Antibiotic Resistance Properties of *Pseudomonas aeruginosa* Isolated From Cases of Superficial Infections at the Emergency Unit

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Abstract

**Background:** *Pseudomonas aeruginosa*, a ubiquitous opportunistic pathogen, is one of the major causative agents of human superficial infections. Infections due to these bacteria are difficult to heal and cause serious economic issues.

**Objectives:** The present study was carried out to investigate the antibiotic resistance pattern of *P. aeruginosa* isolated from cases of superficial infections referred to the emergency health care units of Iranian Hospitals.

**Materials and Methods:** Three hundred swab samples were collected from patients with superficial infections. Samples were cultured and those that were *P. aeruginosa* positive were analyzed by the disk diffusion method.

**Results:** One hundred and seventy-two out of 300 swab samples (57.3%) were positive for *P. aeruginosa*. The results of the culture technique were also confirmed using the polymerase chain reaction (PCR). Females had a higher prevalence of *P. aeruginosa* than males, patients older than 70 years were the most infected age group and finally burn infections had the highest prevalence of bacteria.

*P. aeruginosa* strains had the highest levels of resistance against ampicillin (93%), gentamycin (89.5%), ciprofloxacin (82.5%) and amikacin (77.3%). The most effective drugs were meropenem (2.3%), imipenem (2.9%), polymyxin B (21.5%) and cotrimoxazole (31.9%).

**Conclusions:** It is logical to primarily prescribe meropenem, imipenem, polymyxin B and cotrimoxazole in the cases of superficial infections caused by *P. aeruginosa*. Medical practitioners should be aware of the presence of such levels of antibiotic resistance in cases of superficial infections in Iran.

**Keywords:** Antibiotic Resistance, Superficial Infection, Emergency Health Care Units, Iran, *Pseudomonas aeruginosa*

1. Background

Superficial infections such as burns, wounds, and post-surgical site infections are important causes of emergency health care-associated problems all around the world. Superficial infections cause longer hospital stays, more expensive hospitalizations and increased mortality (1). The annual superficial infection care products market is projected to reach $15.3 billion by 2010 (1). *Pseudomonas aeruginosa* is a non-fermentative, aerobic, gram-negative rod shape bacteria, which substantially contribute to wound-related morbidity and mortality worldwide. They are widely distributed, mostly in hospital environments and are one of the most important agents of hospital-acquired superficial infections, ecthyma gangrenosum and black necrotic lesions (2, 3). Superficial infections caused by *P. aeruginosa* are one of the most prevalent causes of hospitalization and emergency health care references all around the world (2-6).

Treatment of superficial infections caused by *P. aeruginosa* often requires antibiotic therapy yet the levels of antibiotic resistance in the rough strains of these bacteria have increased over time (7-11). Therefore, it is essential to study the levels of antibiotic resistance in the *P. aeruginosa* isolates of each region and even each hospital.

In the recent years, the growing incidence of *P. aeruginosa* has been of particular concern. The incidence of *P. aeruginosa* in superficial and wound infections is becoming more serious in developing countries like Iran (12, 13). This issue is of higher importance for females and elders, due to their relatively lower levels of immune system.

2. Objectives

The present study was carried out in order to study the antibiotic resistance pattern of *P. aeruginosa* isolated from cases of superficial infections referred to the emergency health care units of Iranian Hospitals.
3. Materials and Methods

3.1. Ethical Considerations

Ethical committees of the educational hospitals approved the general principles and framework of the present investigation. Written informed consent was obtained from all of the study patients or their parents. Personal information of all patients remained confidential.

3.2. Sample Collection

From June 2014 to October 2015, a total of 300 swab samples were taken from patients with superficial infections referred to the emergency health care units of Iranian hospitals. Swab samples were taken from various types of superficial infections including wound (n = 110), burn (n = 90) and post-surgical site (n = 100) infections. Personal information like age and gender were recorded for each sample and all samples were transferred to the laboratory in a cooler with an ice pack.

3.3. Pseudomonas aeruginosa Isolation

Swab samples were inoculated on blood, MacConkey (Merck, Germany) and nutrient agar (Merck, Germany) and incubated at 37°C for 24 hours. Colonies that produced pyoverdin, pyocyanin and pyorubin pigments were transferred to nutrient agar and subcultured more than one time to obtain pure cultures. The isolates were identified using conventional biochemical tests such as motility, oxidase, catalase, citrate utilization, gelatinase, alkaline protease production, triple sugar iron agar, oxidative-fermentative, indole, lecithinase production and hemolysin production.

