Genotyping of Pseudomonas aeruginosa strains isolated from burn patients by RAPD-PCR

Fatemeh Nanvazadeh a, Azar Dokht Khosravi b,c,* , Mohammad Reza Zolfaghari a, Najmeh Parhizgari b

a Department of Microbiology, Faculty of Basic Sciences, Qom Branch, Islamic Azad University, Qom, Iran
b Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
c Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

ABSTRACT

Background: Pseudomonas aeruginosa is one of the important causes of nosocomial infections that easily gains resistance to many antibiotics. This opportunistic pathogen is a major health hazard particularly in immunodeficient patients, patients in intensive care units (ICU) and burn units with life threatening outcome. The bacterium may be originated from different or common sources, and comprises a high colonization and transmission capacity.

Objective: The aim of present study was to investigate the genotypic variation of Pseudomonas aeruginosa strains isolated from burn patients by using Random Amplified Polymorphic DNA (RAPD) method.

Methods: Totally 70 clinical samples were collected from burn patients in Taleghani Burn Hospital of Ahvaz. Fifty out of total samples were positive for P. aeruginosa by application of conventional culture and biochemical identification tests. DNA was extracted from the isolates and the RAPD-PCR method was applied to the DNA extracts according to standard method using a short single primer of 272. The technique created repetitive electrophoresis patterns which was used for genotypic differentiation.

Results: RAPD-PCR, created 9 genotypic profiles designated as I–IX with base pair length ranging from 180 to 2700. Each genotype showed between 3 and 6 different weight DNA bands. Genotype I was the most prevalent, identified in 10 bacterial isolates (20%). Genotypes I, II and VI were mostly common in patients with more severe burn, and were mainly isolated from wound and blood samples obtained from the same patients.

Conclusion: In present study, we found RAPD-PCR technique as a useful tool for investigation of the genetic variation among P. aeruginosa strains. This is a rapid, low cost, genotypic method with high discriminatory power. The results could assist to screen for the original of infection caused by this organism with subsequent control of colonization and transmission.

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1. Introduction

Pseudomonas aeruginosa (P. aeruginosa) is the major opportunistic pathogen in the family of Pseudomonaceae. In 1882, Gessard isolated it and originally designated as Bacillus pyocyaneus [1]. Today it is identified as an aerobic, motile, non sporulating, ubiquitous gram negative bacterium and a common source of many community acquired and nosocomial infections [2]. P. aeruginosa is among the important cause
of variety of infections such as infection of urinary tract, respiratory, gastrointestinal and soft tissue which affects mainly hospitalized patients with cancer [3], cystic fibrosis and burns [4].

Patients with severe burn present an immunosuppression condition and consequently higher susceptibility to infections by nosocomial pathogens such as P. aeruginosa with a high mortality rate mainly due to the high intrinsic resistance of microorganism to many antimicrobials [5]. P. aeruginosa is the second most common bacterial cause of nosocomial infections with incidence reported 17–26% of wounds infections [6]. It exhibits intrinsic resistance to a lot of different types of chemotherapeutic agents and antibiotics [7]. Prevention and control of P. aeruginosa infections are still among serious nosocomial problems worldwide. Host immunodeficiency, combined with a high incidence of antibiotic resistance, makes treatment of P. aeruginosa infections a serious medical challenge.

Understanding pathogen distribution and relatedness is essential for determining the epidemiology of nosocomial infections and aiding in the design of rational pathogen control methods [8]. PCR-based molecular genotyping of P. aeruginosa is of major importance in the elucidation of transmission routes that compared to phoentyping methods is less affected by environmental factors. In general, molecular methods comprises higher discriminatory power and higher reproducibility than phenotypic tests because of their ability to detect minor genome differences and the higher stability of molecular targets compared with that of phenotypic profiles for some species [9]. Different genotypic systems have been described for characterizing P. aeruginosa isolates. These systems include hybridization with specific probes and pulse-field gel electrophoresis (PFGE) known as gold standard genotypic technique, and have proved to have good specificity and sensitivity. However, their disadvantages are that they are expensive and time-consuming [10]. Among PCR-based genotyping methods, Random Amplified Polymorphic DNA (RAPD) – PCR has received considerable attention in recent years for epidemiological studies, due to its simplicity, rapidity, sensitivity, reproducibility, low cost (no need to expensive specialized instrumentation). It comprises high strain differentiation power and is a definitive method for molecular characterizing of bacteria [11,12]. This rapid methodology has been shown to be as discriminatory as PFGE for typing P. aeruginosa and was recommended for the primary screening of large numbers of isolates because of its efficiency [13,14].

