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

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1. Introduction

Pseudomonas aeruginosa is the cause of 10% of 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 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

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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 MgCl₂, 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 (UVItect, 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.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer</th>
<th>Sequence</th>
<th>Amplicon</th>
<th>Reference</th>
<th>GenBank number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int 1</td>
<td>Forward</td>
<td>5′-ACGAGCGCAAGGTTTCCGT-3′</td>
<td>565</td>
<td>13</td>
<td>AF550415</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5′-GAAAGGTCTGGTCATACATG-3′</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int 2</td>
<td>Forward</td>
<td>5′-GTGCAACGCATTTCGAG-3′</td>
<td>403</td>
<td>13</td>
<td>AP002527</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5′-CAACGGAGTCATGCAGATG-3′</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS-′5</td>
<td>Forward</td>
<td>5′-GGCATACAACGAGCAAG-3′</td>
<td>Variable</td>
<td>28</td>
<td>U12338</td>
</tr>
<tr>
<td>CS-′3</td>
<td>Reverse</td>
<td>5′-AAGCAGACTTGACCTGAT-3′</td>
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</tr>
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</table>
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 on class 1 integron carriage among the burn isolates showed that 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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References