3.4. Antimicrobial Susceptibility Testing of P. aeruginosa Isolates

Pattern of antimicrobial susceptibility was studied using the simple disk diffusion technique. The Mueller-Hinton agar (Merck, Germany) medium was used for this purpose. Antibiotic susceptibility of P. aeruginosa strains against 12 commonly used antibiotics, including norfloxacin (30 µg/disk), cefotaxime (30 µg/disk), ampicillin (10 µg/disk), imipenem (30 µg/disk), cefotaxime (30 µg/disk), chloramphenicol (30 µg/disk), amikacin (30 µg/disk), gentamycin (10 µg/disk), ceftazidime (30 µg/disk) and aztreonam (30 µg/disk), antibiotic agents (Oxoid, UK) was analyzed using the disk diffusion method. Pattern of antibiotic susceptibility was examined according to the Clinical Laboratory Standards Institute protocol (CLSI) (14). P. aeruginosa ATCC 27853 was used as a quality control in all reactions.

3.5. DNA Extraction From the P. aeruginosa Isolates

Total genomic DNA was extracted from the bacterial colonies. A single colony was inoculated in 5 mL of brain heart infusion broth and incubated over night at 37°C. Then 1.5 mL of a saturated culture was harvested with centrifugation for five minutes at 14,000 rpm. The cell pellet was resuspended and lyzed in 200 µL of lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium acetate, 1 mM EDTA, 1% SDS) by vigorous pipetting. To remove most proteins and cell debris, 66 µL of 5M NaCl solution was added to the lysate, mixed well, and then the viscous mixture was centrifuged at 12,000 rpm for 10 minutes at 4°C. After transferring the clear supernatant to a new eppendorf tube, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Following centrifugation at 14,000 rpm for five minutes, the supernatant that was transferred to another eppendorf tube and equal volume of 100% ethanol was added. The tubes were gently inverted five to six times, then centrifuged at 10000 rpm for five minutes. The supernatant was discarded and 1 mL of 96% ethanol (70%) was added to the pellet, andubes were centrifuged at 10000 rpm for five minutes. Finally, the supernatant was discarded and the pellet was dried for 10 minutes at room temperature and was resuspended in 100 µL H2O. The stock was kept at -20°C until use. The DNA concentration was determined by measuring absorbance of the sample at 260 nm, using a spectrophotometer (15).

3.6. Polymerase Chain Reaction Amplification For Confirmation of P. aeruginosa

Genomic DNA extracted from the bacterial colonies was confirmed to be P. aeruginosa using the PCR technique. The PCR mixture contained 200 µM of each dNTP (Fermentas, Germany), PCR buffer (10 mM Tris/HCl, 50 mM KCl, 1.5 mM MgCl2, pH 8.3), DMSO at a final concentration of 4%, 12.5 pmol of each primer (F: 5'- GGCGGATCTTCG-GACCTCA -3' and R: 5'- TCCTTAGAGTGCCCACCG -3', 956 bp) (16), 1 U Taq DNA polymerase (Fermentas, Germany) and 25 ng DNA template. The DNA was amplified in a programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) PCR device using the following protocol: 94°C for one minute, 30 cycles of 94°C for 35 seconds, 58°C for 60 seconds, 72°C for 60 seconds, and 72°C for five minutes. P. aeruginosa ATCC 27853 were used as positive controls and distilled water (D. W, Merck, Germany) was used as a negative control in all PCR reactions.

3.7. Agarose Gel Electrophoresis

Fifteen microliters of PCR products were resolved on a 1.5% agarose gel containing 0.5 mg/mL of SYBR Green in trisborate EDTA buffer at 90 V for 40 minutes, also using suitable molecular weight markers. The products were examined under ultraviolet illumination.

3.8. Statistical Analysis

The results were transferred to a microsoft excel spread-
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sheet (Microsoft Corp., Redmond, WA) for analysis. Statistical analysis was performed using the SPSS/16.0 software (SPSS Inc., Chicago, IL) for significant relationships between incidences of antibiotic resistance of *P. aeruginosa* isolated from the samples of superficial infections. The chi-square test and Fisher’s exact 2-tailed test analysis were performed in this study. Statistical significance was regarded at a *P* < 0.05.

### 4. Results

The present investigation was carried out to study the prevalence of antibiotic resistance of *P. aeruginosa* isolated from various types of superficial infections. Table 1 shows the total distribution of *P. aeruginosa* in the swab samples taken from various types of superficial infections. Of the 300 studied swabs, 172 (57.3) samples were found to be contaminated with *P. aeruginosa*. The results of the culture technique were also confirmed using the PCR method (Figure 1). Swab samples, which were taken from female cases (64.2%), patients older than 70 years (68.5%) and cases of burn infections (66.6%), had the highest prevalence of *P. aeruginosa*. Statistically significant differences were seen in the prevalence of *P. aeruginosa* between male and female cases (*P* = 0.039), younger than 10-years-old and older than 70-years-old patients (*P* = 0.016) and cases of burn infections and wound infections (*P* = 0.041).

