Original Article

Distribution of the Strains of Multidrug-resistant, Extensively Drug-resistant, and Pandrug-resistant *Pseudomonas aeruginosa* Isolates from Burn Patients

Abstract

Background: *Pseudomonas aeruginosa* is an opportunistic and Gram-negative pathogen that is used as the most important factor in burn wound infections, and due to the rapid acquisition of multidrug resistance (MDR), it causes high mortality rates in these sectors. Thus, diagnosis and assessment of antibiotic resistance patterns are very important in these patients. The aim of this study was to evaluate antibiotic resistance pattern and determining *P. aeruginosa* MDR. **Materials and Methods:** In this study, phenotypic, biochemical, and polymerase chain reaction tests were used to identify *P. aeruginosa* from 120 wound burn samples that 96 samples were detected to *P. aeruginosa* species. In the next step, according to the Clinical and Laboratory Standard Institute standard guidelines, antibiogram test was performed by disk diffusion method for amikacin, ciprofloxacin, norfloxacin, gentamicin, cefepime, aztreonam, meropenem, colistin, ceftazidime, and piperacillin-tazobactam antibiotics. Antibiotic data were analyzed by WHONET software; finally, the rate of antibiotic resistance and MDR strains was determined. **Results:** The highest antibiotic resistance belonged to amikacin (94.8%) and norfloxacin (90.6%); in contrast, colistin (8.3%) had the lowest and the MDR strains were MDR (95.8%) and extensively drug resistance (XDR) (87.5%). **Conclusion:** In this study, there was MDR with an alarming rate including MDR (95.8%), XDR (87.5%), and pan-drug resistance (0%). As a result, given antibiotics to patients should be controlled by the antibiogram results to avoid increasing MDR strains.

Keywords: *Antibiotic resistance, burn, Iran, multidrug resistant, Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa is an opportunistic and Gram-negative bacteria found in various environments and hosts such as water, soil, plants, animal, and human beings.[1,2] It has a very large genome that causes more complex features than other bacteria.[3] Because of high versatility with the host and different virulence factors such as exotoxin, elastase, rhamnolipid, pyocyanin, pyochelin, and lectin. *P. aeruginosa* is considered as one of the most dangerous bacteria in the world.[4-6] The bacteria are colonized in the human body in wet areas such as anus, armpits, ears, nose, and throat mucosa. The prevalence of colonization in healthy people is very low, but it is increased in hospitalized patients, especially those treated with broad-spectrum antibiotics.[3,7] Nowadays, nosocomial infections and subsequent antibiotic resistance are one of the serious problems at a global level, so that each year, a large number of patients involved with these infections and lose their lives.[1,8] *P. aeruginosa* is one of the most common bacteria in nosocomial infections, especially in burn units. Burn patients, because of losing the skin barrier, are very vulnerable to infection.[7,9] It can be transmitted through the flora contamination be in touch with different surfaces in hospitals, such as equipment, disinfectant solutions, nurse's hands, and may spread among other patients.[10,11] The ability to use multiple mechanisms, including decreased outer membrane permeability, expression of efflux pump, produces antibiotic degradative enzymes, alginate production and transfer of resistance genes, the bacteria has enabled to show a high level of resistance to the most used antibiotics.[2,12] Multidrug resistance (MDR) to antibiotics against *Pseudomonas* is a common and growing problem in most hospitals as

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P. aeruginosa of MDR is responsible for 4%–60% of nosocomial infections in different parts of the world.[13,14] The use of broad-spectrum antibiotics such as ceftazidime in the burn ward and Intensive Care Units (ICUs) by creating a selective pressure on bacteria likely increases emergence of multidrug-resistant strains including MDR, extensively drug-resistance (XDR), and even pan-drug resistance (PDR). The presence of these strains in the burn wards has become a key issue in infection control. Due to increasing resistance to antibiotics, treatment of these infections has become constantly more complicated and difficult and will follow problems such as increased illness, higher mortality rate, and economic impacts. Hence, for proper treatment, detection of MDR strains is essential.[13-16] With regard to this issue, study of antibiotic resistance and trying to control this problem by new methods of treatment is very important. In this study, we tried to survey the resistance of *P. aeruginosa* isolated from burn unit and to determine MDR patterns of bacteria.

