

Molecular Typing of Multidrug-Resistant *Pseudomonas aeruginosa* Isolates Obtained from Hospitalized Burn Patients by Rep-PCR

ARTICLE INFO

Article Type Original Article

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How to cite this article

Delroshan M., Ghandehari F, Mirzaei R., Hoveida L. Molecular Typing of Multidrug-Resistant *Pseudomonas aeruginosa* Isolates Obtained from Hospitalized Burn Patients by Rep-PCR. Infection Epidemiology and Microbiology. 2023;9(2): 99-106.

Article History

Received: December 13, 2022
Accepted: June 03, 2023
Published: August 19, 2023

ABSTRACT

Backgrounds: Multidrug-resistant *Pseudomonas aeruginosa* is known as a major opportunistic pathogen in burn patients with hospital-acquired infections. The aim of this study was to investigate antibiotic resistance and the capability of (GTG) 5-PCR (polymerase chain reaction) assay for molecular typing of *P. aeruginosa* strains isolated from clinical samples of hospitalized burn patients in southern Iran.

Materials & Methods: This cross-sectional research was carried out on 70 *P. aeruginosa* isolates collected from hospitalized burn patients in southern Iran from June 2020 to January 2021. Antimicrobial susceptibility patterns of the isolates were determined using disk diffusion method. Additionally, repetitive extragenic palindromic-PCR (rep-PCR) method was used to examine the genetic similarities among the strains.

Findings: Antimicrobial susceptibility patterns revealed that the highest antibiotic resistance was against gentamicin (95.8%), followed by imipenem (94.3%) and piperacillin-tazobactam (92.8%), while colistin was the most effective antimicrobial agent. Rep-PCR typing revealed that 60 *P. aeruginosa* strains were classified into 49 GTG5 types (G1-G49), which were then grouped into 12 clusters (A-L) and 10 isolates with unique banding patterns according to the 80% cut off point.

Conclusion: The present study data indicated a substantial resistance to the studied antimicrobial agents, especially the last-resort antimicrobial agents. In addition, rep-PCR analysis revealed that most of the evaluated strains had partial genetic diversity; therefore, infection control activities should be carried out to decrease the colonization of MDR *P. aeruginosa* isolates in the hospital setting.

Keywords: *Pseudomonas aeruginosa*, Drug-resistant, Molecular typing, Burns.

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Introduction

Pseudomonas aeruginosa is one of the most widespread opportunistic bacteria, which often lives in moist environments and causes serious infections in patients and rarely infects healthy hosts [1-5]. Empirical therapies including monotherapy (e.g., carbapenem, ceftazidime, cefepime, piperacillin, or piperacillin-tazobactam) and combination therapy (an antipseudomonal β -lactam with an aminoglycoside or a fluoroquinolone) are used to treat *P. aeruginosa* infected cases [1,3,6]. However, treatment of *P. aeruginosa* infections in burn patients could be a major challenge as this organism is intrinsically resistant to many available antibiotics and rapidly develops resistance to all effective antimicrobial medicines during treatment [1].

Understanding the local molecular epidemiology of *P. aeruginosa* is necessary to control the spread of this bacterium in hospital settings [7]. Among PCR (polymerase chain reaction) based genotyping methods, repetitive extragenic palindromic-PCR (rep-PCR) as a straightforward, reliable, and reproducible typing technique is appropriate for local typing of Gram-negative enteric bacteria [8, 9]. It is an extragenic typing technique that targets bacterial genomic areas of repetitive non-coding sequences [10]. Given the significance of *P. aeruginosa* infections in patients with burn injuries, little information is available about the molecular characteristics of these isolates in nosocomial infections.

Objectives: The purpose of this study was to determine genotypic relationships and distribution of antibiotic resistance among *P. aeruginosa* isolates obtained from burn patients in a teaching burn hospital in Ahvaz, southern Iran.

Materials and Methods

In this cross-sectional study conducted during 7 months from June 2020 to January

2021, a total of 70 *P. aeruginosa* isolates were collected from the specimens of hospitalized burn patients in a teaching hospital in Ahvaz, southern Iran. *P. aeruginosa* strains were isolated from a variety of clinical samples, including biopsy, wound, blood, and sputum specimens.

