Infectious diseases are an important cause of morbidity and mortality throughout life and in this regard, opportunistic pathogens play an important role. *Pseudomonas aeruginosa* is an aerobic gram-negative rod shaped bacteria that belongs to the *Pseudomonadaceae* family (1).

The rapid increase of drug resistance in clinical isolates of this opportunistic pathogen has been of worldwide concerns (2,3). *Pseudomonas aeruginosa* is an opportunistic pathogen in burned and cystic fibrosis patients all over the world, especially because of its natural resistance to many classes of antibiotics and also, for its potential virulence factors plus additional acquired resistance due to plasmids. It is also the most common gram-negative bacteria found in nosocomial infections (4-6).

Many *P. aeruginosa* infections occur after patients have been hospitalized. Several factors that account for the success of *P. aeruginosa* are as follows: it can utilize a wide range of nutrients; many strains have acquired resistance...
factors; and it has tendency to form biofilms that further protects this pathogen from antibiotics and from the host immune defense.

The bacterium's virulence depends on numerous cell-associated and extracellular factors. Cell-to-cell signaling systems control the expression and allow a coordinated cell-density-dependent production of various extracellular virulence factors. *P. aeruginosa* is naturally resistant to a wide range of antibiotics and may show additional resistance after unsuccessful treatment, particularly via modification of a porin (7).

This pathogen is intrinsically resistant to most antibiotics such as β-lactams, quinolones, chloramphenicol, tetracycline, macrolides, trimethoprim–sulfamethoxazole, and rifampin (8). Resistance in *P. aeruginosa* may be due to outer membrane modifications, production of extended-spectrum beta-lactamase and efflux pumps, which confers various levels of resistance to expanded spectrum cephalosporins, such as ceftaxime, ceftazidime, and aztreonam (9,10).

**Aims of the study:** Therefore, the aim of this study was detection and survey of antibiotic resistance patterns of *P. aeruginosa* isolated from patients in Isfahan (located in central Iran).

**Materials & Methods**

A Total of 71 samples were collected (from February to June 2012) from “Al-Zahra” and “Shariati” Hospitals in Isfahan (located in central Iran). Among them, 50 strains of *P. aeruginosa* were isolated. The sample size was calculated using the following formula:

\[ n = \frac{pqz^2}{d^2} \]

where, \( n \) = number of samples; \( pq \) = estimation of population; \( z \) = confidence level; and \( d \) = deviation of the ratios.

Information included patients' gender and age, cause of hospitalization, and origin of clinical samples, were collected. The bacteria were biochemically identified using biochemical tests including oxidation-fermentation, oxidase, catalase, green-blue pigment production, growth at 42°C, and production of colorless colonies on MacConkey agar.

Then, for further tests, the samples were kept in a rich environment, brain-heart infusion broth (BHI; Scharlau, Spain) and 30% glycerol, at -70°C. The strains were selected on the basis of their growth on MacConkey medium, which was shown by producing oxidase-positive lactose non-fermenting colonies. Antibiotic susceptibility was confirmed by Kirby-Bauer disc diffusion method on Mueller-Hinton medium, according to the Clinical Laboratory Standard Institute (CLSI) guidelines (11,12). Paper discs (HiMedia, Mumbai, India) were impregnated with the following antibiotics: oxacillin (1 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg), cefepime (30 mcg), imipenem (10 mcg), gentamicin (10 mcg), tobramycin (10 mcg), amikacin (30 mcg), and ciprofloxacin (5 mcg).

Suspension of bacteria (0.5 McFarland) was prepared and inoculated on Mueller-Hinton agar (HiMedia, Mumbai, India) plates using sterile swabs, and then antibiotic discs were placed on it. The plates were incubated overnight at 37°C for 24 hours.

The diameter of the zone of inhibition was measured and compared to that of standard strain, and the results were interpreted as sensitive, intermediate resistant, or resistant, according to the CLSI guidelines.

Quality control was performed utilizing strains from the Iranian Type Culture Collection (PTCC), *P. aeruginosa* 1074.

**Data analysis:** Susceptibility data were compared by chi-square test using SPSS software version 15 for Windows. Both susceptibility and resistance were calculated as percentages with 95% confidence intervals. The analysis was performed on the cross-tabulated values of the presence of the resistant/intermediate/susceptible isolates, according to the categories of the selected
variable. A p-value of <0.05 was considered to be statistically significant.

Results

Among the 71 samples, a total of 50 \emph{P. aeruginosa} strains were isolated from patients admitted during the study period. Bacteria that were lactose-negative, oxidase positive produced blue-green pigment on Mueller Hinton agar, lactose-negative, and colorless colonies on MacConkey agar were selected as \emph{P. aeruginosa} strains.

Twenty eight patients (56%) were men and 22 patients (44%) were women, and the mean age of all patients was 45 years. The origin of specimens is shown in table 1.

