Original Article

Antibiotic Resistance and RAPD-PCR Genotyping of *Pseudomonas aeruginosa* Clinical Strains Isolated from Intensive Care Unit Patients

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Abstract

Background: *Pseudomonas aeruginosa* is one the most important nosocomial pathogens, especially in immunocompromised patients. Identifying the source of contamination in health centers plays an important role in the control of hospital infections. The aim of this study was to determine antibiotic susceptibility and genetic patterns of *P. aeruginosa* isolated from patients hospitalized in intensive care unit of Masih Daneshvari Hospital, Tehran, Iran.

Materials and Methods: Antibiotic susceptibility of the isolates was examined through 10 antibiotics recommended by Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines using the Kirby-Bauer disc diffusion method. Random amplified polymorphic DNA (RAPD) analysis with the short primer of 272 was used to evaluate genetic relationship among the isolates and the results were analyzed by Gelcompar II software.

Results: Of the antibiotics used, the most sensitive was found in colistin (96.4%) and the highest resistance rates were observed in cefotaxime (94.6%), chloramphenicol (83.9%) and imipenem (71.4%). DNA fingerprinting was able to identify 12 genetic patterns by RAPD-PCR technique.

Conclusion: Antibiotic resistance in isolates of *P. aeruginosa* is rising and there is possibility of occurring outbreaks in the medical centers. Different sources of strains show their constant exchange via intra- and extra-hospital transmission routes. Thus, according to the data of this study, there is a serious need to control sources of infections by physicians and staff when they are working in several sectors to control and prevent the transmission of the bacterium.

Keywords: *Pseudomonas aeruginosa*, multi-drug resistance, RAPD-PC, ICU

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Introduction

Pseudomonas aeruginosa is a major opportunistic Gram-negative pathogen, which is the most often

isolated in intensive care units (ICUs)[1]. According to point-prevalence surveys carried out in the USA, Europe and Asia, *P. aeruginosa* has emerged as a leading cause of infections, accounting for 11-14% of all nosocomial infections[2], which is one of the primary pathogens to cause infections in immunocompromised individuals and other highly vulnerable patients[3]. The spectrum of *P. aeruginosa* infections is broad, including respiratory, gastrointestinal, urinary tract infections, wound infections and burns. All these infections may lead to bacteremia, sepsis and even death[4]. Due to its broad band of natural and acquired antibiotic resistance, only some therapeutics reliably treats multi-drug resistant (MDR) *P. aeruginosa* infections[5]. Therefore, *P. aeruginosa* related infections are usually associated with high morbidity and mortality[6].

It is essential to understand pathogen distribution and its genetic relatedness to determine the epidemiology of hospital-acquired infections (HAIs)[7,8]. There have been various technical approaches to bacterial source tracking, but a consensual single method for field application has not been recognized until now[9]. The adaptability of *P. aeruginosa* to stress and phenotype changes are the main reason for the difficulties in *P. aeruginosa* virulence identification. It has become necessary to use a genotype-based system capable of accurate strain characterization[10]. Genomic fingerprinting techniques are currently viewed as the most exact strategies for the typing of microorganisms. These methods comprise ribotyping, pulsed field gel electrophoresis, and PCR-based fingerprinting methods. Screening by random amplified polymorphic DNA (RAPD–PCR) is a simple approach enabling the determination of clonal relatedness at a high speed and relatively low-cost. On the other hand, emergence and spread of MDR *P. aeruginosa* appear as a great public health concern in many parts of the world, Iran as well[11]. Therefore, the aim of our study was to evaluate antibiotic resistance and genotyping of *P. aeruginosa* in patients hospitalized in ICUs of Masih Daneshvari Hospital by RAPD–PCR.

