

Antibiotic Resistance Pattern and Molecular Typing by PCR-RAPD Analysis in Clinical Isolates of *Pseudomonas aeruginosa* from Motahari Hospital, Tehran, Iran

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ABSTRACT

Background: *Pseudomonas aeruginosa* infection is one of the major challenges in burn patients. This organism is resistant to a wide range of antibiotics. The aim of this study was to investigate the antibiotic susceptibility and genetic relatedness in *Pseudomonas aeruginosa* isolated from patients admitted to Motahari Burn Center, Tehran, Iran.

Methods: This study was conducted on 186 burn patients with *Pseudomonas aeruginosa* colonization following admission in Motahari Burn Center during one year. Antibiotic resistance test was performed by disc diffusion method and genetic relatedness was evaluated by PCR-RAPD analysis.

Results: The highest and lowest percentage of resistance was observed against ceftizoxime and ciprofloxacin. The genotyping study by RAPD PCR technique revealed 57 different genotypes, among which RAPD 5, RAPD 8, and RAPD 9 were the most prevalent patterns and produced by 14%, 9%, and 7% of the isolates, respectively.

Conclusion: In total, no association was found between RAPD genotypes and antibiotic resistance patterns, and death rate.

Keywords: RAPD PCR, *Pseudomonas aeruginosa*, Antibiotic resistance, Genotypes

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Introduction

Burn wound is very susceptible for opportunistic colonization and infection due to disruption in protective function of skin, necrosis, and inducing systemic immunosuppression (1, 2). Although, current techniques have reduced infection in burn patients, some of them may still acquire life threatening nosocomial infection caused by microorganisms such as *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter*, *Klebsiella*, and *Staphylococcus aureus*. *P. aeruginosa* has been reported as the most common organism isolated from burn wound infections (3-6). It is also an opportunistic pathogen that its native resistance to many common antimicrobial agents and ability to acquire additional tolerant to new antibacterial drugs can cause severe complications in immunocompromised diseases (7-10).

Epidemiological studies have a critical role in understanding and elucidating of transmission routes and sources of bacterial infection in nosocomial outbreaks (11). Genotyping methods are now the most accurate and precise techniques for epidemiological investigation of the most microorganism such as *P. aeruginosa*. Among these methods, the most popular methods which have been used for *P. aeruginosa* typing are pulse-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), ribotyping, and random amplified polymorphic DNA (RAPD) (12,13). Despite good specificity and sensitivity, most of these methods are time consuming and expensive. Among the above-mentioned techniques, the RAPD is fast, inexpensive, easy-to-perform, and involves few steps (12, 14, 15). [Nanvazadeh](#) et al. (2013) described genotyping of *P. aeruginosa* strains isolated from burn patients by RAPD-PCR and reported RAPD-PCR method as a useful tool for investigation of the genetic variation (12). This study aimed to assess antibiotic susceptibility and RAPD PCR genotyping pattern of *P. aeruginosa* isolates of patients admitted in Motahari Burn Center, Tehran, Iran.

Materials and Methods

Bacterial source

This cross-sectional study was performed on 186 burn patients with confirmed *P. aeruginosa* infection in their burn wound in Motahari Burn Center, Tehran, Iran, from January 2017 to January 2018. Burn wound surface swab

samples were cultured and *P. aeruginosa* was identified and confirmed by standard biochemical tests including Gram staining, catalase, oxidase, oxidative fermentation (OF), TSI, MR-VP, gelatinase, ability to grow at 42°C, and production of pyocyanin pigment (16).

Antimicrobial susceptibility pattern

Antibacterial susceptibility test for carbenicillin (30 µg), gentamicin (10 µg), cefotaxime (30 µg), imipenem (10 µg), piperacillin (100 µg), ciprofloxacin (5 µg), tobramycin (10 µg), ceftizoxime (30 µg), and amikacin (30 µg) (Mast, UK) was performed by Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) (17).