Materials and Methods

This study was conducted on 120 wounds' samples belong to burn patients with suspected colonies of *P. aeruginosa* collected from the Microbiology Laboratory between January 2015 and June 2015 at Imam Musa Kazim Burn Hospital of Isfahan, Iran. The first step was the use of diagnostic tests including Gram-staining, oxidase, triple sugar iron, sulfide-indole motility, oxidative fermentative (OF), gelatinase, specific medium cetrimide, and incubation at 42°C. Polymerase chain reaction (PCR) of toxA was done with specific primer for the confirmation of phenotypic detection. ToxA primer was used in the experiment consists of 5-CGACCTCTGGAACGAATGC-3 and 5-AGCAGGCACAACACCTTGC-3.[17]

Preparation of genomic DNA

For DNA extraction, two or three colonies of fresh culture of *P. aeruginosa* were dissolved in 300 ml of lysis buffer containing (Tris 100 mmol, Nacl 50 mmol, and EDTA 25 mmol, pH = 7.5) completely. Subsequently, suspension was boiled at 95°C for 10 min. Equal volumes of phenol and chloroform (25:24, pH = 7.5) were added, mixed thoroughly, and centrifuged at 9000 g for 5 min. Aqueous–viscous supernatant was transformed to a fresh microtube; phenol/chloroform (25:24) was added again and centrifuged at 9000 g for 5 min. To DNA precipitation, 600 µl of cold pure ethanol (Merck, Germany) was added and centrifuged at 13,000 g (4°C, 20 min). Obtained DNA after washing with 70% ethanol was stored at –20°C.

ToxA amplifications

PCR for toxA (396 bp) amplification includes the following:

Each reaction was made in 20 µl volume to perform PCR which, respectively, include 10 µl the commercial master mix (containing Taq DNA polymerase, dNTPs, and $MgCl_2$) (Ampliqon Danmark), 1 µl of each primer (Metabion, Germany), 2 µl DNA sample, and 6 µl distilled water that was added to 0.2 microtube. Amplification was performed for 35 cycles as: initial denaturation at 94°C for 4 min, denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 5 min. PCR products were visualized on 1% agarose gel stained with DNA green viewer dye [Figure 1]. In this experiment, the standard strains of *P. aeruginosa* ATCC27853 as a positive control and sterile water were used to control blank. Antibiotic susceptibility of the isolates was ascertained by Kirby-Bauer disk diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines. For antibiogram stages, first to obtain a single colony, samples were cultured on blood agar by streak plate method. In the next step, a freshly prepared bacterial suspension adjusted to 0.5 McFarland unit (1.5 × 107 cells) was streaked for confluent growth on Mueller-Hinton agar plate using a swab, and discs were conducted to a distance of 2 cm from each antibiotic. In this study, following antibiotic disks were used: ciprofloxacin (5 µg), amikacin (30 µg), norfloxacin (10 µg), gentamicin (10 µg), cefepime (30 µg), aztreonam (30 µg), meropenem (10 µg), ceftazidime (30 µg), piperacillin-tazobactam, and colistin (10 µg). The mentioned antibiotics were provided from the two companies of Padtan Teb, Iran, and Mast, England.

Enterococcus faecalis ATCC29212 using trimetoprima sulfametoxazol disk was used to control the quality of Mueller-Hinton agar, and *P. aeruginosa* ATCC27853 was used for antibiotic discs. The obtained results indicated the good qualities of medium and antibiotic discs. Comparing the Iranian and foreign discs showed very little difference. However, the results of antibiotic resistance were extracted according to data from quality control. After incubation

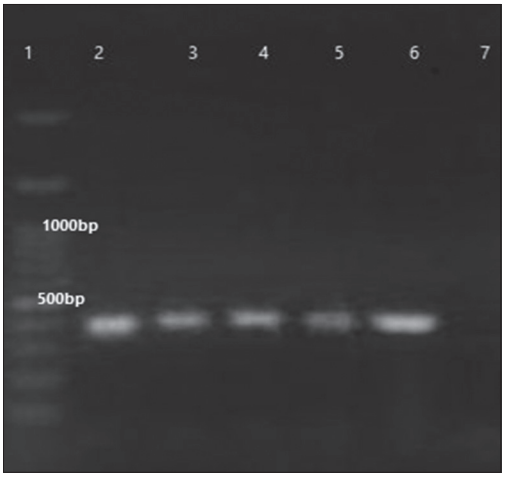

Figure 1: Gel image of representative polymerase chain reaction of toxA gene. Line 1 ladder (1000 bp), line 2–5 clinical specimens, line 6 positive control, line 7 negative

at 37°C for 18–24 h, the diameter around each disk was measured using the ruler and the results were analyzed by WHONET software. Determining the level of antibiotic resistance, MDR strains were found. According to the CLSI, strains are resistant to antibiotic if at least a factor of 3 or more different classes were MDR. Resistance to a factor of all classes except one or two class defined as XDR and strains that were resistant to all antibiotic classes as PDR.

