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Molecular Analysis of *Pseudomonas aeruginosa* Strains Isolated from Burn Patients by Repetitive Extragenic Palindromic-PCR (rep-PCR)

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Abstract

Background: Infections caused by *Pseudomonas aeruginosa* raise an important issue in burn patients. Molecular epidemiologic studies have been used for investigating the genetic features of *P. aeruginosa* and rep-PCR technique has been introduced as a rapid low cost method.
Objectives: This study focused on investigating the genetic similarity and antibiotic resistance pattern of *P. aeruginosa* isolated from the clinical samples of burn patients in a major burn center in Khuzestan Province, Iran.
Methods: In a cross sectional study, a total of 75 strains of *P. aeruginosa* were isolated from burn patients at Taleghani hospital, which is the main burn center in Ahvaz, Iran, during May- September, 2015. Antimicrobial susceptibility of the isolates was detected using the disk diffusion method. Genetic relatedness of the isolates was analyzed by the rep-PCR technique.
Results: Antimicrobial susceptibility testing showed more than 80% of *P. aeruginosa* isolates were resistant to ceftriaxone, cefotaxime, meropenem, piperacillin/tazobactam, ticarcillin, ciprofloxacin, and amikacin. Based on the rep-PCR analysis, 20 different common types and 20 unique patterns were illustrated among *P. aeruginosa* isolates.
Conclusions: According to the findings of our study, there were diverse and high-level resistant *P. aeruginosa* strains in the major burn center in Khuzestan. Therefore, we faced troubles controlling the diverse *P. aeruginosa* clones in the burn patients.

Keywords: *Pseudomonas aeruginosa*, Burn, Antibiotic Resistance, rep-PCR

1. Background

Pseudomonas aeruginosa is a major opportunistic pathogen for hospitalized patients (1). It is most harmful to individuals whose immune systems have been compromised similar to those with in AIDS, cancer, burns, cystic fibrosis, and neutropenia. Several infections can be acquired in the hospital such as wound, burn, urinary tract, and eye and outer ear infections, as well as meningitis and necrotizing pneumonia (2).

Multi-drug-resistant (MDR) *P. aeruginosa* causes 4% - 60% nosocomial infections all around the world and accounts for the mortality and morbidity in burn patients (3). Due to its intrinsic resistance to numerous antibiotic classes and its ability to practically resist to all effective antibiotics, *P. aeruginosa* has been one of the major concerns for nosocomial infections in hospitals in recent years (4).

The effectiveness of surveillance systems has been greatly enhanced by bacterial strain typing, through which significant strategies have been provided to control public health (5).

The diversity of *P. aeruginosa* strains has been frequently investigated through molecular typing methods, including ribotyping, repetitive-element-based PCR (rep-PCR), arbitrarily primed PCR (AP-PCR), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphic (RFLP) DNA analysis, random amplified polymorphic DNA (RAPD) assay, and pulsed-field gel electrophoresis (PFGE) (6).

Repetitive extragenic palindromic-PCR (rep-PCR) is a common method in which the regions between target repetitive non-coding sequences in the bacterial genome are exhibited (7).

The rep-PCR method is an appropriate assay for DNA typing associated with high type ability, stability, rapid turnaround reproducibility, and low complexity and cost (8).

There have been no epidemiological studies regarding *P. aeruginosa* clones based on molecular typing by the rep-PCR method in burn centers in the Khuzestan Province so far. Thus, this study aimed to investigate the genotypic relatedness and antibiotic resistance of clinical isolates of *P. aeruginosa* at the 160-bed Taleghani hospital as a governmental and referral burn center in Ahvaz and the Khuzestan Province of Iran.

2. Methods

2.1. Identification of Pseudomonas aeruginosa

In this cross sectional study, *Pseudomonas* strains were isolated from clinical samples (wound urine, and blood) of hospitalized burn patients from the Taleghani hospital, which is the major burn center in Ahvaz, Iran, during May-September, 2015. *P. aeruginosa* isolates were identified and confirmed by conventional microbiological and biochemical tests. These tests included culturing on Eosin-Methylene Blue (EMB) agar (Biolab, Hungary), Cetrimide agar, blood agar, TSI, oxidation fermentation (OF) test, and pigment production in Mueller Hinton Agar (Merck, Germany) and growth at 42°C.