Random Amplified Polymorphic DNA-Genotyping assay

About 10 colonies of pure isolates of *P. aeruginosa* on Mueller-Hinton Agar were picked up and suspended in 250 µl distilled water in a 1.5 ml microtube and boiled for 10 minutes, then, centrifuged at 12000 g for 10 minutes. The supernatant was collected and transferred to another sterile microtube and stored at -20°C. The reaction solution for RAPD PCR (25 µl) contained: 10 ng genomic DNA, 2.5 mM NaCl, 10 pmol primer (sequence 5'-AGG GGT CTT G-3'), and 2.5 unit Hot Start Taq DNA polymerase. RAPD PCR was performed under following condition: 5 minutes at 95°C for one cycle, then, one minute at 95°C, 2 minutes at 37°C, and 2.5 minutes at 72°C for 45 cycles, and final incubation of 10 minutes at 72°C (18, 19). Amplification was performed and 10 µl of amplification product was load in 1.2% low electroendosmosis agarose gel (Roche, Germany) and electrophoresed at 80 volt for 3 hours. The gel was stained by ethidium bromide and photographed with gel doc UV transilluminator. RAPD PCR patterns were evaluated visually by two independent researchers and genotypes assigned on the basis of differences in the numbers and locations of bands on the RAPD patterns, single band differences among RAPD patterns were ignored (11, 13).

Results

Out of 186 patients, 52 (28%) were female and 134 (72%) were male. The mean age of patients was 32.9 years. Most of patients (75%) had second to third degree burns. Total body

surface area (TBSA) was 31.87% and 75.3% in patients with second and third degree burns, respectively. Body (areas other than the head and neck, arms, and legs) with 22.6% frequency was the most site of burning in patients. Gasoline and gas with 21% for each were the first causes of burn. Mean of hospitalization was 25 days and mortality rate was 30% among patients. Out of

186 clinical isolates, 78% were multidrug-resistant (MDR) *P. aeruginosa* isolates. The percentage of resistance to antibiotics for the isolates were amikacin 86%, piperacillin 80%, gentamicin 86%, tobramycin 86%, imipenem 84%, carbenicillin 88%, ciprofloxacin 76%, cefotaxime 94%, and ceftizoxim 96% (Figure 1).

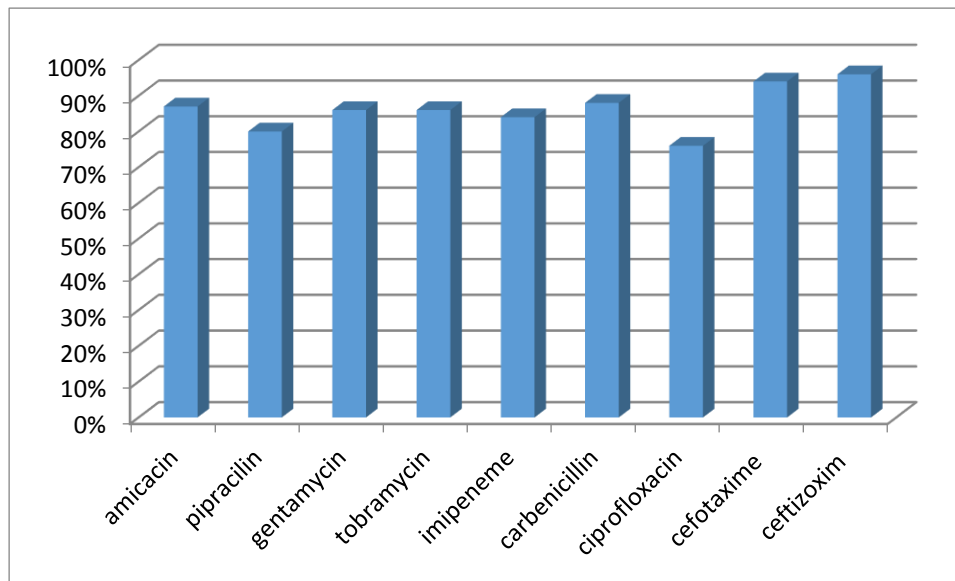


Figure 1. Antimicrobial resistance (%) of *P. aeruginosa* isolated from burn patients to various antibiotics at Motahari Burn Center.

