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Prevalence of class I, II and III integrons in multidrug-resistant and carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates

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ABSTRACT

Integrons are mobile genetic elements located on transposons, plasmids and bacterial chromosomes. These elements play a key role in spreading resistance gene cassettes to various disinfectants and antibiotics and facilitate the global antibiotic resistance crisis. The present study was aimed to evaluate the prevalence of class I, II and III integrons in multidrug-resistant (MDR) and carbapenem-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) strains isolated from patients referring to Ardabil hospitals. A total of 103 confirmed *P. aeruginosa* clinical isolates were included in this study (collected between June 2019 and April 2021). Antibiotic resistance pattern was assessed using Kirby-Bauer disk diffusion method on Mueller–Hinton agar. The presence of *intI1*, *intI2* and *intI3* genes was detected using the set of specific primers by polymerase chain reaction (PCR) assay. The total frequency of class I integron gene was 58.3% in *P. aeruginosa* isolates. No class II and III integrons were detected in *P. aeruginosa* isolates. The prevalence of MDR *P. aeruginosa* strains was 54.3% and the rate of class I integron was significantly higher in MDR *P. aeruginosa* than non-MDR isolates (76.7% *vs.* 23.3%) ($p < 0.05$). Additionally, the frequency of class I integron gene among carbapenem-resistant *P. aeruginosa* strains was as follows: doripenem 44.7%, imipenem 85.5% and meropenem 62.7% ($p < 0.05$). Our results revealed that class I integron was the most prevalent integrons among clinical isolates of drug-resistant *P. aeruginosa* in Ardabil hospitals. These integrons seem to play a significant role in dissemination of drug resistance genes and the emergence of antibiotic-resistant strains, particularly MDR and carbapenem-resistant ones.

1. Introduction

Since the mid-1950s, in which the first cases of bacterial antibiotic resistance were identified, infections caused by drug-resistant bacteria have been becoming a public health crisis all over the world (Morehead and Scarbrough, 2018; Sabbagh et al., 2021; Khademi et al., 2021). If drug-resistant infections problem is not tackled appropriately, its effects on human and global economy will be remarkable by 2050, projected as 10 million deaths every year and 2–3.5% reduction in GDP (Gross Domestic Product) (Neill, 2014). Among antibiotic-resistant bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa*), an opportunistic Gram-negative bacillus, has attracted more attention because it is a causative agent for important community- and hospital-acquired infections (Khademi et al., 2021; Vaez et al., 2018). According to the reports of international organizations (CDC and WHO), carbapenem-resistant as well as multidrug-resistant (MDR) *P. aeruginosa* strains are growing serious threats against human health in hospitals, especially in patients hospitalized in intensive care units (ICUs) and those who use medical devices such as catheters and ventilators (Morehead and Scarbrough, 2018; World Health Organization). Approximately, 13% of *P. aeruginosa*

Abbreviations: P. aeruginosa, *Pseudomonas aeruginosa*; MDR, Multidrug-resistant; PCR, Polymerase chain reaction; ICUs, Intensive care units; CDC, Centers for Disease Control and Prevention; WHO, World Health Organization; CLSI, clinical and laboratory standard institute; *intI1*, Class 1 integrase; *intI2*, Class 2 integrase; *intI3*, Class 3 integrase; ESBLs, Extended-spectrum-β-lactamases; MBL, Metallo-β-lactamases.

