Incidence and Susceptibility Pattern of Metallo-Beta-Lactamase Producers Among Pseudomonas aeruginosa Isolated From Burn Patients at Kurdistan Province

Enayatollah Kalantar1, 2*, Vahideh Torabi3, Himen Salimizand4, Fariborz Soheili5, Rashid Ramezanzadeh4

1School of Health, Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, IR Iran
2Department of Pathobiology, School of Medicine, Alborz University of Medical Sciences, Karaj, IR Iran
3Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, IR Iran
4Department of Microbiology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, IR Iran
5School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, IR Iran

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 7 Apr 2011
Revised: 15 Nov 2011
Accepted: 01 Dec 2011

Keywords:
Kerosene
Pseudomonas aeruginosa
Burns
Therapeutics

ABSTRACT

Background: Infection with metallo-beta-lactamase (MBL)-producing organisms, particularly Pseudomonas aeruginosa, is associated with higher mortality and morbidity rates and health care costs.

Objective: In this study, we aimed to determine the etiology of burns and the incidence and susceptibility patterns of metallo-beta-lactamase producers among P. aeruginosa samples that were isolated from burn patients in Kurdistan province.

Patients and Methods: A total of 176 clinical specimens were received for P. aeruginosa testing. Antimicrobial susceptibility testing, determination of MIC, and detection of MBL-producing P. aeruginosa strains were performed per CLSI guidelines.

Results: During the study period, 145 burn patients were admitted to the burn unit of Tohid Hospital. Their mean age and total body surface area (TBSA) were 29 years and 37.7%, respectively. The mean length of hospital stay was 10 days. Kerosene was the most common cause of burns (60%), followed by gas (30%). In the 176 clinical specimens from burn patients, 100 P. aeruginosa isolates were identified. The antibiotic to which most bacteria were resistant was ampicillin (100%), and the most effective antibiotic was meropenem. Of the 100 P. aeruginosa isolates, 22 (22%) were positive for MBL production, of which 8 were resistant to imipenem.

Conclusions: Of all burn injuries, 87 (60%) patients were caused by kerosene, which is used primarily by people in low-income groups to light lamps. Our results demonstrate the serious threat of the spread of metallo-beta-lactamase producers; thus, systematic surveillance for detecting MBL producers is necessary.

*Corresponding author: Enayatollah Kalantar, Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran, Tel: +98-876631409, Fax: +98-8766644654, E-mail: kalantar_enayat@yahoo.com

Implication for health policy/practice/research/medical education:
An infection among burn patients with MBL resistant P. aeruginosa is associated with significantly higher morbidity, mortality and cost of care.

Please cite this paper as:

Copyright © 2012 Kowsar Corp. All rights reserved.
1. Background

Despite advances in antimicrobial therapies, bacterial infections remain a significant problem in the management of burn victims. Approximately 75% of the mortality following burn injuries is related to infections. Therefore, knowledge of the responsible bacterial flora in burn wounds and its prevalence is crucial in making fast and reliable therapeutic decisions (1). Over the years, *Pseudomonas aeruginosa* has become the most frequently isolated organism in most burn units (2-4). *P. aeruginosa* develops antibiotic resistance very quickly, which complicates the medical treatment of infections.

Due to extensive use of β-lactam antibiotics in clinical practice over the past several decades, various β-lactamases have emerged. One of the most important mechanisms of microbial resistance to β-lactam antibiotics is hydrolysis by β-lactamases. Infection with metallo-beta-lactamase (MBL)-producing organisms is associated with higher mortality and morbidity rates and health care costs (5, 6). *P. aeruginosa* that is associated with hospital infections are often difficult to eradicate, because they are resistant to drugs. Thus, the detection of MBL-producing *P. aeruginosa* is crucial in controlling the spread of resistant strains and optimally treating patients, particularly the critically ill and hospitalized burn patients (7, 8). Little information concerning MBL-producing isolates is available in Kurdistan. To this end, we evaluated the etiology of burns and incidence and susceptibility patterns of metallo-beta-lactamase producers in *P. aeruginosa* samples from burn patients in Kurdistan province.

