



Determination Antimicrobial Resistance Profile and Prevalence of Class 1 and 2 Integron Resistance Gene Cassettes in *Pseudomonas aeruginosa* Strains Isolated from Hospitalized Patients in Markazi Province, Iran

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Abstract

Background: *Pseudomonas aeruginosa* is recognized as a serious opportunistic pathogen that causes infections in hospitalized patients and shows a high level of antibiotic resistance.

Objectives: The current study aimed at investigating the frequency of class 1, 2 integrons in *P. aeruginosa* strains isolated from hospitalized patients in Markazi Province, Iran.

Methods: Totally, 100 clinical isolates of *P. aeruginosa* were collected and identified using standard biochemical tests *P. aeruginosa*. Antibiotic sensitivity test was performed using disk-diffusion (Bauer-Kirby) method. DNA was extracted, then polymerase chain reaction (PCR) was performed for the detection of class 1 and 2 integrons.

Results: Trimethoprim/sulfamethoxazole (76%) and cefotaxime (45%) were the most effective antimicrobial agents in this study. Also, PCR results showed that 95% of *P. aeruginosa* strains carried *int1*, but none of the isolates harbored the *int2* genes. A significant correlation was observed between class 1 integrons and resistance to trimethoprim/sulfamethoxazole and cefotaxime ($P < 0.005$).

Conclusions: The current study recognized a high prevalence of antibiotic resistance also a high presence of class 1 integron. Since integron-carrying elements are responsible for the transmission of antibiotic resistance genes introduced into the hospital is of concern; thus applying comprehensive strategies for prevention and control of infections is necessary.

Keywords: Antibiotic Resistance, Integron, *Pseudomonas aeruginosa*

1. Background

Pseudomonas aeruginosa is a Gram-negative, aerobic bacillus, flagellated, motile which is the main cause of infection in patients with underlying conditions. It is recognized as a serious opportunistic pathogen. Serious infections due to *P. aeruginosa* are life-threatening and associated with the highest rates of mortality and morbidity. In recent years, overuse and misuse of antibiotics made this bacterium resistant to broad-spectrum antibiotics from different groups (1, 2). Generally, resistance mechanisms in *P. aeruginosa* antimicrobial agents can be classified into intrinsic and inherent resistance. In intrinsic resistance, natural or wild cells are resistant to antibiotics through a chromosomal origin. While the inherent resistance is due to gene acquisition such as transposons, plasmids, and integrons by horizontal transfer (1, 3, 4).

Integrons were first described at the 1980s, they are genetic elements. Integrons are a genetic set that are capable of integrating and expressing genes called “gene cassette” and displacing them. All known integrons composed of three key essential components for the procurement of exogenous genes. The *int I* gene encodes an integron integrase (*IntI*) (5). This integrase belongs to the family of tyrosine recombinase (phage integrase) integrase protein that catalyzes recombination between incoming gene cassettes. Promoter and the *att I* (attachment site), which directs transcription of cassette-encoding genes (6).

The most studied integrons are the resistance integrons (RIs) (7). Resistance integrons have been divided into three classes based on differences in the integrase genes, and it seems that each class can acquire the same gene cassettes (8). Class 1 integrons (linkage a Tn 402-like transpo-

son) are widely found in clinical specimens and most antibiotic resistance gene cassettes belong to this class are strongly associated with multiple antibiotic resistance (9). Class 2 integrons are related to Tn7 transposon and associated with resistance to trimethoprim, streptomycin and spectinomycin, while class 3 integrons are rare (10). In recent years, the role of integrons has been identified as a genetic element in the horizontal transfer of antibiotic resistance genes (11-15). Integrons play a key role in the expression and spread of antibiotic resistance genes (16-18). Therefore, identification of these types of antibiotic resistance genes is essential for successful infection control programs and preventing the spread of resistant strains.

2. Objectives

The aim of this study was to investigate the antibiotic resistance pattern and the prevalence of integron class 1 (int1) and 2 integrons (int2) in *P. aeruginosa* strains isolated from hospitalized patients in Markazi Province, Iran.