Results

The patients were including 3rd degree of burning. Of 96 samples that were detected as *P. aeruginosa*, there was more resistant to amikacin (%94.8) and norfloxacin (90.6%) and the least resistance was related to colistin (8.3%). Resistance to other discs contained ciprofloxacin 89.6%, aztreonam 87.5%, meropenem 88.5%, ceftazidime 60.4%, gentamicin %82.3, cefepime 80.2%, and piperacillin-tazobactam 80.2% [Figure 2].

According to the survey of MDR, 95.8% of samples were MDR, 87.5% were XDR, and no PDR were detected [Table 1].

Discussion

Staphylococcus aureus, *Escherichia coli*, *P. aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* are the most common bacteria involved in nosocomial infections. Of these, *P. aeruginosa* is very important in burn ward.[11,12,18] In many reports of burn, this bacteria is considered as the most common cause of hospital infections.[19-22] One of the main problems associated with *P. aeruginosa* is rapid acquisition of MDR, which leads to high morbidity and mortality and treatment complexity, especially in burn centers.[10] Several

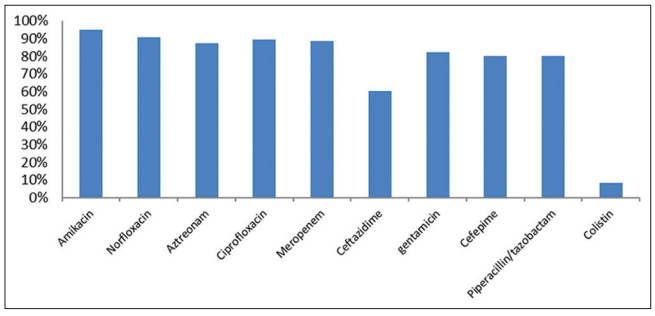

Figure 2: Antibiotic resistance

Table 1: Multidrug resistance (total sample=96)

Sample type	Samples' number/total (%)	Number of samples
Susceptible strains	4.2	4
Multidrug-resistant	95.8	92
Extensively drug-resistant	87.5	84
Pandrug-resistant	0	0

mechanisms have been known for antibiotic resistance, for example, production of enzymes cephalosporinase (due to the presence of AmpC gene), production of beta-lactamase enzymes, reducing the permeability of the outer membranepurine reduction (OprD), synthesis of enzymes such as phosphorylation transferase and acetyl transferase (resistance to aminoglycosides), changes in topoisomerase II and IV (quinolone-resistance) and efflux pumps.[16,23,24] Today, the MDR strains of *P. aeruginosa* are increasing around the world. More than 10% of *P. aeruginosa* strains in worldwide are MDR.[8,25,26] Several studies were conducted in connection with MDR in Iran which for example can be mentioned as the following. In a study by Mirsalehian *et al.*, in 2007 (Tehran), 87.05% of the cases were MDR.[27] In addition, in the studies by Japoni *et al.* from Southern Iran and Ranjbar *et al*. from Tehran, 73% and 100% of MDR have been reported, respectively.[10,28] In our study, 95.8% of the isolates were MDR that 87.5% of them identified as XDR. It can be concluded from comparing the studies in Iran with other countries such as Korea, Italy, Greece, Poland, Egypt, Turkey, Pakistan, India, Bulgaria, and Spain that the rate of MDR in different parts of the world is higher than Iran.[14,25,27,29-36] For example, MDR has been reported in Korea as 50%, Turkey 60%, Egypt 36%, Pakistan 29.24%, Bulgaria 49.8%, Spain 70%, and India 36.2%, which is different from our study and some other reports in Iran. These statistics clearly indicate that the range of effective antibiotics in the treatment of *P. aeruginosa* in burn infections is being extremely limited in Iran and particularly in Isfahan that showed the high rates of MDR. For example, Fazli *et al*. in 2008 examined the samples of respiratory tract, cerebrospinal fluid, stool, burn wounds, etc., that were collected from five treatment hospitals in Isfahan. The rate of MDR was reported as 71.54% in the all samples and it was 100% in burn wounds.[37] These results were similar to Rahimzadeh *et al*.'s study in 2012 on four treatment hospitals in Isfahan. In this study, MDR of burn wounds strains was 100%.[38] Golshani *et al*. in 2013 collected and investigated the clinical samples including urine, respiratory, blood, wound infection, swabs, and nasal mucosa from various hospitals of the province. In this study, 63% of all cases were MDR.[39] Comparing the mentioned statistics with MDR, rate of 95.8% in our study revealed a high level of MDR in *P. aeruginosa* in the most hospitals of Isfahan, with a very higher rate in the burn wound strains. Fortunately, in our study, we did not find PDR strains; however, in the study of Fazeli *et al*., samples related to the ICU were examined which 50% of strains were PDR resistant. The reason for this was overuse of broad-spectrum antibiotics.[40] This can be a great alarm to burn unit strains of Imam Musa Kazim Hospital to become PDR strains. Hence, to avoid this, applying strict monitoring for the use of antibiotics is required. In a number of previous studies, the treatment of MDR strains recommended by carbapenems and aztreonam; however,