2. Patients and Methods

We studied 145 patients who were referred to the burn unit of Tohid Hospital, Sanandaj, from April 2009 to April 2010. A total of 176 clinical specimens (biopsy, urine, blood, pus swab, and sputum) from the burn unit of Tohid Hospital, Sanandaj, were received by the microbiology department to test for the presence of *P. aeruginosa*. The samples of *P. aeruginosa* isolates were based on gram-negative, nonlactose-fermenting, oxidase-positive colonies that oxidized glucose and maltose and grew on cetrimide agar at 42°C (9). The patients’ demographic data, including gender, age, total body surface area (TBSA), cause of burn, and mortality, were recorded.

2.1. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by disc diffusion method of the Kirby Bauer protocol on Muller-Hinton agar (Merck, Germany) (10). The antibiotics were: amikacin, gentamicin, carbencillin, ciprofloxacin, ollaxacin, cepfime, ceftazidime, cefotaxime, ampicillin, imipenem, and meropenem (Mast disks, UK).

2.2. Determination of MIC

The MIC was determined by broth dilution method and is defined as the lowest concentration of an antibiotic that inhibits the visible growth of *Pseudomonas aeruginosa* strains after overnight culture. MICs for predefined gradients of imipenem were interpreted per the CLSI (11). Serial dilutions were prepared from 0.125 to 128 in microplates (NUNC, Denmark).

2.3. Detection of MBL

Detection of MBL-producing *Pseudomonas aeruginosa* strains was performed by double disk synergy test per CLSI guidelines (11). In this method, an overnight cultured strain was prepared at a concentration of 0.5 McFarland and used to inoculate Muller Hinton agar (Merck, Germany), to which was meropenem added at a distance of 10 mm to a blank disk. Next, 5 µl (930 µg) 0.5 M EDTA (935 µg) (Merck, Germany) was spotted onto a blank disk and incubated at 35°C overnight. *P. aeruginosa* ATCC 27853 was used as the negative control.

3. Results and Discussion

Although all burned patients are routinely cleaned with antiseptic solution, we isolated and identified 100 *Pseudomonas aeruginosa* in this study. During the study period, 101 burn patients were admitted to the burn unit of Tohid Hospital. Their mean age was 29 years (range: 4-74 years). The distributions of age and gender by injury and outcome are shown in Table 1; 46.5% was male and 53.5% was female. Pegg et al. (12) observed a ratio of 71.3% males to 28.7% females in a study on burns in Australia. The predominance of males in this case was likely due to the higher incidence of burns in industry in males.

The mean TBSA (Table 2) was 37.7% (range: 5% - 95%), which is much less than what Hosseini et al. (13) noted in Shiraz. In their study, the mean (SD) TBSA that was burned was 56%. Similarly, the mean length of hospital stay was 10 days (range: 4 to 18 days) (Table 3), which is more than in other centers in Iran (14-16).

*P. aeruginosa* has been implicated in the majority of invasive burn wound infections in many burn centers (17, 18).

<table>
<thead>
<tr>
<th>Table 1. Frequency of Burn Patients According to Age and Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age Group, y</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
This bacteria has caused much concern due to the rapid increase in resistance to a variety of antibacterial drugs. MBLs have been identified from clinical isolates worldwide with increasing frequency over the past several years, and strains that produce these enzymes have been responsible for prolonged nosocomial infections (18-20). As seen in Table 4, kerosene was the most common cause of burns (60%), followed by gas (30%), which differs from other studies in Iran (18-20). In our study, in the 176 clinical specimens from burn patients, 100 P. aeruginosa isolates were identified. Table 5 shows the antibiotic resistance patterns of the P. aeruginosa isolates. The antibiotics that the bacteria were most resistant to were ampicillin (100%), ceftazidime (94%), and ceftriaxone (89%), consistent with other reports from Iran (21-23). These antibiotics are available over the counter (OTC) in Sanandaj, potentially increasing resistance rates.

The next step in our test was to analyze the MBL contents of the P. aeruginosa isolates. By CLSI-MBL phenotypic confirmatory test, of the 100 P. aeruginosa isolates, 22 (22%) were positive for MBL production by the double disk synergy test (DDST) (Figure). Of the 22 isolates that were MBL-positive, the MIC of imipenem exceeded 16 µg/ml against 8 (Table 6).