3. Methods

3.1. Study Design and Setting

In this cross-sectional study, a total of 100 non-duplicate isolates of *P. aeruginosa* were obtained from hospitalized patients in Markazi Province, Iran. The following standard conventional biochemical tests were used for the identification of all isolates: catalase and oxidase tests, growth at 42°C, pigment production on Mueller-Hinton agar, Oxidative/fermentation glucose test (OF glucose test).

3.2. Antimicrobial Susceptibility Testing

The Kirby-Bauer's disk antimicrobial diffusion test on Mueller-Hinton agar (Merck, Germany) in accordance with the guidelines of Clinical and Laboratory Standards Institute were used in order to evaluation of antimicrobial susceptibility of *P. aeruginosa* isolates (8). Six antibiotics (MAST, UK) were employed in the study, including cefazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), gentamicin (GEN, 10 µg), sulfamethoxazole /trimethoprim (SXT, 25 µg), imipenem (IPM, 10 µg) and piperacillin/tazobactam (PTZ, 100 µg/10 µg). For quality control of the tests, *P. aeruginosa* ATCC 27853 was used as standard microorganism.

3.3. Molecular Analysis

The DNA of all the isolates was extracted from the bacterial cells by boiling method. In brief, few colonies of overnight culture of *P. aeruginosa* suspended in 1 mL distilled water. Then boiling for 15 min and centrifugation.

The supernatant was stored at -20°C. Moreover, PCR was carried out using a specific primer for detection of class 1 and 2 integrons. To this aim, PCR mixture contained DNA template 2 µL, Mastermix 12.5 µL (Yekta Tajhiz Azma, Iran), distilled water 8.5 µL and each primer 1 µL (Gene Fanavaran, Iran) in a final volume of 25 µL. The primers used in the amplification reactions were as follows (Table 1). PCR experiments for Int1 and Int2 genes were performed (thermocycler: PEQLAB, UK) with 35 cycles consisting of 30 s of denaturation at 94°C, 30s of annealing at 55°C and 30 s extension at 72°C. Finally, PCR products were run on 1.5% agarose gel (w/v) (Merck, Germany) with DNA Ladder 100 bp (CinnaGen, Iran) for 40 min at a constant 80 V using 1X TBE buffer.

3.4. Statistical Analysis

The SPSS software (IBM Corp., USA) version 21.0 was used for statistical analysis. A P value of > 0.05 was considered statistically significant.

4. Results

A total of 100 isolates of *P. aeruginosa* were collected from hospitalized patients in Markazi Province, Iran. Susceptibility patterns against the tested antibiotics are shown in Figure 1. The most prevalent antibiotic resistance included trimethoprim/sulfamethoxazole (76%) and cefotaxime (45%).

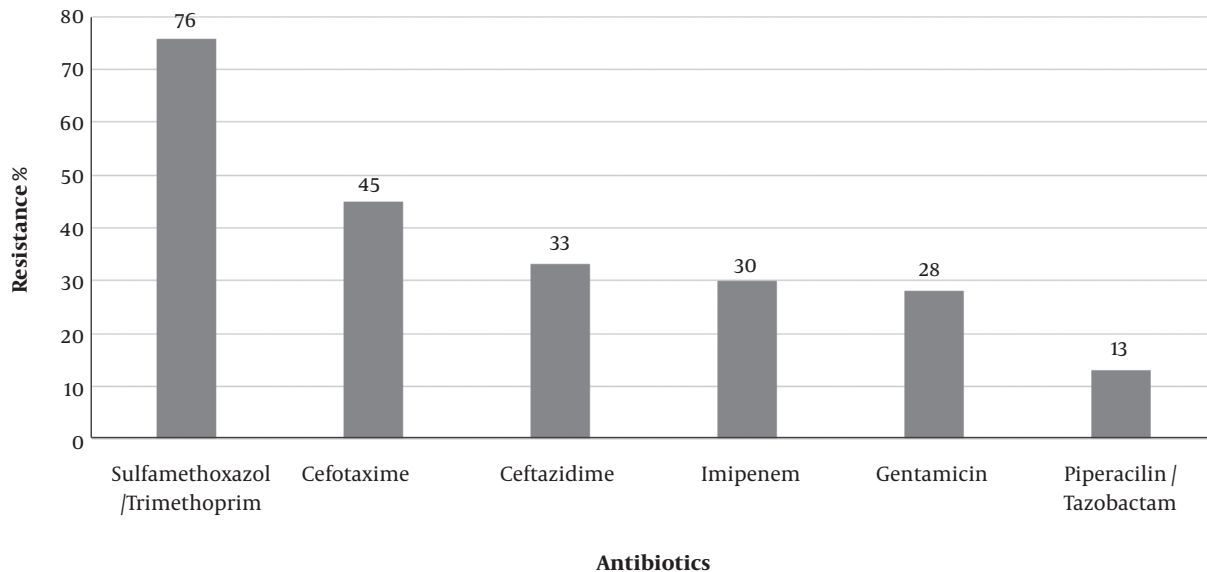
Molecular analysis for the presence of class 1 and 2 integrons showed that 95% *P. aeruginosa* isolates harbored the int1 gene (Figure 2), but none of them demonstrated the int2 genes (Figure 3). The results of chi-square analysis showed a significant association between the presence of class 1 integron and resistance to trimethoprim/sulfamethoxazole and cefotaxime. There was no significant association between resistances to other antibiotic resistance with the presence of class 1 integron (Table 2).

