Antibiotic susceptibility and serotyping of clinical Pseudomonas aeruginosa isolates in northern Lebanon

Hamze M\textsuperscript{1,2}, Mallat H\textsuperscript{1,2}, Dabboussi F \textsuperscript{2}, Achkar M\textsuperscript{3}  

1 Microbiology Laboratory,  
Azm center for biotechnology research Lebanese university  
2 Faculty of public health,  
Lebanese university Tripoli-Lebanon  
3 Head of laboratory department  
Nini hospital  
Maarad street Tripoli-Lebanon  
Correspondence: mhamze@monzerhamze.com

Abstract

Background: Pseudomonas aeruginosa is widely spread in nature, inhabiting soil, water, plants, animals and humans. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia. This study aims to investigate the common serotypes and resistance phenotypes of P. aeruginosa clinical isolates in Tripoli, Northern Lebanon.

Materials and methods: A total of 88 P. aeruginosa were isolated from clinical specimens according to standard protocols. Serotyping of the strains was performed using P. aeruginosa polyvalent and monovalent anti serum kit (Bio-rad®, France). Antibiotic susceptibility of the isolates was determined by disk diffusion method and MIC of imipenem and meropenem was determined using the E-test method (Biodisk-Solna, Sweden).

Results: Most P. aeruginosa isolates were recovered from bronchial alveolar lavage (34%) and urine samples (26.1%). Serotype O11 was the most frequent isolates (16%) followed by serotypes O7 (12.5%) and O2 (11.36%). Most isolates of broncho-alveolar secretions were multidrug resistant. Antibiotic susceptibility of the isolates was the following: 87.5% to meropenem, 80.7% to cefepim, 78.4% to imipenem, 77.4% to ceftazidim, and 66% to ticarcillin.

Conclusion: This study showed that most P. aeruginosa isolates were still susceptible to common used antibiotics and few serotypes determine the epidemiology of P. aeruginosa infection in Northern Lebanon.

Key words: Pseudomonas aeruginosa, serotyping, antibiotic susceptibility.

Introduction

Pseudomonas aeruginosa is a ubiquitous gram-negative bacterium, saprophytic and naturally resistant to a wide range of antibiotics. P. aeruginosa can be considered in many aspects as a typical example of opportunistic pathogen, not virulent in healthy humans but dangerous in vulnerable individuals [1]. It occupies the third place in nosocomial infections after Escherichia coli and Staphylococcus aureus.

P. aeruginosa is distinguished by its great adaptability to different environmental situations, for its ability to acquire resistance to antibiotics and by the multiplicity of its virulence factors that resist host defenses and allow the development of infections especially among patients suffering from burn, cancer, blood disorders, and patients on long-term corticosteroid therapy, in addition to patients that suffered of multiple trauma and who had multiple surgeries and multiple transfusions and invasive procedures [2, 3, 4].
Severe infections with *P. aeruginosa* require the use of bactericidal antibiotics with high doses to reduce the risk of selection of resistant strains. The choice of antibiotics should take into account the host site of the infection, presence of kidney and liver disease, prior antibiotic therapy and especially susceptibility of *P. aeruginosa* isolates [5, 6, 7]. Development of resistance is very common in *P. aeruginosa* during antibiotic treatment. The mechanisms of acquired resistance to ß-lactams are mostly related to extended spectrum ß-lactamases (ESBL), carbapenemases or non-enzymatic (oprD2 porin deficiency, over expression of efflux pump system) [8, 9, 10].

This study investigated the prevalence of serotypes and resistance phenotypes of *P. aeruginosa* isolates in the microbiology department of Nini* Hospital Laboratory in Northern Lebanon.

### Materials and Methods

#### Patient samples

A total of 88 non-repetitive strains of *P. aeruginosa* were included in this study. These were isolated from urine, bronchial alveolar lavage, ear discharge, stool, pus -wound, blood and nasal secretions at the microbiology department of Nini hospital laboratory in Tripoli, northern Lebanon during the period (19-01-2010 to 30-07-2011). The study was carried out in collaboration with the microbiology laboratory of Azm center for biotechnology research, Lebanese University.

#### Identification and serotyping of *P. aeruginosa* isolates

All *P. aeruginosa* isolates were identified according to bacteriology standard protocol proposed by the Remic group of French Society of Microbiology [11]. These includes culture characteristics of growth at 37˚ C and 41˚ C, and using biochemical characteristics (Rapid IDNF system, Remel, USA). King A and King B media (Bio-Rad®, France) were inoculated to demonstrate the production of pigments (pyocyanin and pyoverdin). *P. aeruginosa* strain ATCC10145 was used as control. Serotyping of isolates was determined by slide agglutination technique using specific polyvalent and monovalent antisera according to recommendation of the manufacturer’s protocol (grouping for pyocyanic bacilli, Bio-Rad®, France [12]).