RAPD reactions are PCR reactions, but use a short (8-12mers) single primer, that could attach randomly to the several DNA sequences in the bacterial genome and create repetitive electrophoresis patterns which is used for genotypic differentiation. If a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA segments on the gel. The other difference between this method and ordinary PCR is using of two different annealing temperatures [15,16].

Since the bacterium is a major cause of nosocomial infections, particularly in burn patients, so the knowledge of the spread of P. aeruginosa strains is of epidemiological importance in order to monitor the spreading of the strains. The aim of this study was then to determine the RAPD genotyping of P. aeruginosa strains isolated from burn patients to characterize their genetic diversity.

2. Materials and methods

In total 70 clinical samples including wound discharge, blood and urine, were collected from burn patients hospitalized in Taleghani Burn Hospital in Ahvaz, Iran, between January and October 2011.

The sampling procedure from wound included swabs that were taken from clinically deep areas of the burn wounds when clinical signs of wound infection occurred. Blood culture was taken from patients with the suspicious of sepsis. The samples were immediately transferred to the laboratory. The isolates were identified as P. aeruginosa by application of culture and standard biochemical tests including SIM, MRVP, Oxidation Fermentation (OF) tests and pigment production in Mueller Hinton agar (Himedia, India) [17]. Each isolate was obtained from a different patient except for 6 patients who had blood cultures simultaneously. P. aeruginosa isolates were preserved at −70 °C in Tryptic Soy Broth medium (Himedia, India) supplemented 15% glycerol until further processing.

Bacterial Genomic DNA was extracted from 200 µl of suspension culture cells (10^10–10^8) prepared from a single bacterial colony using DNA extraction kit (Bioneer, South Korea).

RAPD-PCR was performed as described previously [13]. In brief, DNA amplification was performed on a thermo cycler (Bio Rad, USA) in a final volume of 25 µl containing 10× PCR buffer (2.5 µl), 50 mM MgCl₂ (1 µl), 10 mM dNTP mix (1 µl), 50 µM primer 272 [1 µl] (3'-AGCGGCCCA- 5') [8], Taq polymerase [0.5 µl] (Qiagen, USA), double distilled water (17.5 µl) and 1 µl genomic DNA equivalent to 40 ng. The cycling conditions were as follows: initial denaturation at 96 °C for 2 min followed by 3 cycles of denaturation at 94 °C for 1 min, annealing at 36 °C for 2 min, extension at 72 °C for 2 min and 29 more cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min.

The RAPD-PCR products were loaded on a 1.5% (w/vol) agarose gel with 0.5 mg/ml of Ethidium bromide, and were analyzed by gel electrophoresis and banding patterns were observed in Gel-Documentation system (Uvitec, UK). A 1 kilobase DNA ladder (Fermentas, Canada) was used as a molecular size standard. The RAPD fingerprints were analyzed and genotypes were assigned on the basis of number and weight of band differences. Each reaction was repeated at least three times for reproducibility. The minor non reproducible reactions were excluded from the study. For making sure whether each patient was colonized by a unique strain of P. aeruginosa or more strains were colonized in the same patient, the multiple colonies were screened by RAPD and we found out that each individual was colonized with a only one certain P. aeruginosa strain. SPSS software (SPSS Inc. no. 13) was used for data analysis.
3. Results

In current study, 70 clinical samples were isolated from burn patients with different burn degrees. The patients were divided into 2 groups on the basis of degree of burn from deep partial-thickness (degree 2) to full-thickness injury (degree 3), which 28(40%) out of total patients with burn infection had a degree 3 burn. The total body surface area (TBSA) burn ranged from 12 to 51%, with a mean of 28.1%. The most common cause of burn in studied patients were gas explosion (35.7%) and exposure to flame (28.5%). Other minor burn causes were electricity, scald, and hot objects.