Table 2. Percentage of Total Body Surface Area (TBSA) of Burn Patients in Tohid Hospital

<table>
<thead>
<tr>
<th>TBSA</th>
<th>Age Group, y</th>
<th>0 - 10</th>
<th>11 - 20</th>
<th>21 - 30</th>
<th>31 - 40</th>
<th>41 - 50</th>
<th>&gt; 51</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 (NO %)</td>
<td>02</td>
<td>05</td>
<td>01</td>
<td>03</td>
<td>01</td>
<td>01</td>
<td></td>
</tr>
<tr>
<td>10 -30 (NO %)</td>
<td>02</td>
<td>08</td>
<td>08</td>
<td>07</td>
<td>06</td>
<td>05</td>
<td></td>
</tr>
<tr>
<td>31 - 60 (NO %)</td>
<td>01</td>
<td>05</td>
<td>08</td>
<td>05</td>
<td>04</td>
<td>01</td>
<td></td>
</tr>
<tr>
<td>&gt; 60 (NO %)</td>
<td>00</td>
<td>05</td>
<td>05</td>
<td>02</td>
<td>03</td>
<td>01</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Length of Hospital Stay According to Percentage of Total Body Surface Area (TBSA)

<table>
<thead>
<tr>
<th>Hospital Stay (Day)</th>
<th>Number</th>
<th>Percent, NO %</th>
<th>Number</th>
<th>Percent, NO %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 7</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>7 - 4</td>
<td>31</td>
<td>31</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>15 - 30</td>
<td>07</td>
<td>07</td>
<td>07</td>
<td>07</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Etiology of Burn Patients According to Sex

<table>
<thead>
<tr>
<th>Male</th>
<th>Petrol</th>
<th>Kerosene</th>
<th>Gas</th>
<th>Chemicals</th>
<th>Electricity</th>
</tr>
</thead>
<tbody>
<tr>
<td>04</td>
<td>24</td>
<td>18</td>
<td>01</td>
<td>03</td>
<td></td>
</tr>
<tr>
<td>01</td>
<td>35</td>
<td>12</td>
<td>01</td>
<td>01</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>05</td>
<td>59</td>
<td>30</td>
<td>02</td>
<td>04</td>
</tr>
</tbody>
</table>

Table 5. In vitro Antibiotic Resistance Pattern of Infection-Associated P. aeruginosa

<table>
<thead>
<tr>
<th>Resistance, NO. (%)</th>
<th>Amikacin</th>
<th>Gentamicin</th>
<th>Carbenicillin</th>
<th>Ciprofloxacin</th>
<th>Ofloxacin</th>
<th>Cefepime</th>
<th>Cefazidime</th>
<th>Cefotaxime</th>
<th>Ampicillin</th>
<th>Imipenem</th>
<th>Meropenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>52 (52)</td>
<td>54 (54)</td>
<td>76 (76)</td>
<td>31 (31)</td>
<td>82 (82)</td>
<td>72 (72)</td>
<td>96 (96)</td>
<td>88 (88)</td>
<td>100 (100)</td>
<td>21 (21)</td>
<td>14 (14)</td>
</tr>
</tbody>
</table>

Figure. Phenotypic Detection of MBLs by DDST Among P. aeruginosa Isolates in Burn Patients at Tohid Hospital - Sanandaj
The acquisition of MBL in \textit{P. aeruginosa} has been well established, but MBL has not been studied extensively in our region. With the occurrence of MBL in \textit{P. aeruginosa} increasing worldwide (24, 25), there is a tremendous need to detect MBLs in clinical isolates of \textit{P. aeruginosa}. In this study, the DDST successfully identified 22 (22\%) of 100 \textit{P. aeruginosa} isolates as positive for MBL production, less than that in Mihani and Khosravi's study in Ahvaz (26).

Of all burn injuries in this study, 87 (60\%) were caused by kerosene, which is used primarily by people in low-income groups to light lamps. This study demonstrates the serious threat of the spread of metallo-beta-lactamase producers with regard to antibiotic resistance; therefore, systematic surveillance for detecting MBL producers is necessary. Infections in burn patients with MBL-resistant \textit{P. aeruginosa} are associated with significantly greater morbidity, mortality and cost of care.

Acknowledgments

None declared.

Financial disclosure

None declared.

Funding/Support

This work was supported financially by a grant from Kurdistan University of Medical sciences, Sanandaj, Iran.

References