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ABSTRACT

**Background & Objective:** Infections due to burn wounds are serious because of their effects on the course of the disease and its consequences. The rate of burn wound infection is very high in developing countries. The purpose of this study was to identify common bacterial agents causing burn wound infection and determine antimicrobial susceptibility patterns in a burn hospital, Isfahan, Iran.

**Materials & Methods:** This cross-sectional study was conducted from 2017 to 2018 on all patients with burn wound infection. Burn wounds suspected of infection were collected aseptically and traditional bacteriological methods were used to identify the causes of infection. Antimicrobial resistance test was done by the disk diffusion method in accordance with CLSI recommendations.

**Results:** From the total of 1500 wound culture, 957 (63.8%) samples were detected as positive. The highest rate of infection was in the ICU ward and the lowest was in the restoration ward. The most common gram-negative bacteria were *Acinetobacter baumannii* (34.9%) with the highest and the lowest antibiotic resistance to Ceftazidime and Tobramycin, respectively. Among recovered Gram-positive isolates, *Staphylococcus aureus* (10.2%) were the predominant isolates with the highest and the lowest antibiotic resistance to Penicillin and Vancomycin, respectively.

**Conclusion:** Due to the variable nature of antibiotic susceptibility patterns and pathogens causing burn wound infection, continuous evaluation, detection of dominant bacterial infections and sensitivity patterns to locally available antibiotics in burn wound patients in order to modify the drug regimen for proper antibiotic treatment is important and seems reasonable.

**Keywords:** Antimicrobial resistance pattern, Bacterial infection, Burn patients

**Introduction**

Infections are a major public health problem in burn patients due to features such as the loss of the first line of defense against microbial invasion, the presence of abandoned and vascular tissue, changes in the specific and non-specific components of the immune system, and hospitalization. The hospital provides a good environment for infections to grow (1). Burn lesions are sterile from the beginning, but 48 hours later the wounds are colonized by gram-positive skin flora such as β-hemolytic streptococcus and *Staphylococcus aureus* bacteria (2,3). After 2 to 3 days, the wounds are colonized with gram-negative bacteria in the patient’s respiratory tract and gastrointestinal tract like the *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*, as well as in the microorganisms of the hospital environment or hospital staff (4-9).

The world health organization (WHO) has estimated that the burn injury leads to annual 265,000 deaths, half of which occurred in the WHO south-east Asia region (10). Long-term administration of antibiotics leads to the development of multidrug-resistant strains, which mainly belong to the: *S. aureus, P. aeruginosa*, and *Acinetobacter baumannii* (11). Multidrug-resistant organisms cause infections that are very difficult to treat because antimicrobial drugs are slightly effective in combating them (12). Since effective new antibiotics have not yet been developed, especially for gram-negative bacteria, efforts should be made to maintain the activity of existing antimicrobial drugs, fight against antimicrobial resistance and provide effective measures to control infection to prevent infection of patients with drug-resistant strains (13).

*P. aeruginosa* and *A. baumannii*, particularly in developing countries, are the most significant and common causes of important infections in burn patients (14-22). When *P. aeruginosa* and *A. baumannii* infect burn wounds, treatment becomes very complicated, especially if there is multidrug resistance and the
mortality rate among patients is approximately 40-50\% (14,15,23,24). The problem of treating drug-resistant infections is increasing day by day (15-17). The major problem of infection control especially in developing countries is appearance of multi drug-resistant strains in burn wound infections. The aim of the present study was to investigate the prevalence of burn wound infections (BWI) in one of the main burn hospitals in the country and the measure antimicrobial resistance in this hospital for active treatment and emergency medicine.

## Materials and Methods

### Sample Collection:

The current study was conducted over a 12-month period (March 2017 to April 2018) at Imam Musa Kazem burn hospital in Isfahan, Iran. About 1500 burn wound samples were collected from the hospitalized patients (784 men and 716 women) in different wards of the hospital.

Sampling of the burn wound was performed after removing the dressings and removing local antibacterial agents and wound cleaning by washing with sterile saline solution (0.9\% NaCl).

In order to get enough cellular material for culture, the end of two sterile swabs were transmitted by at least one centimeter of the open wound. Adequate pressure should be applied to the tip of the swabs to bleed a little into the underlying tissue (24).

### Sample Processing

The first swab was used for direct smear and gram staining to examine bacteria and diagnose PMNL, which is an important feature in the case of bacterial infection, which must be distinguished from bacterial cloning (23).

### Culture

The swabs were cultured as streak on blood and MacConkey agar plates and incubated for 24 h of aerobic incubation at 37°C. Conventional bacteriological methods (Gram-stain, catalase test, oxidase test, indole test, Methyl red (MR) and Voges–Proskauer (VP) test, Citrate test, urease test, coagulase test, Novobiocin resistance, Hemolysis type, Susceptibility to Bacitracin, Optochin, Hydrolysis of cAMP, Hydrolysis by Bile esculin, Growth in 6.5\% NaCl, oxidative-fermentative (OF) test, triple sugar iron agar (TSIA)) were done for the isolation and identification of the bacteria (25).

