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Emerging Carbapenem-Resistant *Pseudomonas aeruginosa* Isolates Carrying bla_{IMP} Among Burn Patients in Isfahan, Iran

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Abstract

Background: Metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa* is a significant pathogen in burn patients.
Objectives: The aim of this study was to determine the prevalence of carbapenem-resistant *P. aeruginosa* isolates, including those resistant to imipenemase (IMP), in a burn unit in Isfahan, Iran.
Patients and Methods: One hundred and fifty *P. aeruginosa* isolates from burn patients were tested for antibiotic susceptibility by the disc diffusion method in accordance with CLSI guidelines. Production of MBL was identified with the EDTA disk method. DNA was purified from the MBL-positive isolates, and detection of the bla_{IMP} gene was performed with PCR.
Results: Fifty-seven out of 150 (38%) isolates were multi-drug resistant (MDR), and 93 (62%) were extensively-drug resistant (XDR). Among all isolates, the resistance rate to ciprofloxacin, tobramycin, imipenem, meropenem, amikacin, ceftazidime, and cefepime was higher than 90%, while the resistance rates to piperacillin/tazobactam and aztreonam were 70.7% and 86%, respectively. Colistin and polymyxin B remained the most effective studied antibiotics. All of the imipenem-resistant *P. aeruginosa* isolates were MBL-positive, and 107 out of 144 (74.3%) of the MBL isolates were positive for the bla_{IMP} gene.
Conclusions: The results of this study show that the rate of *P. aeruginosa*-caused burn wound infections was very high, and many of the isolates were resistant to three or more classes of antimicrobials. Such extensive resistance to antimicrobial classes is important because few treatment options remain for patients with burn wound infections. bla_{IMP}-producing *P. aeruginosa* isolates are a rising threat in burn-care units, and should be controlled by conducting infection-control assessments.

Keywords: Antibiotic Resistance, Carbapenem-Resistant, Metallo-Beta-Lactamase, Burn, *P. aeruginosa*

1. Background

A burn is described as a traumatic injury to the skin or other organic tissue, mainly caused by thermal or other acute exposures. *Pseudomonas aeruginosa* skin infections in burn injuries carry a very high mortality rate in burn units, even with aggressive antibiotic therapy (1). Nosocomial infections are widespread in burn patients because of the characteristic aspects of the disease, changes in the specific and nonspecific components of the immune system, extended hospitalization, and various invasive diagnostic and therapeutic procedures (2-4). Emerging multidrug-resistant (MDR) strains of *P. aeruginosa* have recently caused an unexpected rise in burn wound infections, sepsis, and associated deaths worldwide (5). Microbial factors, such as type, virulence, and bacterial count (> 105 organisms per gram of tissue) increase the risk of an invasive wound infection. Prolonged use of antibiotics, frequently in combination, has led to the selection of MDR *P. aeruginosa* strains. These may cause infections that are very difficult to treat, as there are few antimicrobial agents available to eradicate them. Even resistance to carbapenems in burn patients can be considerable.

The risk factors for acquisition of imipenem-resistant *P. aeruginosa* include carbapenem use, broad-spectrum antibiotic use, extended length of hospitalization, the presence of imipenem-resistant *P. aeruginosa* in the unit, and the previous presence of imipenem-sensitive *P. aeruginosa* in the patient (6). For empiric therapy prior to the acces-

sibility of susceptibility testing results in burn patients with serious *P. aeruginosa* wound infections, a combination of two antibiotics is suggested because of the high organism load and the chance of infection with, or development of, resistant organisms. Possible regimens include aztreonam, ciprofloxacin, ceftazidime, cefepime, or an antipseudomonal carbapenem plus an aminoglycoside. Colistin has been gradually increasingly used in the management of severe infections when no other choices are available. Consideration of *P. aeruginosa* in burn patients is important because it causes severe hospital-acquired infections, is often antibiotic-resistant (thus complicating the choice of therapy), and is associated with a high mortality rate.

2. Objectives

The aim of this study was to determine the prevalence of carbapenem-resistant *P. aeruginosa* isolates, including those resistant to imipenemase (IMP), in a burn unit in Isfahan, Iran.

