resistance)	Burn isolates (% resistance)	Nonburn isolates (% resistance)	Significance
Ticarcillin	79.5	100	31.4	P = 0.000
Carbenicillin	82.1	100	40.0	P = 0.000
Piperacillin	72.3	98.7	14.3	P = 0.000
Aztreonam	75.9	98.7	25.7	P = 0.000
Tobramycin	77.7	97.4	34.3	P = 0.000
Cefepime	76.8	96.1	34.3	P = 0.000
Meropenem	71.4	96.1	17.1	P = 0.000
Amikacin	77.7	94.8	40.0	P = 0.000
Ciprofloxacin	77.7	94.8	40.0	P = 0.000
Cotrimoxazole	93.7	93.5	94.3	P > 0.500*
Imipenem	70.3	92.2	22.8	P = 0.000
Piperacillin/tazobactam	63.4	87.0	11.4	P = 0.000
Ceftazidime	67.8	69.8	20.0	P = 0.000

* = Not significant.

Amplifications of the class 1 integron variable regions revealed three gene cassette arrays (0.8, 1.3, and 1.7 kb). The majority of the isolates (68.3%) carried the 0.8-kb fragment, 10.1% harbored the 1.3-kb fragment, and 51.9% had the 1.7-kb fragment. Furthermore, 17.7% of isolates harbored both 0.8-kb and 1.7-kb fragments, 5.1% carried both 1.3-kb and 1.7-kb fragments, and 5.1% had all three amplicons.

4. Discussion

Antibiotic resistance integrons, mostly class 1 integrons, have been suspected to serve as reservoirs for antibiotic resistance genes within bacterial pathogens. Several studies have reported the presence of class 1 integrons among clinical isolates of *P. aeruginosa* (19,20). Class 1 integron carriage in *P. aeruginosa* clinical isolates in China has been reported within the range of 38% to 45.8% (12,13,20,21). Brazilian studies have also shown high rates of class 1 integron carriage (41.5% and 63.5%) in *P. aeruginosa* clinical isolates (15,22). Similarly, studies in Malaysia, Thailand, and Nigeria have shown that 60%, 69.3%, and 57% of *P. aeruginosa* clinical isolates harbored class 1 integrons, respectively (23–25). Two reports from Iran have shown the presence of class 1 integrons in 56.3% and 82.6% of *P. aeruginosa* clinical isolates, respectively (18,26). We also found a high rate of integron carriage in our burn isolates of *P. aeruginosa* (70.5%). In fact, integron

carriage was 4.8-fold higher in the burn isolates compared to the nonburn strains (84.8% vs. 17.7%). The present study also showed a significant association between multidrug resistance and integron carriage among the burn isolates, suggesting the role of integrons as the source of resistance genes. In fact, 92.4% of the MDR isolates carried class 1 integrons compared to 7.6% of the non-MDR burn strains ($P < 0.05$). A recent Iranian study reported that 69.2% of MDR burn isolates of *P. aeruginosa* harbored class 1 integrons (27). Characterization of the IVRs of the class 1 integron in the present study showed three gene cassettes, similar to the results reported by Shahcheraghi et al. in clinical isolates of *P. aeruginosa* (14).

Class 2 integron carriage in *P. aeruginosa* was first shown in 2009 in China (19) and later in Malaysia in 2011 (23). Our results showed that three burn isolates (2.7%) harbored the class 2 integron, among which one also carried a class 1 integron. To our knowledge, this is first report from Iran on class 2 integron carriage as well as the coexistence of class 1 and 2 integrons in *P. aeruginosa* clinical isolates. The significant association between multidrug resistance and class 1 integron carriage, as well as the emergence of the class 2 integron in *P. aeruginosa* burn isolates, suggests a potential dissemination of integron-mediated MDR genes by horizontal gene transfer among these organisms. Since integrons are usually located on mobile genetic elements such as plasmids, the rising levels

of drug resistance in these bacterial pathogens could be explained (15,16).

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