Fifty out of total samples were positive for P. aeruginosa. The isolates were belonged to 28 male (56%) and 22 (44%) female patients. The patients age ranged from 1 to 50 years, which the majority of patients were in age group of 15–20 years (26%). Most of P. aeruginosa strains were isolated from wound discharge (80%), following by blood (12%) and urine (8%). In 6 patients blood culture and wound discharge were positive for the same organism (8.57%) simultaneously. Other positive cultures were isolated from samples of different patients. The origin of the isolates was from samples of hospitalized patients in men ward (52%), women ward (36%) and pediatrics (12%).

RAPD fingerprinting of 50 P. aeruginosa isolates, revealed 9 genotypic profiles (I to IX) comprised of 13 DNA fragments with length of 180 to 2700 bp. Each genotype showed between 3 and 6 different weight DNA bands (Fig. 1). The most common genotype was I appeared in 10 isolates (20%), while genotype VIII was the least one appeared in only 2 P. aeruginosa isolates (4%).

The distribution of genotypes according to patients’ sex were as follows: I, II and IV were predominant in females, while V, VII and IX were seen entirely in male patients. Genotypes III and VI showed identical distribution among both sex. In total genotypic polymorphism of isolates was higher in males (32%) compared to females (23%). In relation to patients age, genotype I was seen in all age groups. Genotype VIII, appeared in isolates belonged to only 2 male patients, genotypes V and VII similarly showed the lower frequencies, appeared in 3 patients (V in one 1.5 year infant and 2 adult males and VII in 3 adult males).

Table 1 represents the genotypic profiles in relation to type of clinical sample and patients’ burn severity. According to presented data, genotypes I, II and VI were mostly common in patients with more severe burn, and were mainly prevalent in wound and blood samples obtained from the same patients. The most genotypic variation were seen among P. aeruginosa isolated from wound samples comprised all 9 genotypes.

<table>
<thead>
<tr>
<th>Degree of burn (geno-type no.)</th>
<th>Type of clinical sample (genotype no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 3</td>
<td>Wound Blood Urine</td>
</tr>
<tr>
<td>I(20)</td>
<td>3 7 6 2 2</td>
</tr>
<tr>
<td>II(16)</td>
<td>3 5 7 1 0</td>
</tr>
<tr>
<td>III(8)</td>
<td>3 1 3 0 1</td>
</tr>
<tr>
<td>IV(12)</td>
<td>4 2 5 0 1</td>
</tr>
<tr>
<td>V(6)</td>
<td>3 0 3 0 0</td>
</tr>
<tr>
<td>VI(16)</td>
<td>2 6 5 3 0</td>
</tr>
<tr>
<td>VII(8)</td>
<td>2 2 4 0 0</td>
</tr>
<tr>
<td>VIII(4)</td>
<td>2 0 2 0 0</td>
</tr>
<tr>
<td>IX(10)</td>
<td>4 1 5 0 0</td>
</tr>
</tbody>
</table>

4. Discussion

In order to conduct epidemiological studies regarding the distribution of P. aeruginosa isolates in the certain setting, rapid molecular methods such as RAPD-PCR have been shown to be useful for genetic system that has shown great specificity and sensitivity to define bacterial isolates [18]. Utilization of RAPDs for source tracking in the hospitals of developing countries could be a valuable technique especially since there is minimal expenditure for equipment. There are newer technologies and methodologies that have been developed [19,20], but could be cost prohibitive in certain parts of the world.

RAPDs technique have previously been used in the burn population for source-tracking of P. aeruginosa [14,21–24]. De Vos et al. utilized this technique for typing of P. aeruginosa strains in the burn population 16 years ago and the authors emphasized on the usefulness of the technique in routine microbiology laboratory in combination with other techniques as a tool for controlling nosocomial infections, critical in burn units [14]. The technique was also used by Renders et al., for typing of P. aeruginosa and they concluded that RAPD can be used as a first screen in epidemiological characterization of P. aeruginosa [21].