RAPD PCR analysis revealed 57 different genotype patterns, which were named as RAPD1 to RAPD57 (Figure 2). The most common genotyping patterns were RAPD5, RAPD8, and RAPD9, which were isolated from 14%, 9%, and 7% of the patients, respectively. There was no

correlation between the RAPD genotypes and antibiotic resistance patterns. Also, there was no correlation between RAPD genotypes and antibiotic resistance patterns, and death rate.

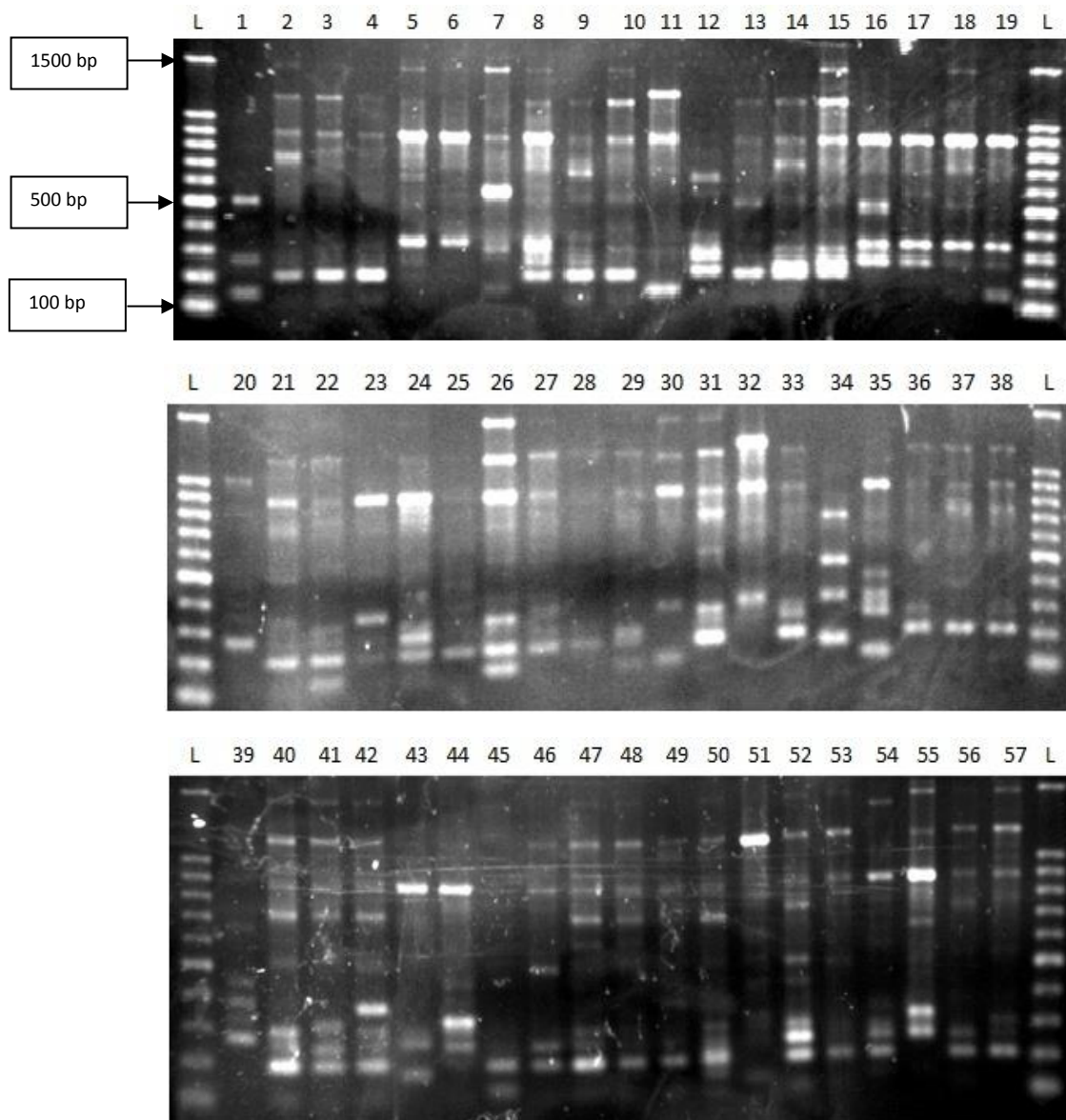


Figure 2. RAPD PCR amplification patterns for *P. aeruginosa* isolated from burn patients. Lanes L: DNA ladder (100-1500 bp).

Discussion

P. aeruginosa is one of the major causes of nosocomial infection and outbreak in burn units (20). One of the most important challenges of *P. aeruginosa* is its inherently resistance to many antibiotics and ability to acquire resistance to almost all effective antibacterial drugs, which make *P. aeruginosa* to be multidrug resistant (21, 22). Increasing rates of MDR *P. aeruginosa* have been reported in different hospitals of Iran (23-25).

In the present study, 78% of all the isolates were MDR *P. aeruginosa*. The prevalence of MDR *P. aeruginosa* in Iran is higher than that in developed countries. Abbassi Ghaleh Sorkh et al. (2017) described a decrease in the

susceptibility pattern in *P. aeruginosa* collected from Taleghani Burn hospital in Khuzestan Province, Iran, and reported that most of the strains were MDR *P. aeruginosa* (19).

Moreover, the mortality rates in burn patients from Motahari Burn Center have been reported to vary from 53% in 1998-1999 (10), 10.6% in 2005-2009 among patients aged 14 years and younger (26), 12% during July 2010-December 2010 (27), to 10.7% during March 2010-April 2011 (28). During the present study period, the mortality rate was 30%. Some of the variations in mortality rates are due to that death in burn patients depending on different factors such as age, delayed admission, pre-hospital care, length of hospital stay, inhalation injury, burning

severity, and percentage of total body surface area (TBSA) (29).

