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 main 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 burn, wound 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* are 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 pyruran 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 liquefaction, urease production, nitrate reduction, 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 resistance was studied using the simple disk diffusion technique. The Mueller-Hinton agar (Merck, Germany) medium was used for this purpose. Antibiotic resistance of P. aeruginosa strains against 12 commonly used antibiotics, including norfloxacin (30 μg/disk), ampicillin (10 μg/disk), imipenem (30 μg/disk), gentamycin (10 μg/disk), ciprofloxacin (5 μg/disk), cefepime (30 μg/disk), cotrimoxazole (30 μg/disk), polymyxin B (300 μg/disk), meropenem (10 μg/disk), amikacin (30 μg/disk), cefozadime (30 μg/disk) and aztreonam (30 μg/disk) antibiotic agents (Oxoid, UK) was analyzed using the Clinical Laboratory Standard Institute protocol (CLSI) (14). P. aeruginosa ATCC 27853 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 1%, 1 U Taq DNA polymerase (Fermentas, Germany), PCR buffer (10 mM Tris/HCl, 50 mM KCl, 1.5 mM MgCl2, pH 8.3), DMSO at a final concentration 1%, 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) PC 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 then 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.

3.6. 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'-GGGGATCTTCTG-GACCTCA -3' and R: 5'-TCTTCTTTGTGCCCACCCG -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) PC 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 then 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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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 the Swab Samples Taken From Various Types of Superficial Infections

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

<sup>a</sup>Values are expressed as No. (%).
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%) (2), Bhattacharjee et al. (2006) (32%) (4), Oguntibeju and Nwobu (2004) (33.2%) (5), Masaadeh and Jaran (2009) (27.78%) (6) and Siguan et al. (1990) (18.8%) (17).

High prevalence of *P. aeruginosa* in the clinical samples of our study maybe due to the fact that the type of samples (swabs samples of the site of infection) and health care managements 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 despite 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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Table 2. Antibiotic Resistance Pattern of *P. aeruginosa* Isolated From Patients Referred to the Emergency Units of Several Iranian Hospitals

<table>
<thead>
<tr>
<th>Different Criteria</th>
<th>Number of Positive</th>
<th>Antibiotic Resistance Pattern a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nor</td>
<td>AMP</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>82</td>
<td>35 (42.6)</td>
</tr>
<tr>
<td>Female</td>
<td>90</td>
<td>29 (32.2)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>25</td>
<td>4 (16)</td>
</tr>
<tr>
<td>10-30</td>
<td>28</td>
<td>9 (32.1)</td>
</tr>
<tr>
<td>30-50</td>
<td>31</td>
<td>12 (38.7)</td>
</tr>
<tr>
<td>50-70</td>
<td>40</td>
<td>17 (42.5)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>48</td>
<td>22 (45.8)</td>
</tr>
<tr>
<td>Type of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound</td>
<td>50</td>
<td>18 (36)</td>
</tr>
<tr>
<td>Burn</td>
<td>60</td>
<td>25 (41.6)</td>
</tr>
<tr>
<td>Post-surgical site</td>
<td>62</td>
<td>21 (33.8)</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>64 (37.2)</td>
</tr>
</tbody>
</table>

Abbreviations: AMP, ampicillin (10 µg/disk); AMK, amikacin (30 µg/disk); Azt, aztreonam (30 µg/disk); Cef, cefepime (30 µg/disk); Cfta, ceftazidime (30 µg/disk); CIP, ciprofloxacin (5 µg/disk); Cotr, cotrimoxazole (30 µg/disk); Gen, gentamycin (10 µg/disk); IMP, imipenem (30 U/disk); Merop, meropenem (10 µg/disk); Nor, norfloxacin (30 µg/disk); Pol B, polymyxin B (300 U/disk).

aValues are expressed as No. (%).
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We found that *P. aeruginosa* isolates had the highest levels of resistance against ampicillin (93%), gentamicin (89.5%), ciprofloxacin (82.5%) and amikacin (77.3%). In a study conducted in Nepal (27), there was no resistance against ampicillin, gentamicin, norfloxacin and ofloxacin. The prevalence of resistance against ceftriaxone, cephalaxin, ciprofloxacin and cotrimoxazole were 50%, 100%, 50% and 100%, respectively. An Indian investigation revealed that the *P. aeruginosa* isolates of wound swab samples harbored the highest levels of resistance against tobramycin (66.3%), ciprofloxacin (87.93%), ceftazidime (73.27%), cefixime (84.48%), gentamycin (78.44%), amikacin (21.55%) and ofloxacin (87.93%), which was similar to our findings. In a study, which was conducted in Ethiopia (28), 40% of *P. aeruginosa* isolates were resistant to seven antibiotics including amoxicillin, ampicillin, ciprofloxacin, norfloxacin and gentamicin. In the cases of burn infections (29), 70% of *P. aeruginosa* isolates were positive for metallo-beta-lactamase, with high prevalence of antibiotic resistance against ceftazidime (70%), chloramphenicol (68%) and gentamicin (62.5%). A recent Iranian investigation (30) revealed that the *P. aeruginosa* strains isolated from the site of burn infections were resistant to cloxacillin (91.8%), cotrimoxazole (86%), cefazolin (83.7%), carbencillin (74.4%), piperacillin (69.9%), ceftazidime (68.8%), ciprofloxacin (66.3%), tobramycin (58.2%), amikacin (48.8%) and gentamicin (37.2%), while the most effective antibiotic was imipenem with a resistance rate of 23.3%, which was similar to our results.

Irregular and unethical antibiotic prescription and even self-treatment by strong antibiotics cause such high levels of resistance in the *P. aeruginosa* strains of our investigation. Differences in the idea of medical practitioners in antibiotic prescription cause variations in the levels of antibiotic resistance against different antibiotics. In addition, the differences in the bactericidal activities of antibiotics and also difference in difficulty in developing resistance against various antibiotics are two other reasons for differences in the levels of antibiotic resistance.

The present study was one of the most extensive prevalence reports of *P. aeruginosa* and its antibiotic resistance pattern in the burn, post-surgical site and wound infection samples of emergency health care units of Iranian Hospitals. Our results showed that resistant strains of *P. aeruginosa* had a high prevalence in patients older than 70-years-old and especially in the samples taken from the site of burn infections. In keeping with this, the prevalence of these bacteria in cases of wound and post-surgical infections and also other studied groups were considerable. Hence, judicious use of antibiotics is required by clinicians. Also, because of the variation of resistance pattern in each hospital, it is important for each region and even hospital to formulate their own antibiotic policy, according to their local resistance pattern. We recommend the initial prescription of meropenem, imipenem and polymyxin B antibiotics for treatment of the cases of superficial infections in Iran.

**Footnote**

**Authors’ Contribution:** Koorosh 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.

**References**