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nosocomial infections are caused by MDR strains, which are difficult to treat with common antibiotics, except for carbapenems and third-generation cephalosporins (Safarirad et al., 2021). However, resistance to these antibiotics is also increasing (Safarirad et al., 2021; Vaez et al., 2018). Various antibiotic resistance mechanisms have been identified in *P. aeruginosa* strains including inhibition of drug entrance, enzymatic degradation, efflux pumps and alteration in target site of drug, but integron-mediated resistance is known for rapid bacterial adaptation to antibiotic selection pressures (Driscoll et al., 2007). Integrons are mobile genetic elements which were identified for the first time in the late 1980s. Similar to transposons, bacteriophages and plasmids, integrons carry and spread the antibiotic resistance gene cassettes, which is more common in Gram-negative bacteria (Sabbagh et al., 2021). Integrons are ubiquitous and located on transposons, plasmids and 17% of the bacterial chromosomes (Sabbagh et al., 2021). There are more than 9 classes of integrons in Gram-negative bacteria, among which 4 are found in clinical isolates (Sabbagh et al., 2021). Hitherto, there has been no information on integron-positive *P. aeruginosa* strains isolated from clinical specimens in Ardabil, northwest of Iran. Therefore, the purpose of this study was to investigate the frequency of class I, II and III integrons among drug-resistant, especially MDR and carbapenem-resistant, *P. aeruginosa* strains isolated from hospitalized patients in northwest of Iran. As a secondary aim, we also assessed the correlation between the presence of integron genes and resistance to different antibiotics.

2. Methods

2.1. P. aeruginosa specimens and drug susceptibility testing

A total of 103 clinical isolates of *P. aeruginosa* were collected from different specimens (urine, blood, wound, sputum and cerebrospinal fluid) in Ardabil hospitals (*Imam* Reza, *Imam* Khomeini, Bu-Ali, Alavi, Sabalan, Fatemi and Ghaem) during the period of June 2019 to April 2021. The clinical specimens were obtained from ICU, emergency, internal medicine, neurology and pediatric wards and cultured on cetrimide agar (Conda, Pronasida, Spain), and then identified using microbiology laboratory tests (colony morphology, pigment production, oxidase and Gram staining) and an amplification-based method using a universal primer that targets genes coding for 16S-23S rRNA internal transcribed spacer (ITS) (Bazghandi et al., 2021b). As previously reported, the prevalence of MDR and carbapenem-resistant *P. aeruginosa* strains was assessed according to the Clinical and Laboratory Standards Institute (CLSI) guideline using Kirby-Bauer disk diffusion method on Mueller–Hinton agar (Conda, Pronasida, Spain) (Bazghandi et al., 2021b; CLSI, 2018).

2.2. Polymerase chain reaction assay

Bacterial DNA was extracted using a simple boiling method (Safarirad et al., 2021) and then used for the amplification of three integron genes, *i.e. intI1*, *intI2* and *intI3*, using polymerase chain reaction (PCR) technique. The optimum conditions for PCR and sequence of primers used in the current study are presented in Table 1 (Delarampour et al., 2020). A volume of 25 μl of the reaction mixture was used in PCR, containing 20 μl of master mix (Ampliqon, Denmark), 2 μl of forward and reverse primers (10 μmol/l) and also 3 μl of template DNA. Finally, 5 μl of PCR amplicon products was used for detection of integron genes using loading on 1% agarose gel electrophoresis. The results were then confirmed by sequencing. The quality control of all experiments was performed by *P. aeruginosa* ATCC 27853 standard strain.

2.3. Statistical analysis

The association between drug resistance and presence of various integron genes in *P. aeruginosa* strains was evaluated using the Chi-square test in SPSS software (version 16). A p-value of <0.05 was considered as statistically significant.

3. Results

Overall, 60 out of 103 clinical isolates (58.3%) were class I integron-positive *P. aeruginosa*. No class II and III integrons were detected among *P. aeruginosa* isolates.

In this study, MDR phenotype was identified in 54.3% ($n = 56$) of *P. aeruginosa* strains. The prevalence of class I integron gene was significantly higher in MDR *P. aeruginosa* than non-MDR isolates ($n = 46$, 76.7% *vs.* $n = 14$, 23.3%, respectively; $p < 0.05$). Additionally, the frequency of class I integron gene among doripenem-, imipenem- and meropenem-resistant *P. aeruginosa* strains was 44.7%, 85.5% and 62.7%, respectively ($p < 0.05$). On the other hand, isolates containing class I integron gene were significantly resistant against all tested antibiotics compared with the negative ones ($p < 0.05$), except for aztreonam (Table 3). Table 2 shows the distribution of *P. aeruginosa* class I integron according to sex, clinical specimen type and hospital ward. There was no significant association between the presence of class I integron gene in *P. aeruginosa* isolates and the above-mentioned variables ($p > 0.05$).