The specimens were cultured on MacConkey agar and blood agar (Merck, Germany) and incubated at 37 °C for 24 hrs. *P. aeruginosa* isolates were identified based on standard microbiological tests and the presence of the *tox-A* gene (genotypic method) [11].

All *P. aeruginosa* isolates were investigated for susceptibility against six antibiotics by Kirby-Bauer disk diffusion susceptibility method according to Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines [12].

The tested antibiotics (Mast Group Ltd., UK.) were imipenem (10 μ g), gentamicin (30 μ g), ciprofloxacin (5 μ g), ceftazidime (30 μ g), piperacillin-tazobactam (100/10 μ g), and colistin (10 μ g). *P. aeruginosa* ATCC 27853 was also tested as the quality control. E-test was used to evaluate the antimicrobial susceptibility of *P. aeruginosa* isolates against colistin. Non-susceptibility to at least one agent in three or more antibiotic chemical classes was defined as multidrug resistance (MDR). Genomic DNA was extracted by a simple boiling method according to the method previously described [13]. PCR was performed to detect the *toxA* gene using specific primers [14]. PCR amplification included an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 60 s, annealing at 68 °C for 45 s, and extension at 72 °C for 1 min. The final extension step was performed at 72 °C for 5 min. After electrophoresis of amplification products on 1% agarose gel (with safe stain dye (CinnaGen Co. Iran)), the presence of expected fragments was analyzed.

All *P. aeruginosa* isolates were analyzed by rep-PCR, and the primer sequence was 5'-

GTG GTG GTG GTG GTG-3'. For molecular typing of *P. aeruginosa* isolates, rep-PCR was done as described previously [14]. Genetic relationships among *P. aeruginosa* isolates were analyzed using GelJ software Version 2.0, and isolates with a similarity coefficient equal to or above 80% were classified into the same genotypes.

Findings

During the 7-month study period, 70 *P. aeruginosa* isolates were obtained. Overall, out of 70 confirmed *P. aeruginosa* isolates from various clinical specimens, 51.5 and 48.5% were obtained from male and female patients, respectively. Most of the isolates were obtained from burn-wound biopsy samples (63%), followed by blood culture (27%), wound swab (5.7%), and sputum (4.3%) specimens. According to the findings, 51.4% of the strains were isolated from patients in the intensive care unit (ICU).

Among 70 *P. aeruginosa* isolates, resistance to gentamicin was the most prevalent (95.8%), followed by imipenem (94.3%) and piperacillin–tazobactam (92.8%). None of the isolates were found to be resistant to colistin. According to MDR definition, 66 (94%) isolates were identified as MDR, most of which belonged to the ICU ward. Moreover, 52 (74%) isolates were resistant

to all antibiotics, except colistin, and only one out of 70 isolates was susceptible to all tested antibiotics. The susceptibility rates of the isolates to the studied antibiotics are presented in Table 1.

Dendrogram and gel electrophoresis images of rep-PCR products are showed in Figure 1. The number of fragments varied from 3 to 10 per strain, and the size of rep-PCR bands ranged from 100 to 1.5 kb. In this study, out of 70 *P. aeruginosa* isolates, 10 isolates did not show any product in the reactions and thereby were non-typeable. Rep-PCR typing revealed that 60 *P. aeruginosa* strains were classified into 49 GTG5 types (G1-G49). According to the 80% cut off point, these types were grouped into 12 clusters (A-L) and 10 isolates with unique banding patterns and distinct genotypes not belonging to any cluster. Some *P. aeruginosa* strains were 100% similar in terms of electrophoretic band pattern, and as a result, these strains were classified in a GTG, such as G24, G34, and G40. Cluster A was the most common rep-type, including 11 (18.3%) isolates, followed by cluster B (eight isolates, 13.3%) and C (seven isolates, 11.6%). However, the rep-PCR analysis results showed high genetic diversity among most of the examined strains. The distribution of rep-types in different hospital wards is shown in Table 2.