Molecular typing of microbial pathogens is one of the best way for better understanding of nosocomial infections epidemiology and routes of bacterial transmission in hospitals (30). One of the most popular of these techniques is RAPD PCR, which has been recommended as the first screening genotyping method because of rapidity, simplicity, and good discriminatory power (12, 31). In a previous study conducted in this center, O antigens were used for serotyping of *P. aeruginosa*, which 21% of the isolates were reported nontypeable and 13% were polyagglutinable (7). In another study conducted by Douglas et al. (2001) in the center, 14.4% of the isolates were reported to be nontypeable, indicating that it is not a valuable technique for epidemiological analysis of *P. aeruginosa* (13). Douglas et al. (2001) confirmed *P. aeruginosa* as a clonal strain by pulse-field gel electrophoresis (PFGE) in a burns unit (13). Also, Khosravi et al. (2016) reported high levels of genotype by ERIC-PCR in multidrug-resistant strains of *P. Aeruginosa* isolated from burn and wound infections (12).

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Conclusion

In this study, RAPD PCR was used for genotyping of *P. aeruginosa* isolates and 57 different genotypes were identified, which is an indication of good discriminatory power of this typing method. The most recovered genotypes were RAPD 5, RAPD 8, and RAPD 9 with percentage of 14%, %9, and %7, respectively. The high frequency rates of some special genotypes (RAPD 5, RAPD 8, and RAPD 9) in the study burn center can be caused by cross infection between patients. In conclusion, this study demonstrated that use of RAPD PCR analysis is very effective in tracking and control of sources and routes of transmission of hospital infections, especially for *P. aeruginosa*.

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Conflict of interests

The authors declare that they have no conflict of interests.

Financial disclosure

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