4. Discussion

Class I integron is one of the most prevalent integrons among clinically important Gram-negative bacteria (22-55%) (Deng et al., 2015). We investigated the frequency of class I, II and III integrons among clinical isolates of *P. aeruginosa*. In the current study, 58.3% of *P. aeruginosa* isolates harbored class I integron, which is higher than those

Table 1
Polymerase chain reaction assay.

Gene	Oligonucleotide sequence (5′ to 3′)	Thermal cycling condition for amplification	Amplicon size (bp)
intI1	F: CCTCCCGCACGATGATC R: TCCACGCATCGTCAGGC	Initial denaturation at 94 °C for 6 min (1 cycle) Denaturation at 94 °C for 45 sec Annealing at 55 °C for 45 sec Extension at 72 °C for 1 min (35 cycles)	280
intI2	F: TTATTGCTGGGATTAGGC R: ACGGCTACCCTCTGTTATC	Initial denaturation at 95 °C for 5 min (1 cycle) Denaturation at 94 °C for 1 min Annealing at 55 °C for 45 sec Extension at 72 °C for 1 min (35cycles)	233
intI3	F: AGTGGGTGGCGAATGAGTG R: TGTTCTTGTATCGGCAGGTG	Initial denaturation at 95 °C for 5 min (1 cycle) Denaturation at 94 °C for 1 min Annealing at 57 °C for 45 sec Extension at 72 °C for 1 min (35 cycles)	600

Abbreviation: *intI1*: class 1 integrase, *intI2*: class 2 integrase, *intI3*: class 3 integrase.

Table 2
Prevalence of class I integron in *P. aeruginosa* isolates based on sex, clinical specimen type and hospital ward.

Sex[a] ($n = 98$)	Class I integron-positive 55 (56.1%)	Class I integron-negative 43 (43.9%)	*p* value
Male	29 (52.7)	21 (48.8)	0.702
Female	26 (47.3)	22 (51.2)	

Specimen ($n = 100$)	Class I integron-positive 57 (57%)	Class I integron-negative 43 (43%)	*p* value
Urine	30 (52.6)	24 (55.8)	0.092
Blood	5 (8.8)	10 (23.3)	
Wound	6 (10.5)	5 (11.6)	
Sputum	15 (26.3)	4 (9.3)	
Cerebrospinal fluid	1 (1.8)	0 (0)	

Ward ($n = 84$)	Class I integron-positive 48 (57.1%)	Class I integron-negative 36 (42.9%)	*p* value
ICU	15 (31.2)	12 (33.2)	0.696
Emergency	8 (16.7)	6 (16.7)	
Internal medicine	13 (27.1)	13 (36.1)	
Neurology	11 (22.9)	4 (11.1)	
Pediatric	1 (2.1)	1 (2.8)	

Hospital ($n = 89$)	Class I integron-positive 53 (59.6%)	Class I integron-negative 36 (40.4%)	*p* value
Imam Reza	7 (13.2)	2 (5.6)	0.085
Imam Khomeini	16 (30.2)	16 (44.4)	
Alavi	27 (50.9)	11 (30.6)	
Bu-Ali	2 (3.8)	5 (13.9)	
Others	1 (1.9)	2 (5.6)	

[a] For some samples, related information was not available and therefore omitted from the analysis.

Table 3
Distribution of class I integron in drug-resistant *P. aeruginosa* isolates.