Table 1) Antibiotic susceptibility pattern of *P. aeruginosa* isolates obtained from burn patients in this study

Antimicrobial Agent	Sensitive No (%)	Intermediate No (%)	Resistant No (%)
Gentamicin	2 (2.8)	1 (1.4)	67 (95.8)
Imipenem	4 (5.7)	-	66 (94.3)
Piperacillin-tazobactam	5 (7.2)	-	65 (92.8)
Ceftazidime	9 (12.8)	-	61 (87.2)
Ciprofloxacin	11 (15.7)	2 (2.8)	57 (81.5)
Colistin	70 (100)	-	-

Table 2) Frequency of rep types of *Pseudomonas aeruginosa* isolates in different hospital wards

Wards (No.)	Rep-Types (No. of Isolates)												
	A	B	C	D	E	F	G	H	I	J	K	L	Unique
ICU (29)	7	4	4	1	2	3		2			2	2	2
Pediatric (10)	1	1	1				2					1	4
Men (10)	1	1		2	1				1				4
Surgery (6)	1		2						1	1		1	
Women (5)	1	2		1						1			
Total (60)	11	8	7	4	3	3	2	2	2	2	2	4	10

Discussion

Due to the intrinsic and acquired antimicrobial resistance of *P. aeruginosa* strains and the emergence of MDR isolates in clinical settings, it is difficult to identify an antibiotic that could effectively treat infections caused by these bacteria [15]. This study investigated the antibiotic susceptibility and (GTG) 5-PCR fingerprinting of *P. aeruginosa* strains isolated from hospitalized burn patients in a burn center in Ahvaz, Iran. In this study, in addition to phenotypic methods, PCR was used to identify the *toxA* gene, which is specific to *P. aeruginosa*, and only the strains that harbored this gene were included in the study. Exotoxin A is regulated by the *toxA* gene, leading to inhibition of protein biosynthesis and tissue damage in the host. Therefore, the exotoxin A gene was used to detect and confirm *P. aeruginosa* isolates. Identification of the *toxA* gene as a genotypic method has previously been shown in other studies [16, 17].

In this study, *P. aeruginosa* strains were mostly isolated from burn-wound biopsy (63%) and blood culture (27%) samples, and more than half of them were obtained from the ICU ward. This finding is in agreement with the finding of another study by Ranjbar et al. (2011), where most of the isolates were

obtained from wound infection [18]. In this regard, Faghri et al. (2018) reported that *P. aeruginosa* strains were typically isolated from the ICU ward (50%) and different clinical samples [11].

In the present research, colistin was the most effective antibacterial agent with a sensitivity rate of 100% based on the antibiotic susceptibility pattern, while the majority of *P. aeruginosa* isolates showed high antibiotic resistance to other antimicrobials. In accordance with these findings, Goli et al. (2017) and Mobaraki et al. (2018) in the northwest of Iran and Khosravi et al. (2017) in Ahvaz have also reported colistin as the most effective antibiotic in the treatment and management of nosocomial infections [19-21]. In another study performed by Fazeli et al. (2014) [22] in Isfahan, a lower resistance rate was reported against cepheims and quinolones. In their study, 63 and 63.1% of the isolates were resistant to ciprofloxacin and ceftazidime, respectively, while in the present study, resistance to ciprofloxacin and ceftazidime was higher, which shows the increasing trend of antibiotic resistance in hospitalized patients. This may also be related to the type of infection; for example, in a study performed by Kashfi et al. (2017) on patients with burn infection, antibiotic

resistance to ciprofloxacin and ceftazidime was reported to be 94 and 75%, respectively [23]. In the present study, the highest antibiotic resistance was observed against gentamicin (95.7%), which is consistent with the results of the studies conducted by Banar et al. (2016) and Zarei-Yazdeli et al. (2018) [24, 25]. Different gentamicin resistance rates have been reported in different studies; for example, in the studies performed by Song et al. (2001) and Singh et al. (2003), resistance to gentamicin was observed in 20 and 31% of the isolates, respectively, while in the study conducted by Coetzee and colleagues (2013), gentamicin resistance rate was 92% [26-28]. This difference in gentamicin resistance rates in different studies is probably due to variation in the type of infection, sample examined, or geographical area.