5. Discussion

The genus *Pseudomonas* contains more than 140 species in which *P. aeruginosa* is an opportunistic pathogen that causes severe acute and chronic infections (20, 21). In recent years, overuse of antibiotics leads to a significantly increased bacterial resistance to broad-spectrum antibiotics. Antibiotic resistance is a growing concern for the public's health (2, 22). The presence of mobile genetic elements (Integrons) can be regarded as one of the most important factors for the occurrence and transmission

Table 1. Sequences of Primers Used in PCR

Gene	Nucleotide Sequence (5' to 3')	Amplicon Size, bp	References
Int1	F: CAG TGG ACA TAA GCC TGT TC	160	(19)
	R: CCC GAG GCA TAG ACT GTA		
Int2	F: TTG CGA GTA TCC ATA ACC TG	288	
	R: TTA CCT GCA CTG GAT TAA GC		

**Figure 1.** Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolates isolated from hospitalized patients in Markazi Province, Iran**Table 2.** The Correlation between Presence of Class 1 Integrons and Antibiotic Resistance^a

Antibiotics	Integron-Positive Isolates (N = 95)			Integron-Negative Isolates (N = 5)			P Value
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant	
IPM	65 (68.42)	2 (21.05)	28 (29.47)	3 (60)	0	2 (40)	0.848
SXT	13 (13.68)	8 (8.42)	74 (77.9)	3 (60)	0 (0)	2 (40)	0.021
CTX	8 (8.42)	45 (47.37)	42 (44.21)	1 (20)	1 (20)	3 (60)	0.018
GEN	67 (70.52)	2 (2.1)	26 (27.36)	3 (60)	0 (0)	2 (40)	0.798
PTZ	65 (68.42)	17 (17.91)	13 (13.68)	5 (100)	0 (0)	0 (0)	0.324
CAZ	63 (66.31)	0 (0)	32 (33.68)	3 (60)	1 (20)	1 (20)	0.231

Abbreviations: CAZ, ceftazidime; CTX, cefotaxime; GEN, gentamicin; IPM, imipenem; PTZ, piperacillin/tazobactam; SXT, sulfamethoxazole/trimethoprim.

^aValues are expressed as No. (%).

of bacterial resistance. The present study investigated antimicrobial resistance profile and prevalence of class 1 and 2 integron resistance gene cassettes in *P. aeruginosa* strains isolated from hospitalized patients in Markazi Province, Iran.

Based on the results of this study, the highest resistance level was observed in our study to trimethoprim/sulfamethoxazole (76%). Drug resistance has been

vigorously studied in *P. aeruginosa* strains isolated from clinical specimens, several of these studies are discussed here. Similar investigations were conducted in many parts of Iran for resistance to trimethoprim/sulfamethoxazole of *P. aeruginosa* that ranged from 57/58% to 96/4% (23-25). This study showed that gentamicin and imipenem were effective antibiotics for *P. aeruginosa* so that only 28% and 30% isolates were resistant to gentamicin and imipenem,

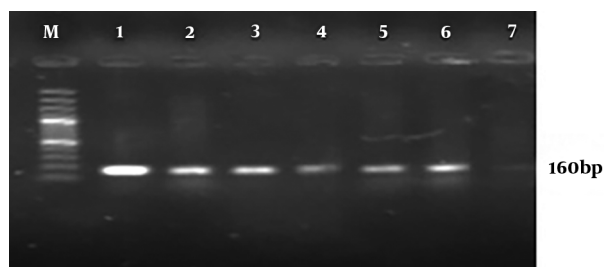


Figure 2. Electrophoresis of Int1 gene PCR product on 1.5% agarose. Lane M, 100 bp DNA ladder; lane 1, positive control; lane 2 - 6: positive strains; lane 7, negative control.