#### Antibiotic susceptibility test

Susceptibility of isolates to antibiotics was determined by the disk diffusion method according to the recommendations of the French Society of Microbiology Antibiogram Committee (Comité de l’Antibigramme de la Société Française de Microbiologie: CA-SFM). Muller-Hinton agar (Bio-Rad®, France) and antibiotic disks (Bio-Rad®, France) were used as follows: ticarcillin (75 µg), piperacillin (75 µg), piperacillin+tazobactam (75/10 µg), ticarcillin+clavulanic acid (75/10 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10µg), cefazidime (30 µg), cefepime (30 µg), amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), netilmicin(30 µg), fosfomycin(50 µg), ciprofloxacin(30 µg), pefloxacin(5 µg), ofloxacin(5 µg), colistin(50 µg). (Bio-Rad®, France).

### Table 1. Distribution of *P. aeruginosa* serotypes isolates.

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. of Strain</th>
<th>%</th>
<th>O1</th>
<th>O2</th>
<th>O3</th>
<th>O4</th>
<th>O5</th>
<th>O6</th>
<th>O7</th>
<th>O8</th>
<th>O9</th>
<th>O10</th>
<th>O11</th>
<th>O14</th>
<th>O15</th>
<th>O16</th>
<th>Divers</th>
<th>Non typeable strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>23</td>
<td>26.1</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>5</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Broncho-alveolar secretions</td>
<td>30</td>
<td>34.1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Pus from wound</td>
<td>20</td>
<td>22.7</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ear discharge</td>
<td>8</td>
<td>9.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Biological fluid</td>
<td>3</td>
<td>3.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Determination of MICs for imipenem and meropenem was done using E-test strips (Biodisk-Solna, Sweden) and as proposed by the manufacturer guidelines.

**Results**

The distribution of 88 *P. aeruginosa* serotypes isolates according the source of their isolation is shown in Table 1. Broncho-alveolar secretions accounted for 34.1% followed by urine samples 26.1% and pus 22.7%. Serotype O7 was the predominant (12.5%) followed by serotype O2 (11.36%).

Table 2 shows that isolates from bronchial alveolar lavage were commonly resistant to antibiotics including 43.3% were resistant to ticarcillin and 33.3% to imipenem. *P. aeruginosa* isolates from urine and other samples were generally less antibiotic resistant.

Antibiotic susceptibility of frequently isolated *P. aeruginosa* serotypes (54 strains) are shown in Table 3. No specific strain serotype is considered highly resistant than the other.

The MIC results of 88 *P. aeruginosa* isolates to imipenem and meropenem are demonstrated in Table 4. A total of 19 strains had MICs <0.047 to meropenem and 44 strains had MICs <0.5 tomeropenem and 5 strains to imipenem.

**Table 2. Antibiotic resistance of 88 *P. aeruginosa* isolates**

<table>
<thead>
<tr>
<th>Source/</th>
<th>Antibiotic</th>
<th>Urine</th>
<th>Broncho-alveolar</th>
<th>Pus -wound</th>
<th>Other body sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(N=23)</td>
<td>secretions (n=30)</td>
<td>(n=20)</td>
<td>(n=15)</td>
</tr>
<tr>
<td>TIC</td>
<td>17.4</td>
<td>43.3</td>
<td>30</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>PIP</td>
<td>17.4</td>
<td>43.3</td>
<td>20</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>T2P</td>
<td>17.4</td>
<td>20</td>
<td>10</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>TCC</td>
<td>17.4</td>
<td>40</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>ATM</td>
<td>13</td>
<td>16.7</td>
<td>15</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>IMP</td>
<td>8.7</td>
<td>33.3</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>MRP</td>
<td>4.35</td>
<td>23.3</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CAZ</td>
<td>17.4</td>
<td>33.3</td>
<td>10</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>FEP</td>
<td>17.4</td>
<td>26.6</td>
<td>15</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>AN</td>
<td>8.7</td>
<td>20</td>
<td>5</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>17.4</td>
<td>36.7</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Antibiotic resistance of frequently isolated *P. aeruginosa* serotypes.

<table>
<thead>
<tr>
<th>Serotype (No. Strains)</th>
<th>%</th>
<th>TIC</th>
<th>PIP</th>
<th>TZP</th>
<th>TCC</th>
<th>ATM</th>
<th>IMP</th>
<th>MRP</th>
<th>CAZ</th>
<th>FEP</th>
<th>AN</th>
<th>GM</th>
<th>TM</th>
<th>NET</th>
<th>FOS</th>
<th>CIP</th>
<th>PEF</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2 (10)</td>
<td>R</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>O4 (7)</td>
<td>R</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>O5 (6)</td>
<td>R</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O7 (11)</td>
<td>R</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>O10 (6)</td>
<td>R</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>O11 (14)</td>
<td>R</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

R: Resistance.