Antibiotic agent (tested isolates)[a]	Class I integron positive n (%)	Class I integron negative n (%)	Total resistance n (%)	*p* value
Piperacillin (92)	38 (69.1)	8 (21.6)	46 (50)	0.000
Piperacillin-tazobactam (90)	29 (56.9)	5 (12.8)	34 (37.8)	0.000
Ticarcillin-clavulanate (66)	44 (100)	16 (72.7)	60 (90.9)	0.000
Ceftazidime (97)	37 (63.8)	6 (15.4)	43 (44.3)	0.000
Cefepime (98)	38 (66.7)	8 (19.5)	46 (46.9)	0.000
Aztreonam (80)	12 (27.9)	6 (16.2)	18 (22.5)	0.212
Doripenem (83)	21 (44.7)	2 (5.6)	23 (27.7)	0.000
Imipenem (95)	47 (85.5)	11 (27.5)	58 (61.1)	0.000
Meropenem (99)	37 (62.7)	5 (12.5)	42 (42.4)	0.000
Gentamicin (101)	35 (60.3)	4 (9.3)	39 (38.6)	0.000
Tobramycin (102)	35 (59.3)	4 (9.3)	39 (38.2)	0.000
Amikacin (93)	36 (63.2)	5 (13.9)	41 (44.1)	0.000
Netilmicin (73)	26 (56.5)	5 (18.5)	31 (42.5)	0.002
Ciprofloxacin (98)	46 (83.6)	6 (14)	52 (53.1)	0.000
Lomefloxacin (87)	46 (86.8)	12 (35.3)	58 (66.7)	0.000
Norfloxacin (100)	46 (80.7)	7 (16.3)	53 (53)	0.000
Levofloxacin (100)	46 (79.3)	6 (14.3)	52 (52)	0.000
Ofloxacin (98)	54 (90)	17 (44.7)	71 (72.4)	0.000

[a] Isolates with intermediate susceptibility were excluded from the analysis.

reported from Isfahan (55.5%), Hamadan (57%), Guilan (30%) and Tabriz (27.5%), and also lower than Ahvaz (95.7%), Shiraz and Kerman (95.2%) and Yazd (82.6%) (Faghri et al., 2018; Alikhani et al., 2017; Ebrahimpour et al., 2018; Mobaraki et al., 2018; Khosravi et al., 2017; Sharifi et al., 2019; Zarei-Yazdeli et al., 2018). Class I integron confers resistance to important antibiotics, which are involved in the treatment of *P. aeruginosa* infections. One of these important antibiotics are carbapenems, which are drugs of choice for the treatment of infections caused by MDR *P. aeruginosa* strains (Safarirad et al., 2021). However, it has been shown that the frequency of carbapenem-resistant *P. aeruginosa* strains was high in Ardabil (doripenem 33.3%, imipenem 66.7% and meropenem 42.9%) (Bazghandi et al., 2021a). Hence, it is necessary to gain knowledge about the mechanisms associated with carbapenem resistance in *P. aeruginosa* strains. It seems that class I integrons play an important role in the emergence of carbapenem-resistant *P. aeruginosa* strains through the spread of resistance gene cassettes, thereby leading to the failure of MDR *P. aeruginosa* infection treatment. In the current study, the prevalence of class I integron gene among carbapenem-resistant *P. aeruginosa* strains was high (doripenem 44.7%, imipenem 85.5% and meropenem 62.7%) ($p < 0.05$) (Table 3). Similar results in accordance with this study were reported by Yousefi et al. (2010) and Fonseca et al. (2005). Over 40 genes are identified in class I integrons, which seem to be associated with resistance to various antiseptics, disinfectants and antibiotics (β-lactams, aminoglycosides, chloramphenicol, macrolides and sulfonamides) (Sabbagh et al., 2021; Deng et al., 2015). Some of these resistance gene cassettes are the *qacEΔ1* gene (resistance to quaternary ammonium salts), *sul1*gene (resistance to sulfonamides), *aadA* gene (resistance to streptomycin-spectinomycin), *aadB* and *aadA2* (resistance to aminoglycosides), bla_{CARB-2} (resistance to carbenicillin) and *dfrA17-aadA5* and *dfrA12-gcuFaadA2* (resistance to trimethoprim) (Sabbagh et al., 2021; Deng et al., 2015; Domingues et al., 2015). Our previous study showed that 42.1% of integron I-positive *P. aeruginosa* strains isolated from Ardabil hospitals harbored *qacEΔ1* gene (unpublished data). However, data on other genes associated with *P. aeruginosa* resistance to antimicrobial agents located in class I integron is not available yet. In addition, genes encoding ESBLs (extended-spectrum-β-lactamases), hydrolyse third- and fourth-generation cephalosporins, and those encoding MBLs (metallo-β-lactamases), hydrolyse carbapenems, are also harbored in class I integrons (Yousefi et al., 2010). Studies showed that Verona integron-mediated (VIM) MBL is involved in the emergence of carbapenem-resistant *P. aeruginosa* strains (Karampatakis et al., 2018). Karampatakis et al. reported that genes encoding some subtypes of this carbapenemase are located in class I integron. Our previous study indicated that 6.6% of imipenem-resistant *P. aeruginosa* isolates collected from Ardabil hospitals harbored the bla_{VIM-1} gene (Safarirad et al., 2021).