2.2. Antibiotic Susceptibility Testing

Antibiotic susceptibilities to ticarcillin (TIC 75 μg), cefotaxime (CTX 30 μg), ceftriaxone (CRP 30 μg), ceftazidime (CEF 30 μg), imipenem (IMP 10 μg), meropenem (MEM 10 μg), aztreonam (ATM 30 μg), piperacillin + tazobactam (TZP 100/10 μg), amikacin (AK 30 μg), ciprofloxacin (CIP 5 μg), azithromycin (AZM 15 μg), and colistin (COL 10μg) (Rosco, Denmark) were determined by the disk diffusion method, according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI 2015).

2.3. DNA Extraction and rep-PCR

Genomic DNAs were extracted from *P. aeruginosa* isolates by a DNA extraction kit (Cinapure DNA, CinaClon, Iran) based on the manufacturer's instructions. rep-PCR was carried out in a thermocyler (Peq-Star, Germany) using the primer rep-F: 5'- ICGICTTATCIGGCCTAC-3' and rep-R: 5'- IIIICGICGICATCIGGC -3' according to the following protocol: initial denaturation (95°C for 2 minutes) followed by 45 cycles of denaturation (95°C for 30 seconds), annealing (38°C for 1 minutes), extension (72°C for 2 minutes), and a final cycle of extension at 72°C for 16 minutes. The PCR products were loaded on a 1% agarose gel at 70 V for 1 hour, and the banding patterns were observed in a gel documentation system. A 1-kilobase DNA ladder (Cinaclone, Iran) was used as a molecular size standard.

2.4. Statistical Analysis of rep-PCR Results

The patterns of bands were compared and clustered by Dice and unweight by Dice and paired group (UPGMA) method by online data analysis service (inslico.ehu.es), respectively.

3. Results

In the current study, of the 75 *P. aeruginosa* isolates from the clinical wound (n = 52), urine, (n = 4), and blood (n = 19) samples of the hospitalized burn patients, 75 clinical samples were isolated from burn patients with different burn degrees. A total of 18.6% (n = 14) and 81.3% (n = 61) of *P. aeruginosa* strains were isolated from male and female patients, respectively. The patients' ages ranged from 3 to 90 years, the majority (32%) of whom were between the ages of 14 to 24 years (Table 1).

The hospital wards involved in the *P. aeruginosa* infection were women; 58.6% (n = 44), followed by ICU; 28% (n = 21), men; 9.3% (n = 7), pediatric; 2.6% (n = 2), and graft wards; 1.3% (n = 1). Distribution of the 75 *P. aeruginosa* isolates, according to the clinical sample types, were as follows: blood; 69.3% (n = 52), wound; 25.3% (n = 19), and urine; (n = 4).

P. aeruginosa strains revealed the highest levels of resistance to ceftriaxone (94.6%) followed by cefotaxime (90.6%), piperacillin/tazobactam (88%), and meropenem (88%), with the lowest resistance to azithromycin (6.6 %) (Figure 1).

Analysis of genetic linkage showed 50% to 100% similarity among *P. aeruginosa* isolates. The length sizes of Rep fragments ranged from 100 bp to 1000 bp. Totally, 2 to 18 bands were detected on the gel electrophoresis of rep-PCR products. Based on rep-PCR typing there was genetic diversity among *P. aeruginosa* isolates, which 40 different rep fingerprints were detected by this technique (Figure 2).

Rep profiles were compared and clustered by the Dice method and UPGMA, respectively. The rep-PCR results revealed diversity among *P. auerogins*a isolates in the burn hospital under study. Totally, 40 different Rep profiles, including 20 common types of A-T and 20 unique types not belonging to any clusters, were identified. Type C was the most common Rep type including 8 isolates. Some strains belonged to similar types isolated from different sources and hospitals wards (Figure 2).