Table 4. MICs of *P. aeruginosa* isolates to imipenem and meropenem*

<table>
<thead>
<tr>
<th>Result</th>
<th>MIC/ E-test</th>
<th>Imipenem/ No. of tested strains</th>
<th>Result</th>
<th>MIC/E-test</th>
<th>Meropenem/ No. of tested strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>&lt;0.047g/ml</td>
<td>0</td>
<td>Susceptible</td>
<td>&lt;0.047g/ml</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>&lt;0.5g/ml</td>
<td>5</td>
<td></td>
<td>&lt;0.5g/ml</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>&lt;1g/ml</td>
<td>10</td>
<td></td>
<td>&lt;1g/ml</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>≤2g/ml</td>
<td>40</td>
<td></td>
<td>≤2g/ml</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>≤4g/ml</td>
<td>14</td>
<td>Intermediate</td>
<td>≤4g/ml</td>
<td>2</td>
</tr>
<tr>
<td>Intermediate</td>
<td>≤8g/ml</td>
<td>5</td>
<td></td>
<td>≤8g/ml</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>≤16g/ml</td>
<td>0</td>
<td></td>
<td>≤16g/ml</td>
<td>0</td>
</tr>
<tr>
<td>Resistance</td>
<td>≤32g/ml</td>
<td>0</td>
<td>Resistance</td>
<td>≤32g/ml</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&gt;32g/ml</td>
<td>14</td>
<td></td>
<td>&gt;32g/ml</td>
<td>9</td>
</tr>
<tr>
<td>Total % of resistance</td>
<td>15.9</td>
<td></td>
<td>Total % of resistance</td>
<td>10.22</td>
<td></td>
</tr>
<tr>
<td>Total % of intermediate</td>
<td>5.7</td>
<td></td>
<td>Total % of intermediate</td>
<td>2.27</td>
<td></td>
</tr>
</tbody>
</table>

* Critical values according to CA-SFM (Comité de l’Antibigramme de la Société Française de Microbiologie: CA-SFM).
Discussion

A previous study conducted during 1997 in two medical centers of north Lebanon has found that the majority of P. aeruginosa isolates were recovered from pus (41.8%) and urine samples (30.9%) [13]. While this study showed that the majority of P. aeruginosa were isolated from broncho-alveolar secretions (34.1%) and urine samples (26.1%). A similar study from Tunis reported that out of 1368 P. aeruginosa strains isolated, the majority was recovered from pus (52.9%), followed by broncho-alveolar secretions (19.5%), urine (10.6%) and blood cultures (5%) [14]. Vachée et al. study [15] reported from three medical centers in France that P. aeruginosa was frequently isolated from urine samples followed by broncho-alveolar secretions. It is well known that P. aeruginosa infection initiated often following using urinary catheters and intubation of patients [16].

P. aeruginosa serotypes 011,07 and 02 accounted for approximately 40% of isolates. This results indicate that these serotypes were commonly infected our hospitalized patients. Moreover, serotypes O5 and O11 were frequently isolated from urine samples, whereas serotypes O7, O4 and O11 were mostly detected in broncho-alveolar secretions. Serotyping or genotyping of P. aeruginosa clinical isolates can help to understand the epidemiology of its nosocomial infections in any hospital. In addition, it is important to identify the source of infection in these patients in order to apply hospital infection control measurements [16].

A previous study of Hamze and Sarkis [13] has reported that P. aeruginosa serotype O11 was the most common isolates from patients, and their study has found the common occurrence of serotypes O12 and O13 which were not found in this study. The study of Vachée et al. [15] in France has reported that the two most common serotypes were serotypes O6 (19%) and O11 (13%).

The present study demonstrated the relationship between antibiotic resistance and P. aeruginosa serotypes. It has been found that few numbers of serotypes O2 and O11 isolates were multidrug resistant, including their resistance to imipenem and meropenem. However, most P. aeruginosa isolates were moderate susceptible to imipenem (60.1%) and meropenem (73.3%).

Generally, several factors favor the selection of multi-resistant strains in hospitalized patients, including nosocomial infection and previous antibiotic therapy or hospitalization and the use of intensive care procedures [17, 18].

The overall antibiotic susceptibility of P. aeruginosa isolates in this study and the previous study of Hamze and Sarkis which was carried in 1997 [13] showed that there was no serious increase in antibiotic resistance to the following antibiotics: ticarcillin, piperacillin, piperacillin+tazobactam, cefepim, imipenem, tobramycin, gentamicin, amikacin, netilmicin, but there was a definite increase in resistance to pefloxacin (62.6% versus 29.8%), and ofloxacin (60.3% versus 29.8%), respectively. Based on these results, we observed the presence of 50 different phenotypes, and the major phenotype resistance (13strains) was observed with fosfomycin. In Tunis, Ben Abdallah et al. [14] has reported less occurrence of antibiotic resistance among P. aeruginosa isolated during the period 2002 to 2005, particularly to most commonly prescribed drugs; ticarcillin, ceftazidim, imipenem, gentamicin, amikacin and ciprofloxacin.

In conclusion, this study showed the importance of serotyping of P. aeruginosa isolated from clinical sources. The knowledge of serotypes may guide the choice of antibiotic therapy 24 hours before susceptibility testing results. Also, this study should alert health care professionals on the increasing rate of P. aeruginosa strains resistant to useful carbapenems and fluoroquinolones.

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