In this study we could revealed 9 genotypic profiles in 50 tested P. aeruginosa isolates. Using a short primer in this technique was lead to amplification of several DNA fragments throughout the bacterial genome, made the more accurate genotypic discrimination as previously stated in a similar study [15]. There are various primers have been used in

![Fig. 1 – RAPD-PCR fingerprints of P. aeruginosa isolates from burn patients using primer 272. M: DNA molecular weight marker (1 kilo base), –C: negative control, 1–9: Samples.](image-url)
RAPD-PCR so far, with the most common primers of 272 and 280 in several studies [8,25–27]. We used primer 272, since according to previous works, these primers could generate more genotypic patterns, reproducible profiles, and comprises higher discriminatory power [25]. Eftekhar et al., showed that primer 272 generated larger numbers of bands within the fingerprints and was shown to be more discriminating compared to primer 280 [27].

In present study a high polymorphism (18%) with 9 different genotypes were generated. This was in concordant to the study of Akjani et al., in which they revealed 5–11 genotypes for P. aeruginosa isolates compared to what they have shown for S. aureus and E. coli [6]. There are different reports on the percentage of P. aeruginosa polymorphisms. In study of Nazik et al. [8] on P. aeruginosa isolates from cystic fibrosis, the higher polymorphisms of 43% (21 genotypes) were reported. The reason for higher polymorphism in their study compared to present work, may be attributed to the fact that patients with cystic fibrosis are severely immunocompromised and the virulence of P. aeruginosa may be more complicated with more strains’ genotypic variation. Moreover, in their study two more primers of ERIC2 and M13 were used and this may the reasons for generation of more genotypic diversity. Besides in the study of Salimi et al., [24], eight different genotypes with the polymorphism of 6% was reported for P. aeruginosa isolates from burn patients. Though the number of revealed genotypes was close to our study, however less polymorphisms was seen probably due to high standard quality control program in the burn hospital where their work undertaken, so resulted in less genetic variation among the isolates.

The results confirmed that P. aeruginosa genotypes are distributed in all hospital wards and the most prevalent genotypes of I, II and VI were seen in 26 (52%) isolates, mostly belonged to patients with burn degree 3 (18/26[69.2%]) of which, 6 had the positive blood culture for the same genotypes. Although the clinical outcome of patients showed that the mortality rate was highest among patients with burn degree 3 infected by the prevalent genotypes of I, II, VI (6/70 [8.57%]), however is not easy to find out whether there is any correlation between certain genotypes and strain virulence, severity of burn or rate of patients death.

The wide genotypic variation in this study was not expected, since they were colonized the patients in the same hospital and adjacent wards, for instance, in pediatrics ward, 6 different patients were colonized with 5 distinct P. aeruginosa genotypes. Though more genotypic diversity (24 genotypes) in P. aeruginosa isolates was previously reported by Tazumi et al. [16], however the origin of isolates were not from human infections and this may explains the higher clonal diversity. It is a serious concern that our burn patients were colonized by P. aeruginosa at an early age and probably bacterial populations are maintained for extended period of time, make the infection more severe with more difficult bacterial eradication.

The most predominant genetic variation was seen among isolates from wound discharge (all 9 genotypes). Blood and urine samples were revealed 3 genotypic profiles. However due to the lower number of these samples, it is difficult to make any judgment about their genomic variation. Despite the predominance of some genotypes in a certain gender (IV in female and IX in male), the correlation between genetic variation of the isolates and patients age were not statistically significant. An interesting idea is that we might use this technique for P. aeruginosa strains directly in clinical samples with no need to culturing. However, some genotyping techniques at present are performed on the bacteria propagated by culture, since for the method, a certain amount of bacterial DNA is needed which is difficult to get it from sparse bacteria within the clinical samples directly.

In conclusion, the present study showed a relative high polymorphism among P. aeruginosa isolates from the same hospital. We believe that the genotypic identification and characterization of P. aeruginosa strains isolated from burn patients provided followed-up for several years, could be an accurate way to study the epidemiology of infection by this bacterium and allows a better assessment of prophylactic, therapeutic, and control measures against bacterial populations.

Conflict of interest declaration

The authors declare that there is no conflict of interest in submitted study.

Conflict of interest

None

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References