Table 2 shows the antibiotic resistance pattern of *P. aeruginosa* isolated from various types of superficial infections. We found that the *P. aeruginosa* strains of superficial infections harbored the highest levels of resistance against ampicillin (93%), gentamycin (89.5%), ciprofloxacin (82.5%) and amikacin (77.3%), and also the lowest levels of resistance against meropenem (2.3%), imipenem (2.9%), polymyxin B (21.5%) and cotrimoxazole (31.9%). *P. aeruginosa* strains of males had a higher prevalence of antibiotic resistance than females (*P* = 0.026). Statistically significant differences were seen between the type of infection and prevalence of antibiotic resistance (*P* = 0.044), and also between the age of patients and prevalence of antibiotic resistance (*P* = 0.032).

Table 1. Total Distribution of *P. aeruginosa* in Swab Samples Taken From Various Types of Superficial Infections

| Different Criteria      | No Samples | *P. aeruginosa*<sup>a</sup> |
|------------------------|------------|-----------------------------|
| Gender                 |            |                             |
| Male                   | 160        | 82 (51.2)                   |
| Female                 | 140        | 90 (64.2)                   |
| Age, y                 |            |                             |
| <10                    | 40         | 25 (62.5)                   |
| 10-30                  | 60         | 28 (46.6)                   |
| 30-50                  | 60         | 31 (51.6)                   |
| >50                    | 70         | 40 (57.1)                   |
| >70                    | 70         | 48 (68.5)                   |
| Type of infection      |            |                             |
| Wound                  | 110        | 50 (45.4)                   |
| Burn                   | 90         | 60 (66.6)                   |
| Post-surgical site     | 100        | 62 (62)                     |
| Total                  | 300        | 172 (57.3)                  |

<sup>a</sup>Values are expressed as No. (%).

**Figure 1.** Gel Electrophoresis for the Amplification of *P. aeruginosa* in the Swab Samples Taken From Superficial Infections

M, 100 bp ladder; 1, Positive samples (956 bp); 2, Positive control (*P. aeruginosa* ATCC 27853); 3, Negative control (distilled water (D.W, Merck, Germany)).
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5. Discussion

The results of the present study showed that *P. aeruginosa* has a higher prevalence in various types of superficial infections. Overall, 62.6% of the swab samples were positive for *P. aeruginosa*. To the best of our knowledge, this finding is the highest prevalence of *P. aeruginosa* in swab samples of superficial infections. Lower prevalence rate of *P. aeruginosa* in human superficial infections have been reported previously by Ranjan et al. (2010) (27.7%) (6) and Siguran et al. (1990) (18.8%) (17).

High prevalence of *P. aeruginosa* in the clinical samples of our study may be due to the fact that the type of samples (site of the infection) and health care management is different with those of other investigations. In fact, the presence of environmental pollution, especially in the hospital environment as well as contaminated and lack of optimal disinfection of instruments and equipment of hospitals are the main reasons for the high prevalence of *P. aeruginosa* (62%) in post-surgical site infections of our study. Low levels of healthcare management in Iranian healthcare units and hospitals have been recognized from the results of our study and the results of various previous Iranian investigations (13, 18, 19). Higher sensitivities of female skin are a reason for the higher prevalence of *P. aeruginosa* in their superficial infections. Similar results were reported by Okon et al. (2009) (20) and Mulu et al. (2012) (21). Al-Hasan et al. (2008) (22) and Khan et al. (2008) (7) reported a higher prevalence of *P. aeruginosa* clinical infections in males than females, which were different to our results. Their reason for the high prevalence of bacteria in males is that they are more in contact with the polluted outside home environment. Also they do exhausting and hard work outside the home. Therefore, they are more prone to get superficial infections.

Aging, decrease in the levels of keratin skin cells and reduction in the level of immunity are reasonable factors for the higher prevalence of *P. aeruginosa* in older than 70-years-old patients. High prevalence of *P. aeruginosa* in old patients has been reported previously (23-25). In spite of the results of a previous investigation, which showed a high prevalence of *P. aeruginosa* in children (26), the results of our study showed that less than ten years old patients had a lower prevalence of bacteria. One possible explanation for this finding is that the age range of younger than ten-year-old patients of our study was eight to ten years. On the other hand, there were no younger than eight-year-old pediatrics in our study population.

Our study also focused on the prevalence of antibiotic resistance in *P. aeruginosa* strains of superficial infec-
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Footnote

Authors’ Contribution: Koorkosh Ahmadi and Amir Masoud Hashemian contributed to critically revising the manuscript for important intellectual content and final approval of the version to be published. Seyyed Mohsen Pouryaghobi and Reza Akhavan contributed to the conception of the work and the acquisition of data. Sara Rozmna and Ehsan Bolvardi contributed to the design and drafting of the work.

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