3. Patients and Methods

This descriptive study was conducted at the Imam Mousa Kazem Burns Hospital in Isfahan, Iran. The data were obtained from September 2013 to August 2014. A questionnaire was completed to collect the patients' data. Samples from the burn wounds were collected from all patients and were cultured on sheep blood agar, MacConkey, and chocolate agar, and incubated for 24 - 48 hours at 37°C. The isolates were confirmed to the species level by gram staining, catalase and oxidase testing, O/F (oxidation fermentation), pyocyanin pigment production, and growth at 42°C. The API 20E/NE (bioMerieux, France) was used. Antimicrobial susceptibility was determined by the disk diffusion method according to the guidelines of the clinical laboratory standards institute (CLSI) (7). The following antibiotic disks (Mast Group Ltd., Merseyside, UK) were used for susceptibility testing: imipenem (10 μg), meropenem (30 μg), aztreonam (10 μg), cefepime (30 μg), ceftazidime (30 μg), amikacin (30 μg), tobramycin (30 μg), ciprofloxacin (30 μg), piperacillin/tazobactam (100 μg), colistin (30 μg), and polymyxin B (300 units). The quality control of antibiotic susceptibility was determined by *P. aeruginosa* ATCC27853. The isolates were classified as MDR if they were resistant to more than three classes of antimicrobial drugs. Extensively-drug resistant (XDR) was defined as bacterial isolates susceptible to only one or two categories. Pan-drug resistant (PDR) was defined as non-susceptibility to all agents in all of the antimicrobial categories. Imipenem-resistant isolates were screened for MBL production. The double-disk synergy test (DDST) was performed for identification of MBLs by imipenem (10 μg) alone and in combination with EDTA (750 μg/disk) (ROSCO, Denmark). An increased zone diameter of $\geq$ 7 mm around the imipenem plus EDTA disk compared to that of the imipenem disk alone was considered positive for MBL production. DNA templates were prepared by the boiling method, and the polymerase chain reaction (PCR) amplification for bla_{IMP} was performed with the primers MIP-F (5'-GAAGGCGTTTATGTTCATAC-3') and IMP-R (5'-GTATGTTTCAAGAGTGATGC-3') for the bla_{IMP} gene under PCR conditions, as described previously, which amplifies a 587-base pair (bp) amplicon (8). The PCR purification kit (Bioneer Co., Korea) was used to purify the PCR products, and sequencing of the forward strand was performed by Bioneer Co. (Korea). The nucleotide sequences were analyzed with Chromas 1.45 and MEGA-4 software, and with BLAST on the NCBI website.

3.1. Statistical Analysis

The statistical analysis was performed with SPSS (version 19, Chicago, IL, USA).

4. Results

The demographic data are presented in Table 1. The mean age of the studied patients was 25.8 $\pm$ 16.4 years, ranging from 3 to 75 years. The mean body surface area burn (BSAB) was 35.85% $\pm$ 7.58%. Eighty-one cases out of the total of 150 enrolled patients in this study were males (54%). Fifty-seven (38%) isolates were considered MDR. Ninety-three isolates (62%) were XDR, and none were PDR. The antibiotic susceptibility of the 150 *P. aeruginosa* isolates is described in Table 2. Twenty-eight resistant phenotypes were identified, of which the predominant resistance pattern was amikacin, ciprofloxacin, imipenem, meropenem, aztreonam, cefepime, ceftazidime, tobramycin, ciprofloxacin, and piperacillin/tazobactam (45% of isolates). Of the isolates, 144 (96%) produced MBLs, and 107 (74.3%) of the MBL producers were positive for the bla_{IMP} gene. Figure 1 shows the bla_{IMP} PCR products of *P. aeruginosa* isolates from the burn patients.

5. Discussion

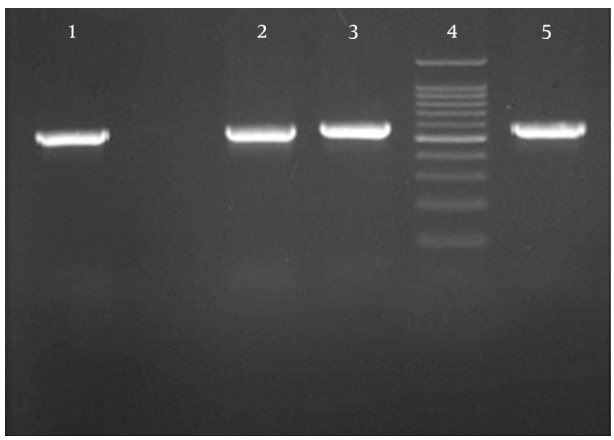
Pseudomonas aeruginosa remains the most frequent Gram-negative microorganism isolated from burn wounds, and it is complicated both to treat and to control it, due to its prolonged environmental survival time and its ability to develop resistance to multiple antimicrobial agents. *P. aeruginosa* infections mostly occur in burn

Table 1. Demographic Information for Patients with Burn Wound Infections in This Study

Characteristic	No. (%)
Age range, y	3 - 75
Mean age, years, ± SD	25.8 ± 16.4
Length of stay range, d	10 - 35
Mean length of stay, days, ± SD	12.8 - 7.33
Length of stay, days	
< 13	81 (54.2)
14 - 20	52 (34.6)
> 20	17 (11.2)
Sex	
Male	81 (54)
Female	69 (46)
BSAB	
< 25%	19 (13)
26% - 40%	104 (69)
41%	27 (8)

Abbreviaion: BSAB, body surface area burn.