### Antibiotic Susceptibility Tests:

Antimicrobial resistance tests were done according to clinical laboratory standards institute guidelines via Kirby-Bauer disk diffusion method. Their interpretation was based on the CLSI-2018 tables (26). Antibiotics included Ciprofloxacin (5 \(\mu\)g), Amikacin (30\(\mu\)g), Cefazidime (30\(\mu\)g), Nalidixic acid (30\(\mu\)g), Pipercillin (100\(\mu\)g), Imipenem (10\(\mu\)g), Meropenem (10\(\mu\)g), and Vancomycin (30\(\mu\)g), Ceftriaxone (30\(\mu\)g), Penicillin (10 units), Pipercillin – Tazobactam (100/10\(\mu\)g), Tobramycin (10\(\mu\)g) (American Bidi). The test was performed on Mueller-Hinton agar (Merck, Germany). E. coli ATCC 25922 strain was used as a control (27).

Approval to conduct the study was obtained from the Research Ethics Committee of Isfahan University of Medical Sciences (ID- number: IR.MUI.RESEA RCH.REC.1397.114).

### Data Analysis:

Data were analyzed via SPSS 24. (SPSS Inc., IL., USA). P-value\(\leq\)0.05 was considered statistically significant.

### Results

Of the 1500 cultured samples, 957 (63.8\%) included bacterial isolates and 543 wound swabs were (36.2\%) sterile.

#### Bacterial Colonization Pattern of Burn Wounds Infections:

Different types of bacterial isolates were isolated from burn wound infection and biopsy of burn wound infection, of which 146 were Gram positive and 815 were Gram negative.

The results clearly showed that A. baumannii with 34.7\% frequency and P. aeruginosa with 29.6\% frequency were the most common Gram-negative bacterial isolates and S. aureus with 10.2\% frequency was the most common Gram-positive bacterial isolate (Table 1).

The prevalence of bacterial isolates among different wards of the hospital showed that burn wound infection was the most frequent in ICU patients (50.2\%) and the least in restoration patients (6.6\%) (Table 2).

According to the results, 50.7\% of isolates showed MDR resistance. The results of antibiotic susceptibility tests showed that the most resistant isolates to 14 antibiotics examined were A. baumannii and P. aeruginosa isolates (Table 3).

#### Antibiotic Resistances of A. baumannii:

Among the A. baumannii isolates, the highest resistance to Ciprofloxacin (91.9\%) and then to Meropenem (81.75\%) were observed. The highest sensitivity to Tobramycin (56.9\%) was reported (Figure 1).

#### Antibiotic Resistance of P. aeruginosa:

Among P. aeruginosa isolates, the highest resistance to Cefazidime (95.6\%) and then to Ciprofloxacin (92.8\%) were seen. The highest sensitivity to Amikacin (57.9\%) was found (Figure 1).
Antibiotic Resistance of *S. aureus*:

Among *S. aureus* isolates, the highest resistance were seen to Penicillin (63.2%) and then to Ciprofloxacin (52%). The highest sensitivity was found to be to Vancomycin (96%) (Figure 2).

Table 1. Rate of bacterial pathogens isolated in burn patients

<table>
<thead>
<tr>
<th>Types of bacteria</th>
<th>Percentage (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. baumannii</em></td>
<td>34.9%(334)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>29.8%(285)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>10.2%(98)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>8.3%(80)</td>
</tr>
<tr>
<td>Other non-fermenting Gram-negative bacilli</td>
<td>5.2%(50)</td>
</tr>
<tr>
<td><em>Coagulase-negative Staphylococcus sp.</em></td>
<td>4.8%(46)</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>3.4%(33)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1.2%(10)</td>
</tr>
<tr>
<td>Others Enterobacteriaceae</td>
<td>1.3%(12)</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>0.09(9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100%(957)</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of bacterial infection among different wards of the hospital

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Wards</th>
<th>ICU</th>
<th>Emergency</th>
<th>Burn</th>
<th>Restoration</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. baumannii</em></td>
<td></td>
<td>229</td>
<td>54</td>
<td>43</td>
<td>8</td>
<td>334</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td>171</td>
<td>57</td>
<td>50</td>
<td>7</td>
<td>285</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>19</td>
<td>44</td>
<td>30</td>
<td>5</td>
<td>98</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td></td>
<td>27</td>
<td>14</td>
<td>14</td>
<td>25</td>
<td>80</td>
</tr>
<tr>
<td>Other non-fermenting Gram-negative bacilli</td>
<td></td>
<td>19</td>
<td>11</td>
<td>16</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td><em>Coagulase-negative Staphylococcus sp.</em></td>
<td></td>
<td>8</td>
<td>14</td>
<td>17</td>
<td>7</td>
<td>46</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td></td>
<td>5</td>
<td>12</td>
<td>10</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Others Enterobacteriaceae</td>
<td></td>
<td>0</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td></td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>481</td>
<td>223</td>
<td>189</td>
<td>64</td>
<td>957</td>
</tr>
</tbody>
</table>