Methods

Pseudomonas aeruginosa isolates

This study has been submitted and approved by the ethics committee in the School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RETECH.REC.1395.840). A total of 56 non-duplicate clinical isolates of *P. aeruginosa* were obtained from patients hospitalized in ICUs of Masih Daneshvari Hospital, Tehran, Iran from March 2015 to May 2019. The *P. aeruginosa* strains isolated from clinical specimens of sputum, bronchial lavage and blood of hospitalized patients were used in the study. The specimens immediately transported to the microbiology laboratory of the Department of Microbiology of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Blood culture was also carried out in patients with a suspicion of sepsis. Identification of *P. aeruginosa* was carried out using conventional methods. The tests used were a Kligler iron agar slant, growth in 42°C, catalase and oxidase tests, sugar fermentation, Simmons' citrate agar slant, urea hydrolysis slant, methyl red/Voges–Proskauer test, and motility test. Isolates identified as *P. aeruginosa* were preserved at –70°C in trypticase soy broth (Merck) supplemented with 20% glycerol until further analysis[12].

Antibiotic susceptibility testing

The antimicrobial susceptibility profile of the isolated *P. aeruginosa* was determined by using antibiotic containing disks on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines[13]. The disks contained the following antibiotics were purchased from MAST Diagnostics (Merseyside, UK): cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin (100 µg), piperacillin/tazobactam (100/10 µg), doripenem (10 µg), ciprofloxacin (5 µg), ticarcillin (75 µg), colistin (10 µg).

Random amplified polymorphic DNA typing (RAPD-PCR)

In order to accomplish the genotyping of *P. aeruginosa*, bacterial genomic DNA was extracted from 200 µl of suspension cultured cells prepared from a single purified colony using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instruction. RAPD-PCR was done as previously described[7]. Briefly, DNA amplification was

accomplished on a thermocycler (Eppendorf, Hamburg, Germany) in a final volume of 25 µl containing 1× PCR buffer, 2 mM $MgCl_2$, 1 mM dNTP mix, 50 mM primer 272 (3'-AGCGGGCCAA-5'), 1-unit Taq polymerase (Qiagen NV, Venlo, the Netherlands), double distilled water, and genomic DNA equivalent to 40 ng. The amplification conditions were as follows: initial four cycles of denaturation at 94°C for 5 minutes, annealing at 36°C for 5 minutes, extension at 72°C for 5 minutes followed, by 30 more cycles of denaturation at 94°C for 1 minute, annealing at 36°C for 1 minute, and extension at 72°C for 2 minutes. The RAPD-PCR products were then electrophoresed in 1.5% (w/vol) agarose gel along with a 100-bp plus DNA ladder (SinaClon, Iran) as molecular size standard and banding patterns were visualized and photographed in gel documentation system (Uvitec, Cambridge, UK).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) software version 22 (SPSS Inc., Chicago, IL, USA) was used for data analysis. RAPD-PCR fingerprinting profile was analyzed with Gelcompar II software, version 6.5 (Applied Maths, Sint-Martens-Latem, Belgium). Comparison of the data among various group was performed by Chi-square test. *P* values <0.05 were considered as statistically significant.

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Results

A total of 56 *P. aeruginosa* isolates were obtained from sputum (43, 76.8%), bronchial lavage (11, 19.6%), and blood (2, 3.6%). Of the patients, 34 (60.7%) were males and 22 (39.3%) were females. The median age of the patients was 42 years (IQR: 9-86 years).

Antibiotic resistance

Percentage of antibiotic resistance in 56 evaluated *P. aeruginosa* has been shown in Table 1. Of 10