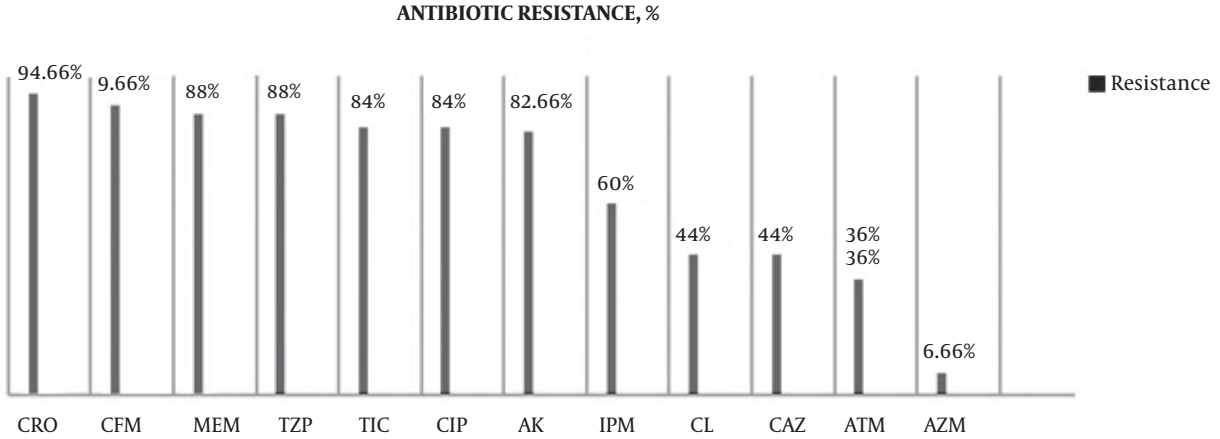
4. Discussion

An essential matter for infection control would be regular monitoring of antibiotic profiles in terms of susceptibility and bacterial infectious agents based on their genetic relatedness. 75 isolates of *P. aeruginosa* were investigated using disk diffusion and rep-PCR methods for a better understanding of antibiotic resistance rates and *P. aeruginosa* clone diversity in a major burn center in Khuzestan.

Table 1. Frequency (%) of the Burn Patients Involved in *P. aeruginosa* According Different Age Groups

Age groups, yr	3 - 13	14 - 24	25 - 35	36 - 46	47 - 57	58 - 68	69 - 79	80 - 90
Rate (%)	12	32	30.66	6.66	5.33	6.66	0	6.66

Figure 1. Rates (%) of Antibiotic Resistance to *P. aeruginosa* in the Burn Patients



Ceftriaxone (CRO), Cefotaxime (CFM), Meropenem (MEM), Piperacillin / Tazobactam (TZP), Ticarcillin (TIC), Ciprofloxacin (CIP), Amikacin (AK), Imipenem (IPM), Colistin (CL), Ceftazidime (CAZ), Aztreonam (ATM), Azithromycin (AZM).

Pseudomonas aeruginosa is significantly able to acquire a variety of resistance determinants. Despite efficient policies for infection control, *P. aeruginosa* infection can be effectively treated by only limited classes of antibiotics in hospitals. In the present study, 12 different antibiotics were investigated as anti psedudomonal agents according to their extensive uses in the hospital. Among the antibiotics, azithromycin was known as the most effective antibiotic against *P. aeruginosa*, while the other drugs showed a decrease in the susceptibility pattern, respectively. Most of *P. aeruginosa* strains in our study were multidrug resistant. A majority of *P. aeruginosa* isolates isolated from various samples was resistant to ceftriaxone followed by cefotaxime, meropenem, and piperacillin/tazobactam. Our results represented that antibiotics might have been used in an unsuitable mode in burn hospitals. Therefore, it was possible that highly resistant Pseudomonas strains could infect burn patients in the hospitals. The results of our research were similar to some reports from Iran and other countries (9-11). Contrary to our findings, in 2013, Alikhani et al. reported a higher resistance to aztreonam in 27% of *P. aeruginosa* isolates (12). Additionally, Gad et al., discovered a high resistance to azithromycin (84%) in this regard (13). In 2016, Sharma showed that all (100%) of the *P. aeruginosa* isolates were sensitive to Imipenem and Meropenem (14).