The results of the antibiotic resistance test (Kirby-Bauer disc diffusion method) are shown in table 2. As the results of this study showed, to the higest resistance rate was found for oxacillin (100%), followed by ceftriaxone (76%), amikacin (70%), cefepime (68%), ceftazidime (68%), tobramycin (62%), and gentamicin (60%). The lowest resistance rates were found for ciprofloxacin (58%), and imipenem (58%).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>Ear swab</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Trachea</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Wound</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Pus</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 2) Antibiotic resistance pattern in \emph{Pseudomonas aeruginosa} strains isolated from clinical specimens

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Semi Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>14</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>Cefepime</td>
<td>13</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>14</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>12</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>20</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>20</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Imipenem</td>
<td>19</td>
<td>38</td>
<td>2</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>16</td>
<td>32</td>
<td>3</td>
</tr>
</tbody>
</table>

Discussion

\emph{P. aeruginosa} isolates due to inherent resistance to many antibacterial agents, are associated with a higher morbidity and mortality. This resistance is due to the synergy between multi-drug efflux systems or \(\beta\)-lactamase and low outer membrane permeability (13). Also resistance may be due to the production of metallo-\(\beta\)-lactamases (MBL), which can be chromosomally encoded or plasmid mediated. The carbapenem hydrolyzing enzyme may be class B metallo-\(\beta\)-lactamases, class D oxacillinase, or class A clavulanic acid inhibitory enzymes (14). In addition, \emph{P. aeruginosa} resistance to imipenem is attributed to diminished expression of certain outer membrane proteins (15).

Maximum resistant isolates of \emph{P. aeruginosa} were isolated from urine samples. In our study, all of the isolates of \emph{P. aeruginosa} were resistant to oxacillin (100%) followed by ceftriaxone (76%), amikacin (70%), ceftazidime (68%), cefepime (68%), tobramycin (62%), gentamicin (60%), ciprofloxacin (58%), and imipenem (58%).

In our study, more than 38% of isolates were sensitive to imipenem and 58% were resistant to this antibiotic. However, in a study by Fazeli et al. (2011) in Isfahan hospitals, it was reported that among 79 isolates of \emph{P. aeruginosa}...
aeruginosa isolated from burned patients, resistance to imipenem were observed in 94.9%, ceftazidime in 100%, ciprofloxacin in 98%, and tobramycin in 95% of isolates. Therefore, in contrast to our results, the prevalence of resistance was higher in isolates of burns (20). Also, Forozesh fard et al. (2011) reported that among 11 P. aeruginosa isolates taken from cystic fibrosis patients of Al-zahra hospital, all isolates were susceptible to imipenem, ticarcillin, ciprofloxacin and piperacillin. The lowest scale of susceptibility belonged to ceftazidime (72.2%) followed by tobramycin (45.4%) (22). It seems that the prevalence of resistance genes in patients with cystic fibrosis is lower than other patients.

Strateva et al. (2007) reported that in clinical isolates of P. aeruginosa, the resistance to clavulanic acid was 53% and to ticarcillin was 8.22% (16). Mohajeri (2004) reported antibiotic resistance to amikacin by nearly 38% (17). Streit et al. showed that the most active agents tested against P. aeruginosa were amikacin, cefepime, tobramycin, meropenem and piperacillin/tazobactam (3.1-13.0% resistance), in the United States (18). Cefepime, ceftazidime and levofloxacin were more resistant antibiotics against P. aeruginosa, in lower respiratory tract infections (LRTI) patients, with resistance of 36.27, 35.30, and 32.35 percent, respectively. In Shahcheraghi et al.’s study in Tehran, the minimum and maximum antibiotic resistance was 9% for imipenem and 97% for ceftizoxime. Also, 42% of the isolates were resistant to ceftazidime. Thirty five percent of isolates were resistance to amikacin and the result for other antibiotics was as follows: piperacillin-tazobactam, 34%; ciprofloxacin, 41%; ceftazidime, 42%; and piperacillin, 55% (19).

In Arak hospitals (2012), Rahimi and colleagues reported, that among 100 isolates of P. aeruginosa, resistance rate to ceftazidime, tobramycin, gentamicin, amikacin imipenem, and ciprofloxacin was 53%, 31%, 38%, 36%, 12%, and 46%, respectively (21). So, it seems that, rates of antibiotic resistance in Isfahan, were higher than those in Arak. This may be due to high antibiotic usage in this region.

Rajat Rakesh et al. (2012) isolated 100 strains of P. aeruginosa and found that resistance to tobramycin, gentamicin, piperacillin, ciprofloxacin, ceftazidime was 68%, 50%, 63%, 49%, and 43%, respectively (23). The results of our study were almost similar with those of Rajat Pakesh et al.’s study in India.

Comparing the results of various researches, we can say that the rate of antibiotic resistance in burned patients in Isfahan is higher than other patients, as demonstrated in the study by Fazeli et al. high consumption of antibiotics leads to the emergence of resistant strains of bacteria and as a result, treatments cannot be effective.

Comparison of the results of various studies, it can be stated that antibiotic resistance is different from one hospital to another, one city to another, and one country to another. Therefore, it is suggested that such studies should be carried out in different hospitals of the city.

Footnotes

Acknowledgments:
We thank the members of Islamic Azad University- Falavarjan branch and also Dr. Ranjbar. The research is part of Master Degree thesis No. 17230507902006.

Funding/Support:
This study was supported by the Islamic Azad University- Falavarjan Branch, Isfahan, Iran.

Conflict of Interest:
The authors declare no conflict of interest.

References


