

Research Article**The Study of Resistant Genes of aac(6^I)-I, aac(3)-III and Integron Class 1 of MDR *Pseudomonas aeruginosa* in Tehran Hospital**Fateme Moradi¹, Abbas. Ali. Imani. Fooladi¹, Abbas Bahador², Mahbobeh Satarzadeh Tabrizi¹, Davoud Esmaeili^{1*}¹Applied Microbiology Research Center, and Microbiology Department, Baqiyatallah University Medical of Sciences, Iran²Departments of Microbiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran***Corresponding author**

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Abstract: *Pseudomonas aeruginosa* is a gram-negative, obligate aerobic bacillus which is widespread in world and causes burned hospital infections in humans. Multidrug resistant strains of *Pseudomonas aeruginosa* (MDR) have been emerged as alarming nosocomial pathogens among burned patients in Tehran (Iran). Integrons play important role in drug resistant in bacteria. Aminoglycosides are common antibiotics which were sometimes used to treatment against pseudomonas infections. The purpose of this study was to determine genes aac(6^I)-I, aac(3)-III and Integron Class1 in MDR *Pseudomonas aeruginosa* isolates of Motahari burned hospital in Tehran during 2013-2014. The bacterial isolates were collected from 100 patients with burn wound infections and *P. aeruginosa* species were identified by standard bacteriological tests. Antimicrobial susceptibility tests were carried out according to Laboratory Standards Institute (CLSI, 2013). PCR was carried out for the detection of class I Integrons and aac(6^I)-I, aac(3)-III genes. Minimum inhibitory concentration (MIC) values of all isolates to aminoglycosides were determined by the micro broth dilution method as were described by the CLSI2013 guideline. A number of 100 hospitalized patients in the burn ward were assessed, resistance rates to various antibiotics were as follows: Gentamycin (83%), Amikacin (87%), Tobramycin (83 %). The PCR results showed that 90% *P. aeruginosa* isolates harboured class 1 integrons. A significant correlation was obtained between the presence of integrons class I and resistance to Gentamycin (p < 0. 01). The results of PCR indicated that rate of frequency resistant genes were aac (6^I)-I (78%), aac (3)-III (93%). Optimum prescription of antimicrobial drugs, control of infection and attention to antibiogram are recommended in order to prevention the increasing outbreak of drug resistant in the burned centers setting in this study. Furthermore, the high frequency of class 1 integrons among MDR isolates might be responsible for dissemination of antibiotic resistance genes. Resistant to aminoglycosides in *P. aeruginosa* remains as major problem in Iran and world. Therefore is a necessary considerable serious surveillance system for prevention drug resistance.

Keywords: *P. aeruginosa*, acc6, aac3, Class 1 integrons**INTRODUCTION**

Increasing of Extensive drug resistant (XDR), Pandrug-1 Resistant (PDR) and multidrug-resistant (MDR) *P. aeruginosa* in hospitalized patients constitute a major public health threat. *Pseudomonas aeruginosa* is a non-fermentative gram negative bacillus. It is widely distributed including hospital environments. It has found to be responsible for about 10-20% of nosocomial infections in intensive-care units (ICUs), Cystic fibrosis, burn and wound infections, etc. It has the ability to rapidly acquire resistance to different broad-spectrum antibiotics and disinfectants. Multidrug-resistant (MDR) and extensive drug resistant (XDR) *P. aeruginosa* has emerged as a cause of morbidity and mortality in burn patients causing 4-60% nosocomial infections in different parts of the world [1,

2]. MDR *P. aeruginosa* releases enzymes such as extended spectrum beta lactamases (ESBLs) and metallo-β-lactamases (MBLs) that inactive beta-lactams and carbapenems [2, 3] and also phosphatase, adenylylase and acetylase that inactive aminoglycosides.

MDR *P. aeruginosa* phenotype is resistant to anti-microbial agents that have been included in three or more anti-Pseudomonas classes (carbapenems, fluoroquinolones, penicillins /cephalosporins and aminoglycosides [4].