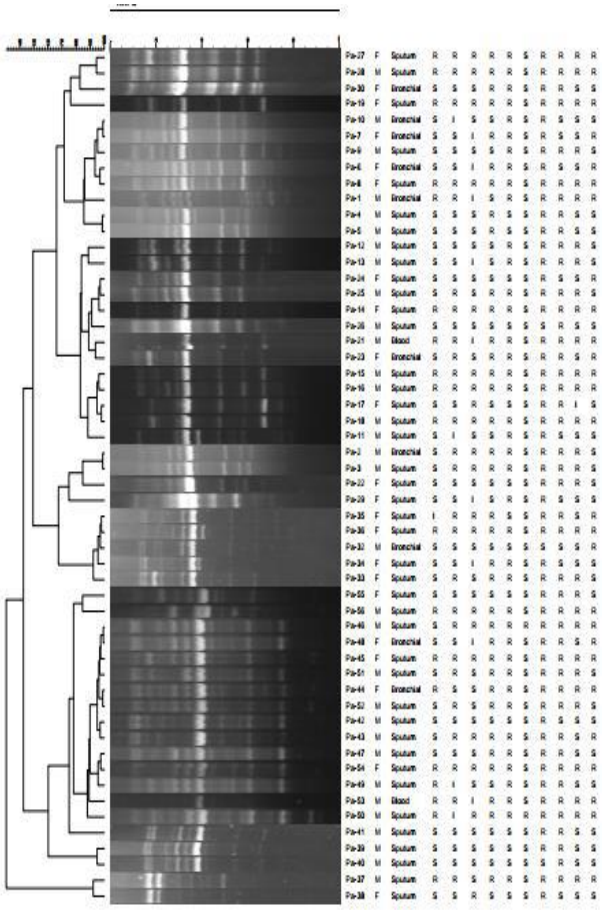

Figure ١. Genotyping of *Pseudomonas aeruginosa* strains isolated from the intensive care unit of Masih Daneshvari Hospital.

antibiotics used, the highest sensitivity was observed to colistin (54, 96.4%) and amikacin (34, 60.7%), and the high frequency of antibiotic resistance was associated with cefotaxime (53, 94.6%) and chloramphenicol (47, 83.9%).

Genotyping

Genomic DNA was extracted from all *P. aeruginosa* isolates, and the genetic patterns of isolates were determined using RAPD-PCR technique using primer 272. Based on the results obtained by RAPD-PCR, 12 patterns were observed among the tested isolates by using a cut off value of 85% as the threshold. Also, no significant relationship was observed by examining the antibiogram in different patterns (P>0.05). RAPD-PCR generated dendrogram of *P. aeruginosa* isolates is illustrated in Fig. 1.

Discussion

Drug resistant *P. aeruginosa* has emerged as one of the most frequently observed pathogens causing nosocomial infections, especially in immunocompromised patients[14]. We tested 56 *P. aeruginosa* strains isolated from different sources collected from patients hospitalized in the ICU, which caused patients to be more susceptible to many infections including urinary, respiratory, and gastrointestinal infections and sepsis[15]. In addition, we characterized and typed *P. aeruginosa* strains isolated from different clinical sources from an Iranian hospital according to their antibiogram and genetic fingerprint.

The current examination demonstrated that the *P. aeruginosa* clinical isolates expressed a high level of resistance to current drugs along with obtaining resistance to newer antibiotics. In the present investigation, a high rate of antibiotic resistance was observed compared to other studies[16,17]. These results might be justified by the extensive usage of antibiotics in Iranian hospitals. Of 10 antibiotics tested from different classes, the highest sensitivity was observed to colistin (96.4%) and amikacin (60.7%); the least sensitivity was associated with gentamicin (50%), ciprofloxacin (44.6%), piperacillin (35.7%), imipenem (38.6%), chloramphenicol (16.1%) and cefotaxime (5.4%). In accordance with our findings, other studies from Iran have likewise indicated that besides colistin which acts as the choice antibiotic for treatment of infections caused by resistant isolates, amikacin is an extremely effective antibiotic as well[18]. Goli et al. uncovered that piperacillin/tazobactam (34% resistant) and colistin (2% resistant) had the most noteworthy measure of activity against MDR strains of *P. aeruginosa* [19]. In a survey that conducted in Latin America during 2004-2015, among 3613 *P. aeruginosa* isolates, the highest susceptibility (72.8%) was seen to amikacin, and 56.8% of the isolates were susceptible to ceftazidime[20]. However, high rates of resistance among the *P. aeruginosa* isolates from burn patients were detected by Talebi et al. in Iran, which the resistance rates to amikacin, cefepime, ciprofloxacin, imipenem, ceftazidime and gentamycin were 83.8%, 85.5%, 88.7%, 93.5%, 95.1% and 95.1%, respectively[21].