Overall, integrons, especially class I type, play an important role in the emergence of MDR strains (Khosravi et al., 2017; Shahandashti et al., 2012). In this study, the prevalence of class I integron was significantly higher in MDR *P. aeruginosa* compared with non-MDR isolates (76.7% *vs.* 23.3%). Similar results were reported from Isfahan (73.6% *vs.* 22.9%) (Faghri et al., 2018) and Guilan (43.3% *vs.* 22.9%) (Ebrahimpour et al., 2018). Additionally, Shahcheraghi et al. showed that all MDR *P. aeruginosa* clinical isolates contained class I integrons (Shahcheraghi et al., 2010).

In addition to carbapenem-resistant and MDR *P. aeruginosa*, we observed a high rate of resistance to various antibiotics among integron-positive isolates (Table 3). Another clinically important integron in Gram-negative bacteria is class II integron (Sabbagh et al., 2021). Gene cassettes available in class II integron that are associated with drug resistance include *dfrA1* (resistance to trimethoprim), *sat1* (resistance to streptomycin) and *aadA1* (resistance to streptomycin/spectinomycin) (Sabbagh et al., 2021). In the present study, the frequency of class II integron was 0%. A similar finding to our results was reported from Ahvaz (0%) (Khosravi et al., 2017). Class III integrons, which contain *qacEΔ1*, *sul1*, bla_{IMP-1} (encode MBL enzymes) and *aacA4* (resistance to tobramycin) genes, are rarely found in *P. aeruginosa* strains (Sabbagh et al., 2021). In this study, *intI3* gene was not found. A similar result was reported by Zarei-Yazdeli et al. in Yazd (Zarei-Yazdeli et al., 2018).

We investigated the prevalence of integrase (*intI*) gene, located in the 5′-conserved segment, but we did not assess the characteristics of some gene cassettes conferring resistance to antibiotics, particularly ESBL-

and MBL-encoding genes, in the internal variable region (IVR) of class I integrons. This was the main limitation in the current study.

5. Conclusion

In the present study, class I integron was found to be the most prevalent integron among clinical isolates of *P. aeruginosa* in Ardabil. Considering the existence of significant association between high prevalence of class I integron and drug resistance in *P. aeruginosa* (especially MDR and carbapenem-resistant) strains, it seems that class I integron plays a significant role in dissemination of antibiotic resistance genes and the emergence of antibiotic-resistant *P. aeruginosa* strains. Therefore, local management of drug-resistant infections caused by *P. aeruginosa* through continuous monitoring of drug resistance trend and evaluation of different drug resistance mechanisms, particularly resistance gene cassettes in class I integron, are strongly recommended.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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