Aminoglycosides are crucial antibiotics that are administered for treatment of a variety of hospital infections [5], These antibiotics exhibited resistances to aminoglycosides, however, have been distributed for

some time, with reports from the 1960s highlighting the general insusceptibilities of *P. aeruginosa* clinical isolates to, e.g., kanamycin [6]. Today, resistance to aminoglycosides with antipseudomonal activities is also all too common and is present in virtually all areas of the world, but particularly in developing countries [7]. Such resistance is seen in respiratory isolates [8], particularly isolates from burn [9], CF patients [10], as well as bloodstream [11], urinary [12], wound [13], eye [14], and aural [15] isolates.

Three classes of integrons (class 1, 2 and 3) have been described and to be associated with resistance gene cassettes. Class 1 is recognized as the most widespread among clinical isolates that is composed of a 5' conserved segment (CS), which includes the integrase gene (*int 1*), the recombination site (*att 1*) and a promoter region (P_{ant}) and a 3' CS usually including the *qacED1* and *sulf1* gene [16].

We made an attempt to determine prevalence of MDR in Tehran Motahari hospital, by considering MDR definition of *P. aeruginosa* as strains which were resistant to antibiotics. The present study investigated the in-vitro activities to four classes antimicrobial agents which commonly were used for treatment *P. aeruginosa* infections in burn patients.

We in this research surveyed susceptibility of *P. aeruginosa* isolates to Integrons and aminoglycoside resistance patterns among burned patients in Tehran. We obtain consent from the patient and hospital management for the publication of this report.

METHODOLOGY

Bacterial isolates

During 201-2014, a total of 100 non-repetitive clinical isolates of *P. aeruginosa* were collected from the burned patients in Motahari hospital. Identification of *P. aeruginosa* isolates, were carried according to Baily & Scott [17]. All the clinical Isolates were kept at -20°C until further testing. We considered a strain as MDR if it was resistant to two or more antibiotic classes. Patients had no bacterial infections at the time of admitted to hospital. All the samples were confirmed as *P. aeruginosa* by biochemical tests.

Disc Diffusion method

Antimicrobial susceptibility tests to various antimicrobial agents were determined by the disc-diffusion method on Mueller Hinton agar recommended by the guidelines of Clinical and Laboratory Standards Institute (CLSI2013) [18]. Briefly, 0.1 ml of a suspension of the test micro organism (1.5×10^8 cfu/ml) was spread on Mueller-Hinton Agar (diameter, 50 mm) (Merck), by the disc diffusion method for the following antimicrobial agents (Mast, Padtab) with concentrations: Tobramycin (10 µg), Gentamicin (10

µg) Amikacin (30 µg), Ciprofloxacin (5 µg), Polymyxin B (300Unit) were then placed on the agar plate and incubated at 37°C for 24 h. The diameters of the zones of inhibition were measured and reported in mm.

Isolates of *P. aeruginosa* were defined as multidrug-resistant (MDR) when the organism was resistant to at least one agent in three or more antimicrobial categories that would otherwise serve as treatments for *P. aeruginosa* infection. An isolate was considered extensively drug-resistance (XDR) when it was non-susceptible to one or more of agent in all but 2 or less the categories. Pan drug resistant (PDR) as defined as non-susceptibility to all antimicrobial agents (19).