Typing of bacteria is vital for monitoring newly emerging pathogens and for examining local outbreaks[22,23]. In this report, we used RAPD-PCR followed by agarose gel electrophoresis technique for the molecular typing of *P. aeruginosa* collected from the patients hospitalized in the ICU of Masih Daneshvari Hospital. The standardization of the reaction conditions has contributed to making RAPD

Table 1: The antibiotic susceptibility patterns of *Pseudomonas aeruginosa* isolates from patients hospitalized in intensive care unit of Masih Daneshvari Hospital using disk diffusion method.

Antibiotic	Resistant No (%)	Sensitive No (%)	Intermediate No (%)
Cefepime	27 (48.2%)	25 (44.6%)	4 (7.1%)
Piperacillin	36 (64.3%)	20 (35.7%)	0 (0%)
Colistin	2 (3.6%)	54 (96.4%)	0 (0%)
Amikacin	21 (37.5%)	34 (60.7%)	1 (1.8%)
Imipenem	40 (71.4%)	16 (28.6%)	0 (0%)
Cefotaxime	53 (94.6%)	3 (5.4%)	0 (0%)
Gentamicin	25 (50%)	25 (50%)	0 (0%)
Chloramphenicol	47 (83.9%)	9 (16.1%)	0 (0%)
Ciprofloxacin	30 (53.6%)	25 (44.6%)	1 (1.8%)
Ceftazidime	22 (39.3%)	25 (44.6%)	9 (16.1%)

typing a trustworthy and reproducible technique[7], with even more discriminatory power than DNA macro-restriction analysis by PFGE for bacterial typing[24]. However, random amplification could simply be applied for the surveillance and prevention of nosocomial infections by clinical microbiology laboratories by enhancing the resolution of the electrophoretic separation and the sensitivity of the staining[24]. The dendrogram of genotyping of *P. aeruginosa* strains showed the presence of 12 patterns among the tested isolates using a cut off value of 85% as the threshold. There are few reports about clonal relationship among hospital isolates of *P. aeruginosa* in Iran. RAPD analysis by Sharifi et al. showed four large clusters consisting of 77 isolates out of 84[24]. Their dendrogram analysis revealed that cluster 3 exhibited the largest fingerprint similarity consisting of 29 isolates. They suggested the possible spread of cluster 3 clones in different wards of hospitals in Shiraz. Taheri et al. studied the genetic similarity among 73 *P. aeruginosa* isolates from Tehran referral hospitals by RAPD analysis and showed 67 different patterns, each containing 2-3 isolates, mostly from ICU[25]. They concluded that most of the isolates were probably originated from the host.

The number of genotypes, as in our case, presumably reflects the various sources of strains and their constant exchange via intra- and extra-hospital routes through transient hand carriage by health care personnel due to contact with contaminated surfaces[26]. These assumptions were supported by the dates of strain isolation, when individual genotypes were found independently on the term and length of patient's hospitalization. Even rare genotypes were recovered either on the first day of hospitalization or after more than 30 days of hospitalization. Taken together, both higher stability and discriminatory power make genotype determination by RAPD analysis a more accurate tool to analyze the epidemiological situation in the hospital than serotyping. We searched for the fast, simple and low-cost approach for profiling *P. aeruginosa* strains and, based on several other studies[27,28], RAPD analyze was finally considered as a suitable method for this purpose, being beneficial even when used separately. RAPD can be used as a first screen in characterization of *P. aeruginosa* strains, allowing quick characterization of molecular differences between various groups of isolates[4]. Yet the following genotypic and/or phenotypic characterization should be used for acquisition of more exact information about particular strains[4].

Conclusion

Based on the results, it can be concluded that our *P. aeruginosa* hospital isolates are highly resistant to different classes of antibiotics and sensitive to colistin, which could be used as an empirical therapy in critically ill patients, especially in patients admitted to ICU. Further studies on a larger number of isolates are required to confirm our observations. We suggest applying strict disinfection policy in a hospital to prevent spreading of infections, also, new classes of antibiotics should replace traditional antibiotic treatment to prevent emergence of resistant strains of *P. aeruginosa*.

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