Taleghani Burn hospital in Ahvaz, the capital of the Khuzestan Province, is a referral center in Southwestern Iran. Therefore, molecular epidemiologic studies of *P. aeruginosa* isolates seemed to be essential in clarifying the epidemiology of this organism in this hospital and to characterize the genetic features and genetic relatedness of *P. aeruginosa* strains isolated from the burn patients. 75 strains were subjected to the rep-PCR analysis. Based on rep-PCR, a high genetic diversity was observed among *P. aeruginosa* isolates. In this study, *P. aeruginosa* isolates were divided into 40 different types, including 20 common and 20 unique types. No clonal distributions of the isolated *Pseudomonas aeruginosa* were detected. This finding indicated diverse sources of Pseudomonas infections in the burn hospital. Thus, there is a need for further investigation on its presumptive clinical and environmental sources.

Khosravi et al., also reported the high level of genotypic heterogeneity in *P. aeruginosa* strains isolated from this burn center have by ERIC-PCR analysis method (15).

Several studies reported genetic diversity and heterogeneity among *P. aeruginosa* clinical isolates using ERIC-PCR, rep-PCR, PFGE, and RAPD-PCR methods in hospitals of Iran as well as other countries (7, 16, 17). This study suggested that the related infection was due to different subtypes of the species. Furthermore, our findings indicated that rep-PCR genotyping might play a significant typing

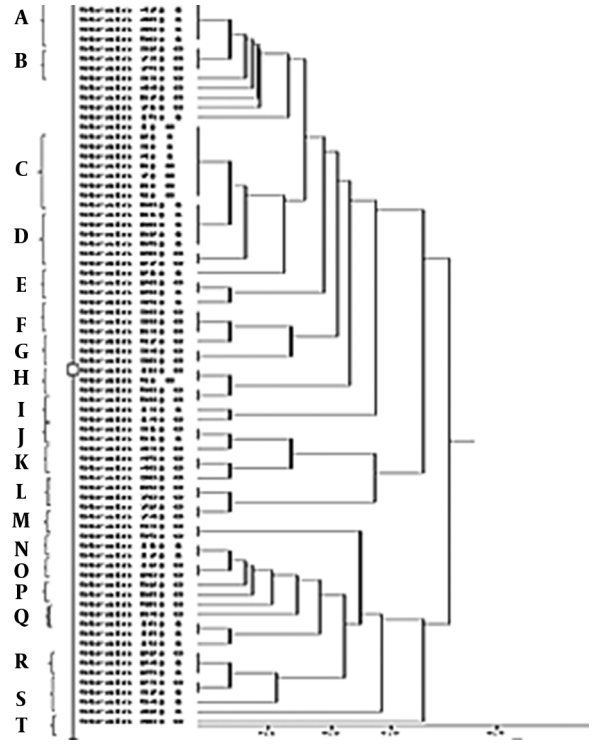


Figure 2. Dendrogram of *P. aeruginosa* Based on rep-PCR Analysis. Isolates are From Burn Infections

method in routine to epidemiological studies in the hospital. Thus, it can be used as the first screening genotyping method for *P. aeruginosa* typing as the discrimination of isolates by the rep-PCR technique was proven to be easily possible at low costs. It should be noted that it was better to analyze and compare the environmental and clinical *P. aeruginosa* isolates in hospitals especially burn centers.

In conclusion, the results of our study revealed that we were facing highly resistant and diverse *Pseudomonas aeruginosa* strains in the burn center in Ahvaz city, which causes concerns and limitations for treatment options of burn patients. Consequently, we have many challenges to control the diverse isolates of *Pseudomonas aeruginosa* and need to pay more attention to control its infections in burn centers.

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Footnotes

Authors' Contribution: Mansooreh Abbassi Ghaleh Sorkh, study concept, drafting of the manuscript, acquisition of data; Leili Shokoohizadeh, study concept and design, acquisition of data, analysis and interpretation of data; Niloufar Rashidi, acquisition of data, critical revision of the manuscript for important intellectual content; Elahe Tajbakhsh, critical revision of the manuscript for important intellectual content.

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