PCR Test

DNA extraction was carried out by commercial DNA extraction kit (CinnaGen, Iran). The presence of *aac(6)-I*, *aac(3)-III* gene was detected by PCR method. PCR was performed in a standard enzyme Taq DNA polymerase. Single primer pair was used to amplify *aac(6)-I*, *aac(3)-III* gene target fragment based on GenBank. The primers sequences as follows: Primer *aac(6)-I* F: CCCAGTGGACATAAGCCTGT and Primer *aac(6)-I* R: GTAACATCGTTGCTGCTCCA. Primer *aac(3)-III* F: CACTTCCAAGAACGCAGACA and Primer *aac(3)-III* R: GTACATGTCCATGCCGAGTG (table 1). We used Pishgam kit for PCR and reactions were performed in a final volume of 25µl according to recipe; Primer (10 µmol, DNA template (50 ng), Master mix (12.5 µl [1X]). The mixtures were incubated for 5 min at 93°C for primary denaturation, 30 sec at 93°C for secondary denaturation of the target DNA and then, annealing at 56°C for 40 sec, and extension at 72°C for 30 sec that 35 cycle was performed. The amplified products were analyzed by electrophoresis on 1% agarose gel (cinnagen) containing 0.1 g of ethidium bromide per ml in TBE buffer. The PCR product was visualized under UV light and photographed. To determine the frequency of Integron class 1 elements sets of primers was used with Primer 3 plus software (version 4.0; <http://primer3.wi.mit.edu/>; accessed 05.06.11) with sequences Int F: ATGCCCGTTCCATACAGAAG, Int R: CGGCCTTGCTGTTCTTCTAC. PCR assays were performed under standard conditions.

RESULTS

In total, 100 *P. aeruginosa* isolates were isolated from patients that were admitted burned units in Tehran (Iran) during 2013-2014 and resistant results following below: Gentamycin 30 µg (83%), Tobramycin (83%), Amikacin (87%), Ciprofloxacin (93%), Ceftazidim (85%) and Polymyxin B (0%). Based on the susceptibility of the isolated *P. aeruginosa* to antibiotics, were observed minimum resistance against Polymyxin B (0%) and maximum resistance to Ciprofloxacin (93%) (Table 2).

The results of PCR screening from one hundred *P. aeruginosa* isolates indicated 90% contain integron gene (Fig. 1, 2).

Table 1: Sequences of primers designed for detection

Primer Name	5' – Sequence - 3'	Detected gene	Molecular Amplicon Reference
aac(6')-I(F) This study	F: CCCAGTGGACATAAGCCTGT	aac(6')-I	229bp
aac(6')-I(R)	R: GTAACATCGTTGCTGCTCCA		
aac(3)-III(F) This study	F: CACTTCCAAGAACGCAGACA	aac(3)-III	222bp
aac(3)-III(R)	R: GTACATGTCCATGCCGAGTG		
Int-1(F) This study	F: ATGCCCGTTCCATACAGAAG	Int-1	184bp
Int -1(R)	R:CGGCCTTGCTGTTCTTCTA		

Table 2: Antimicrobial Susceptibility Pattern of *P. aeruginosa* isolates

Antibiotic	Resistance (%)	PCR
Ceftazidime	(85%)	-
Tobramycin	(83%)	aac(3)-III 93%, aac(6')-I 78%
Gentamicin	(83%)	aac(3)-III 93%, aac(6')-I 78%
Amikacin	(87%)	aac(3)-III 93%, aac(6')-I 78%
Ciprofloxacin	(93%)	-
PolymixinB	(0%)	-

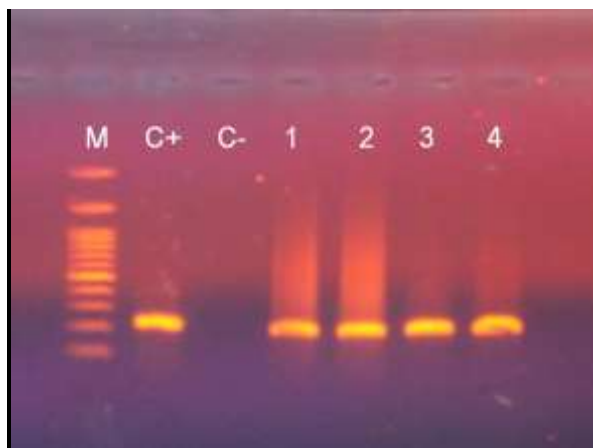


Fig1: Results of PCR for aac(3)-III gene

Line M: Marker 100bp, Line C⁺ positive control, Line C⁻ negative control, Line 1, 2, 3, 4 *P. aeruginosa* isolates