In this study, 66 (94%) isolates were resistant to at least three classes of antibiotics, and they actually exhibited an MDR pattern; also, 52 (74%) strains were resistant to all antibiotics, except colistin. In a meta-analysis study conducted by Vaez et al. (2018) in Iran, the frequency of MDR *P. aeruginosa* in different parts of Iran was 58%, and the highest (100%) and lowest (16%) frequency of MDR *P. aeruginosa* was observed in Tehran and Zahedan, respectively [29]. In a study performed by Tarafdar and colleagues in 2018-2019, similar proportions of MDR *P. aeruginosa* were observed in patients admitted to a teaching hospital in Tehran, and 100% of all isolates were MDR [30].

In contrast to the current study results, Khan and colleagues (2014) reported a lower incidence rate of MDR *P. aeruginosa* isolates in hospitals in Karachi, Pakistan, where 30% of isolates were MDR [31]. Additionally, a research performed in the northeast of Iran found that the prevalence of MDR *P. aeruginosa* was 16.5% [32], which is lower than the present study finding.

An essential matter in infection control is to

determine the genotype and investigate the genetic features and genetic relatedness of *P. aeruginosa* strains isolated from the studied specimens [7, 33]. The rep-PCR analysis results indicated high genetic diversity among most of the examined strains. In this study, 49 GTG5 types were found among 60 *P. aeruginosa* strains typed by rep-PCR, although some of GTG5 types such as G24, G34, and G40 were common in several isolates. Also, in present study, according to the 80% cut off point, these types were grouped into 12 clusters (A-L) and 10 isolates with unique banding patterns. This study results also showed that the highest genetic diversity and distribution of rep types was related to the ICU; on the other hand, most of the isolates that showed the lowest and highest antibiotic resistance (diversity in antibiotic susceptibility) were obtained from the ICU ward, which is consistent with the rep-PCR results. The greater the number and size of clusters in a hospital, the more contamination and circulation of genetically diverse bacteria in the hospital. In the current study, clusters A, B, and C had the highest number of strains, indicating that the strains related to these clusters are more circulating in the hospital. Similarly, Ghaleh Sorkh et al. (2017) found that *P. aeruginosa* strains isolated from this burn center had a significant level of genotypic variability (20 common types of A-T and 20 unique types among 75 strains) according to rep-PCR analysis [8]. In contrast to the present study results, Faridi and Javadpour (2015) in Bandar Abbas reported only seven genotypic clusters among 67 *P. aeruginosa* isolates, indicating less genetic diversity among the isolates [10]. However, the genetic diversity and antibiotic resistance of *P. aeruginosa* strains might help these strains survive in the environment.