Table 3. Antimicrobial resistance pattern

<table>
<thead>
<tr>
<th>ANTIBIOTICS ISOLATES</th>
<th>A. baumannii</th>
<th><em>P. aeruginosa</em></th>
<th><em>S. aureus</em></th>
<th><em>K. pneumoniae</em></th>
<th>ONGINF</th>
<th>CON</th>
<th><em>E. cloacae</em></th>
<th>E. coli</th>
<th>OENT</th>
<th>Enterococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>310 (92.8%)</td>
<td>262 (91.9%)</td>
<td>51 (52%)</td>
<td>53 (66.2%)</td>
<td>37</td>
<td>15</td>
<td>16</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>AN</td>
<td>207 (61.9%)</td>
<td>165 (57.9%)</td>
<td>27 (27.5%)</td>
<td>18 (20%)</td>
<td>28</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>CAZ</td>
<td>314 (95.6%)</td>
<td>197 (69.1%)</td>
<td>*</td>
<td>15 (18.75%)</td>
<td>27</td>
<td>8</td>
<td>*</td>
<td>4</td>
<td>4</td>
<td>*</td>
</tr>
<tr>
<td>NA</td>
<td>*</td>
<td>*</td>
<td>21 (21.4%)</td>
<td>*</td>
<td>5</td>
<td>0</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>4</td>
</tr>
</tbody>
</table>

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**Figure 1.** Resistance of *A. baumannii* and *P. aeruginosa* to antimicrobial drugs.

**Figure 2.** Resistance of *S. aureus* to antimicrobial drug.
**Discussion**

Wound infection is one of the most common burn problems originating from nosocomial infections. The severity of infections in burn wounds is important because of their effects on the course of the disease and subsequently on patients. Most of the burn patients died of infection during the hospitalization. The prevalence of infections in burn wounds is high in developing countries because it is likely to decline the hygiene in poorer socioeconomic status. Malnutrition also plays an important role in the rapid acquisition of infection (28,29). Families and hospital personals who are in closer contact with the patient may also be the source of the infection. Negligence towards the hygiene laws, lack of sterile bandages, clothing and patient care equipment, Long-term catheterization, and inadequate antibiotic treatment are among the most important causes of nosocomial infections (28). In the present study, the most common infections were *A. baumannii* (34.9%), *P. aeruginosa* (29.8%) and *S. aureus* (10.2%). The results were similar to many studies showing that *A. baumannii* and *P. aeruginosa* are the most prevalent bacteria isolated from burn wounds (30); however, these results contradict some other studies showing that *S. aureus* is the most common isolate (28,31). *A. baumannii* is one of the most frequent nosocomial infection bacteria widely isolated from hospital environment and equipment (32). These bacteria have also been widely reported in the ICU ward. In the present study, *A. baumannii* was the most isolated strain of patients admitted to the ICU ward. For the last two decades, *P. aeruginosa* has been a very important pathogen and accounting for a large proportion of nosocomial infections. *P. aeruginosa* infections are particularly prevalent among burn wound patients (33-35). Determining the pattern of antibiotic resistance is very important for epidemiological surveillance programs and treatment alternatives in burn patients. Antibiotic resistance is the most serious causes for concern, as common bacterial isolates resistant to antimicrobial agents are the most important cause of death in burn patients. In the current study *A. baumannii* and *P. aeruginosa* were found to be the most resistant isolates. The highest resistance was seen to Ciprofloxacin and the lowest resistance of *A. baumannii* was seen to Tobramycin and the lowest resistance of *P. aeruginosa* was seen to Piperacillin-Tazobactam. Whereas in Bayram’s study, *A. baumannii* and *P. aeruginosa* resistance to Imipenem was high (30).

*S. aureus* is the third isolate in the list of bacterial isolates. Unlike other studies, the incidence of Penicillin resistance was the highest. Optimal training of burn ward personals, wash and hand hygiene personnel before and after contact with each patient, avoiding crowded appointments, restricting inter-wards hospital exchanges, monitoring the prevention of bacterial infections, attention to antibiotic resistance patterns and effective nosocomial infection control programs all are very important and effective in preventing burn wound infection and its subsequent problems.

**Conclusion**

Due to the variable nature of pathogens causing burn wound infection, continuous evaluation and detection of dominant bacterial infections and their sensitivity patterns, in order to modify the drug regimen for proper antibiotic treatment is important to prevent antibiotic resistance and it seems reasonable. An effective infection control program is required in all wards of the burn hospital to prevent the spread of nosocomial burn wound infections. Staffs from all wards of the hospital need to work closely with the hospital acquired infection.

**Acknowledgments**

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**Conflict of Interest**

Authors declared no conflict of interest.

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