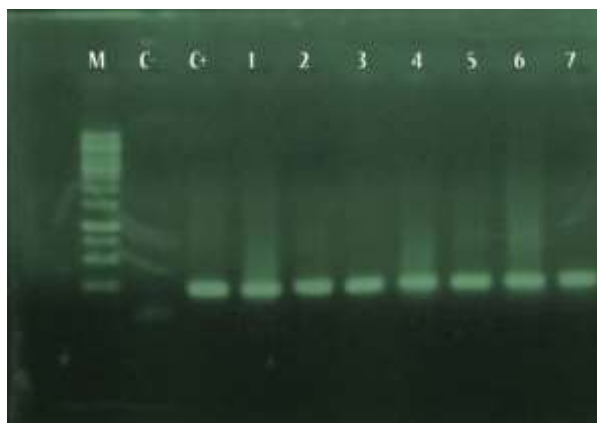


Fig. 2: Results of PCR for aac(6)-I gene

Line M: Marker 1 kb, Line C⁺ positive control, Line C⁻ negative control, Line 1; integron ,lanes;2,3,4,5,6,7 *P. aeruginosa* isolates

DISCUSSION

Nosocomial infections caused by MDR *P. aeruginosa* are currently the most difficult to treatment, and they continue to present serious challenges to clinicians, empirical and therapeutic decisions in burned patient. Outbreaks of MDR, XDR and PDR currently have been reported from worldwide.

In this study, the high prevalence of MDR *P. aeruginosa* isolates from burned patients is consistent with previous reports [17]. The present study revealed that 83% to Tobramycin and Gentamycin; 87% to Amikacin and 85% to Ciprofloxacin , 93 % to Ceftazidime and 0% to Polymyxin B *P. aeruginosa* isolates from burned patients are resistant to Tigecycline and Colistin, respectively, by comparison with the other studies in Iran [17].

P. aeruginosa is one of the most important nosocomial pathogens [18], able to cause severe infections [19], occurring in immunosuppressed patients [20], in patients with serious underlying diseases [21], or subjected to invasive procedures and treatment with antibiotics [22]. *P. aeruginosa* isolates resistant to different classes of antibiotics have been found to be emerging worldwide [23].

This study demonstrated that *P. aeruginosa* was the one serious and major pathogen in burned wards. In a timely manner, antimicrobial resistance surveillance and strict infection control strategies are still lacking in burn ward in Iran [24], despite the alarming emergence of MDR strains, particularly among those isolates that are not susceptible to Polymyxin B. The emergence of aminoglycoside - resistant *P. aeruginosa* in this study might be due to improper. Interestingly, all aminoglycoside resistant isolates were susceptible to Polymyxin B. This is very important for treating serious infections caused by aminoglycoside resistance isolates.

The results of this study are consistent with a recent report in which a number of drugs exhibited

potent activity against Multidrug resistant strains of *P. aeruginosa* (MDR) [25].

Although the presence of Integron class 1 to play a substantial role in antimicrobial resistance in MDR isolates. However, further investigation is required to recover various Integrons in MDR strains isolated from Iran. This Research showed that, it was notified that, the *P. aeruginosa* resistant to aminoglycoside in Tehran is rising dangerously. We have documented those almost more than half strains are resistant to aminoglycosides. Also we observed that resistance mean to aminoglycosides was 84.3%.

CONCLUSION

In conclusion, our data support that Polymyxin B exhibited a potent activity against MDR *P. aeruginosa* isolates from burned patients. *P. aeruginosa* strains isolated from Iranian burned patients are so resistant and this is the first report of spreading integrons and aac genes in MDR isolates among burned patients in Iran. We also show that MDR integrons were present among burned patients in Iran at almost the same time as they were described worldwide [22].

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