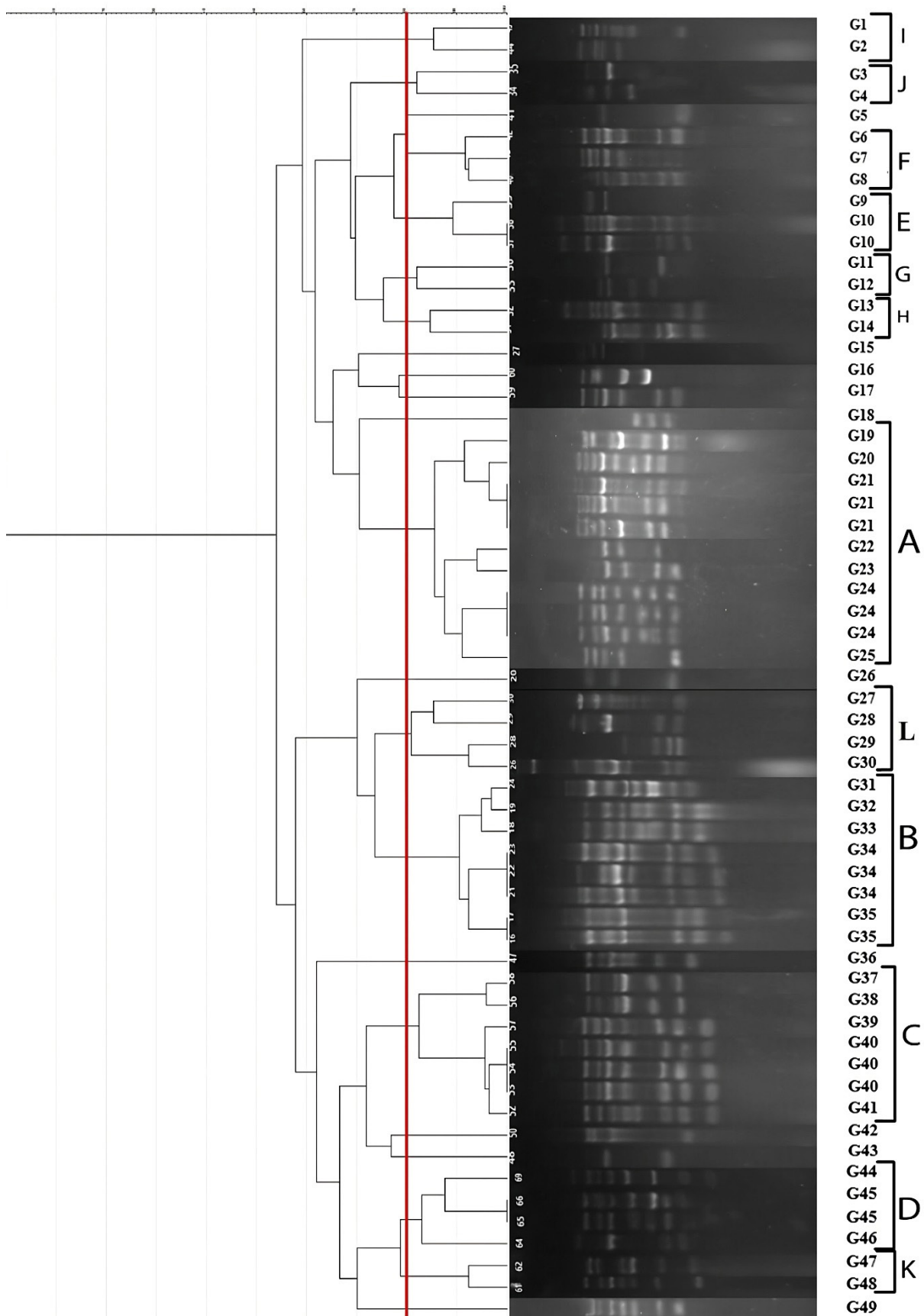


Figure 1) Dendrogram showing relatedness between rep-PCR patterns of 60 *Pseudomonas aeruginosa* strains isolated in this study

Conclusion

In summary, the high rate of antibiotic resistance of *P. aeruginosa* strains in the current study is a serious concern in hospital wards because it is difficult to prevent infections caused by such drug-resistant strains. The rep-PCR analysis results demonstrated high genetic diversity among *P. aeruginosa* strains, and this might complicate the treatment of illnesses caused by this bacterium in hospital settings.

Acknowledgements

Not applicable.

Ethical permissions: The present study was conducted in accordance with the declaration of Helsinki and evaluated and approved by the Ethics Committee of Islamic Azad University, Falavarjan Branch, Isfahan, Iran (IR.IAU.FALA.REC.1398.061). However, the ethics committee waived the need for informed consent since only medical records were used. Also, all samples were obtained from patients as part of routine sampling during their hospitalization.

Authors' contributions: ND and LH conceived, designed, and supervised the study and revised the manuscript; FG, RM, and ND collected and analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

Conflicts of interests: This study was supported in part by a grant from Falavarjan Branch, Islamic Azad University, Isfahan, Iran, [Grant no254565].

Fundings: None declared by authors.

Consent to participate: Not applicable.

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