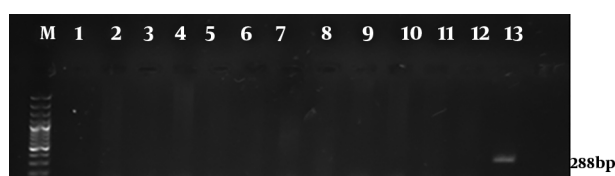


Figure 3. Electrophoresis of Int2 gene PCR product on 1.5% agarose. Lane M, 100 bp DNA ladder; lane 1 - 12, negative *Pseudomonas aeruginosa* strains; lane 13, positive control.

respectively. Reports on Iran show that the resistance rates to gentamicin and imipenem are as follows: Arak 24% and 23%, Zanjan 55.1% and 63.8%, Yazd 63.2% and 62.5%, Tabriz 62% and 46.5% (26-29), respectively. Results of these studies were variable since it depended upon the times or places that the pertinent studies were performed.

In this study, class 1 integron of *P. aeruginosa* isolates was detected in 95% of isolates and class 2 integron was not found. The frequency of different classes of integrons was investigated in published Iranian studies from different regions of Iran. In a study that was conducted in 2015 by Hosseini Pour et al. (30), 92% and 52% of strains had class 1 and 2 integrons, respectively. In the study conducted by Khosravi et al. (31) in 2017, the prevalence of class 1 and 2 integrons was evaluated among 93 *P. aeruginosa* burn isolates. Their results showed that class 1 integron was detected in 95.7% of isolates, while none of them harboured class 2 integron (31). These studies, have similar results to our study. But the results of the following studies differed from our study results. In 2018 Ebrahimpour et al. (29) reported that 30% of *P. aeruginosa* isolates carried class 1 integrons.

In a study conducted by Yousefi et al. (32) in 2010, class 1 integron was detected in 56.3% of the isolates. In a study by Khorramrooz et al. (33), class 1 integron was detected in 35.6% of isolates, whereas class 2 integron was not found. The frequency of class 1 integron in other parts of the world, in previous studies in Nigeria and Brazil, was re-

ported 57%, 41.5%, respectively (34, 35). In a study by Xu et al. (36) in China in 2009, 45.8% and 19.5% of isolates had class 1 and 2 integrons, respectively.

The results of this study, in comparison to similar studies, showed a high prevalence of class 1 integron isolated. The reason for the difference in reported percentages in various studies can be due to differences in geographical location, bacteria strains, overuse and misuse of antibiotics. It should be noted that in the present study, a significant correlation was observed between class 1 integrons and resistance to trimethoprim/sulfamethoxazole and cefotaxime, which could be the reason for the presence of resistance genes to these drugs in this class of integrons. In cases that there was no significant correlation between these genes and other antibiotic resistance, the resulting resistance can be achieved through other ways such as the presence of the resistance genes in resistance plasmids, etc.

5.1. Conclusions

The high presence of class 1 integron in this study and since the integrons are mobile genetic elements and they can transfer between the various species of bacteria involved in Hospital infections, using effective infection control activity and appropriate therapies are necessary to prevent dissemination of them. It is recommended to conduct more detailed studies on the nature of integrons and other resistance mechanisms in these species to provide better therapeutic and control strategies in the future.

Footnotes

Authors' Contribution: Mana Shojapour designed the study, interpretation of the data and manuscript writing. Mahboobe Mirnezami performed the experimental methods on bacterial. Ehsanollah Ghaznavi-Rad and Hamid Abtahi designed the study.

Conflict of Interests: The authors declared no conflict of interest.

Ethical Approval: This research was approved by the Ethics Committee of Arak University of Medical Sciences (ethics code: IR.ARAKMU.REC.1395.463).

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