Figure 1. *bla*$_{IMP}$ PCR Products of *P. aeruginosa* Isolates from Burn Patients



Lane 1, the positive control; lanes 2, 3 and 5, Isolates were positive for *bla*$_{IMP}$ (587-bp amplicons); lane 4, Size standards for the 100 bp ladder.

patients with various risk factors, such as carbapenem use, broad-spectrum antibiotic use, extended length of hospitalization, the presence of imipenem-resistant *P. aeruginosa* in the unit, and a previous history of antibiotic therapy, chiefly with broad-spectrum types, such as third-generation cephalosporins and carbapenems (6). A high rate of antibiotic resistance has been seen with *P. aeruginosa* wound infections in burn patients, which causes difficulties with empiric therapy (1, 9, 10). This study showed high resistance rates to the most clinically relevant antibiotics for the treatment of infections caused by *P. aeruginosa*, with the exception of polymyxin B and colistin, which may be used as the final alternatives for the management of infections caused by this bacterium. In this study, the high resistance rate of *P. aeruginosa* against carbapenems may be the result of excessive and inappropriate use of these antibiotics in our hospital. β-lactams, aminoglycosides, and quinolones are the antibiotics usually used in hospitals for the prevention and treatment of *P. aeruginosa* infections, but their unreasonable use is related to the selection and spread of strains resistant to them. *P. aeruginosa* strains isolated from burn patients hospitalized in a major burn center in Tehran, Iran, showed that 89% were resistant to ticarcillin-clavulanate, 76% to imipenem and gentamicin, and 20% to meropenem (10). Resistance of *P. aeruginosa* to antibiotics is the result of the production of enzymes that inactivate and degrade antibiotics, reducing the membrane permeability and the efflux system (11). The use of imipenem as the first choice of treatment for MDR *P. aeruginosa* in this unit provides a possible explanation for the presence of increasing imipenem-resistant and meropenem-resistant isolates. It is likely that piperacillin/tazobactam is the most efficient drug because it is infrequently used. The results showed that *bla*$_{IMP}$ was significantly correlated with aztreonam resistance in *P. aeruginosa* infections. The production of the IMP-1 enzyme, encoded by the transferable MBL gene, *bla*$_{IMP}$, was first isolated in *P. aeruginosa* in Japan (12). It is important to note that the *bla*$_{IMP}$ gene can be transmitted between gram-negative bacteria, so the increase of MBLs is a risk in antimicrobial therapy with β-lactam antibiotics and carbapenems (11). When carbapenems are used as single agents against originally susceptible isolates of *P. aeruginosa*, resistance may emerge during therapy. The combination of tazobactam with piperacillin results in an enhanced spectrum of activity against many, but not all, organisms containing plasmid-mediated β-lactamases. Aztreonam is a monocyclic β-lactam with good in vitro activity against *P. aeruginosa* infections, but when used alone, resistance may appear. MBL-producing *P. aeruginosa* strains have now emerged in our burn patients. MBLs have also been reported in other parts of Asia, Europe, North America, South America, Australia, Japan, and Iran (13-22). The prevalence of *bla*$_{IMP}$ genes in MBL-producing *P. aeruginosa* isolates from burn wounds in Tehran has been reported at 56.25% (23).

Carbapenem-resistant *P. aeruginosa* is usually resistant to all β-lactams and fluoroquinolones. The intrinsic resistance of these organisms further limits antibiotic selections. Polymyxins are the basis of treatment for

Table 2. Frequency of Resistant Rates to Antimicrobial Agents for 150 *P. aeruginosa* Isolates from Burn Patients[a]

Antibiotic agent	Resistance Pattern		
	R	I	S
Piperacillin/tazobactam, 100/10 μg	106 (70.7)	2 (1.3)	42 (28)
Aztreonam, 30 μg	130 (86)	3 (2)	18 (12)
Cefepime, 30 μg	139 (92.7)	0	11 (7.3)
Ceftazidime, 30 μg	143 (95.3)	0	7 (4.7)
Amikacin, 30 μg	144 (96)	0	6 (4)
Imipenem, 10 μg	144 (96)	0	6 (4)
Meropenem, 10 μg	144 (96)	0	6 (4)
Tobramycin, 10 μg	145 (96.7)	0	5 (3.3)
Ciprofloxacin, 5 μg	145 (96.7)	0	5 (3.3)
Colistin, 10 μg	0	0	150 (100)
Polymyxin B, 300 units	0	0	150 (100)

[a] Data are expressed as No. (%)
Abbreviaion: I, intermediate;R, resistant; S, susceptible.

carbapenem-resistant *P. aeruginosa*, and are usually used in combination with other agents. Burn patients infected with carbapenemase-producing *P. aeruginosa* should be placed on contact-safety measures.

Pseudomonas aeruginosa skin infections that complicate burn injuries are significant infections linked to a very high mortality rate, in spite of antibiotic therapy. The high rate of carbapenem-resistant *P. aeruginosa* in our burn patients complicates empiric antibiotic therapy. In conclusion, we have shown that bla_{IMP}-producing *P. aeruginosa* isolates is a rising threat in our burn care unit, and they should be controlled by conducting infection-control assessments in parallel with the development and implementation of control measures.

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Footnote

Authors' Contribution: Rezvan Moniri and Ahmad Khorshidi were responsible for the study conception and design. Mohsen Radan, Zohreh Norouzi, and Fahimeh Beigi performed data collection. Rezvan Moniri prepared the first draft of the manuscript. Hamidreza Gilasi and Rezvan Moniri performed the data analysis, made critical revisions to the paper for important intellectual content, and supervised the study. Yasaman Dasteh Goli edited the paper.

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