in our study, we faced with 90% resistance to meropenem and aztreonam. Resistance to meropenem was reported in various studies, such as Altoparlak *et al*. from Turkey as 32.5%, Rahimi (Arak) 35%, Mirsalehian (Tehran) 66%, and Fazeli (Isfahan) 62.1%. Resistance to aztreonam has also been shown in two recent studies as 90% and 69.7%, respectively.[40-43] Detection of these two antibiotics resistance and its comparison with our study indicates an increase in resistance to meropenem and high levels resistance to aztreonam. Hence, it seems that the use of these antibiotics in a burn unit of Imam Musa Kazim Hospital was not effective anymore. Among the examples of XDR, colistin was the only antibiotic that the majority of the samples were sensitive to it. Nevertheless, 8.3% of the samples were resistant to this antibiotic. In a study of Adabi *et al*. from Tehran which was conducted in 2015, resistance to colistin was 0%.[44] In another study by Kumar and Srivastava in 2014 on *P. aeruginosa* samples isolated from the patients with the age of 10–70 years, resistance to colistin was reported 3%.[45] We have observed an increased resistance to colistin by examining the data. Of course, evaluating more samples can be helpful in confirming this topic. In the present study, colistin was the most effective antibiotic which can be used in burn wounds to fight infection of *P. aeruginosa*; however, unfortunately, the increasing resistance can expand concerns about the treatment of this disease. It should be noted that susceptibility in burn samples was significantly different from other studies that have been conducted on the samples from a nonburn unit. For example, in the study of Rahimi *et al*. (2012), *P. aeruginosa* was isolated from different parts including urine, sputum, and wound. Susceptibility pattern of the samples was shown for gentamicin 14%, ceftazidime 23%, ciprofloxacin 15%, meropenem 35%, and amikacin 9%.[42] In addition, the study of Shawar *et al.* was performed on samples of patients with cystic fibrosis. Resistance was determined to amikacin 13.1%, gentamicin 19.3%, aztreonam 11.9%, ceftazidime 11.1%, and ciprofloxacin 20.7%.[46] By comparing the pattern of antibiotic resistance in the mentioned studies and our study, we can conclude that resistance in burn unit is much higher than the others. In addition, there is concern that the transition to the other sides of the burn units causes the spread of resistance in these sectors. Although, in these studies, there was found no PDR strains, resistance rate of 87.5% for XDR suggests that the emergence of PDR strains will be very likely in future, so dramatic increase in antibiotic resistance of *P. aeruginosa* in this study such as 8.3% resistance to colistin and 95.8% of MDR should be taken as a serious warning. Therefore, measures such as continuous sterilization of hospital surfaces, increasing awareness of health-care workers, antibiogram tests before prescribing medication, and the use of new diagnostic and therapeutic methods for screening patients are recommended with a major emphasis.

Conclusion

In this study, there was MDR with an alarming rate including MDR (95.8%), XDR (87.5%), and pan-drug resistance (0%). As a result, given antibiotics to patients should be controlled by the antibiogram results to avoid increasing MDR strains.

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Conflicts of interest

There are no conflicts of interest.

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