RESEARCH ARTICLE

Prevalence of aerobic pathogenic bacteria isolated from patients with burn infection and their antimicrobial susceptibility patterns in Al-Najaf City, Iraq- a three-year cross-sectional study. [version 1; peer review: awaiting peer review]

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Abstract

**Background:** Burn infections are one of the most common serious illnesses caused by pathogens, mainly by both gram-negative and gram-positive bacteria. The aim of this study was to detect the prevalence of multi-drug resistant and extended-spectrum β-lactamase-producing (ESBL) bacteria isolated from inpatients with burn infection and the antimicrobials sensitivity patterns of all bacterial isolates during three years.

**Methods:** This cross-sectional study was performed in Al-Najaf Central Hospital in Al-Najaf City, Iraq from January 2015 to December 2017. A total of 295 burns swabs were collected from hospitalized patients with burn infection. All grown bacterial isolates were identified by standardized microbiological tests. Antimicrobials susceptibility testing was done using the disc diffusion method.

Multi-drug, extensive-drug and pan-drug resistant bacteria and extended-spectrum β-lactamase-producing bacteria were determined according to standardized methods and guidelines.

**Results:** Of the 295 burn swabs, 513 different bacteria strains were isolated. *Pseudomonas aeruginosa* was the most common bacteria with 142 isolates (27.6%) followed by methicillin resistance *Staphylococcus aureus* 106 isolates (20.6%), while *Staphylococcus typhi* was the least common bacteria with only 17 isolates (3.3%). 323 (63%) different bacterial strains were isolated from patients who stayed in hospital for 15 days. Most bacterial isolates were resistant to most antimicrobials with high percentages. Out of the 513 bacterial isolates; only 33 isolates (6.4%) were resistant to imipenem 10µg and 464 isolates (90.4%) were multi-drug resistant, 20 isolates (14%) were extensive-drug resistant and 17 isolates (3.3%) were pan-drug resistant. *Pseudomonas aeruginosa* was the most common ESBL-producing bacteria (51 isolates-35.9%).

**Conclusions:** There was a high prevalence of multi-drug resistant bacteria in burn infection in Al-Najaf hospital. *Pseudomonas aeruginosa* was the most common multi-drug resistant bacteria, and the most common of ESBL bacteria causing burn infection over the three years.
Keywords
Burn infection, Pathogenic bacteria, Antimicrobials susceptibility patterns, ESBL.

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Introduction
Burn infection caused by pathogenic bacteria is one of the most common hospital problems worldwide, particularly in developing countries. Fire leads to skin destruction and simultaneous suppression of both humoral and cellular immune system subsequently resulting burn infection. Complications of burn infection are responsible for more than 70% of death cases among inpatients with burns. These infections mainly caused by multidrug resistant gram-negative and gram-positive bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*), Klebsiella pneumoniae (*K. pneumoniae*), and Staphylococcus aureus (*S. aureus*). Non-sterile burns halls and duration of patients stay in hospital in addition to the surface area of burned skin, are the most important factors related to the increase of persistent and multiplication of pathogenic bacteria in the burned areas. Multi-drug resistant (MDR) bacteria is one of the most common pathogens causing burn infection in hospitalized patients worldwide. These pathogens are resistant to at least three different classes of antimicrobials such as, penicillin’s, beta-lactams, cephalosporins, 3rd and 4th generation cephalosporins, aminoglycosides, tetracyclines and quinolones, and is becoming one of the most dangerous health issues in hospitals. In addition, extended-spectrum β-lactamase (ESBL)-producing bacteria are considered as a potent pathogens due to their resistance to a wide range of antimicrobials like, cefotaxime, ceftriaxone and ceftazidime, that lead to difficulty in the treatment of most infections such as burn infection and urinary tract infection. Burn infection is characterized by difficult healing due to administration of unsuitable treatment, long stay in hospital and the contaminates of hospital environments lead to the emergence of new multi-drug resistant bacterial isolates causing dangerous complications such as, bacteremia, septicemia and death. Therefore, we must pay attention to all safety standards in hospitals, especially in burns wards through sterilization, performing antimicrobial susceptibility test on all pathogenic bacteria isolated from burn infections, and keeping the burned skin in sterile conditions to prevent the emergence of these pathogens. According to the above, the aim of this work was to investigate of the prevalence of multi-drug resistant bacteria and extended-spectrum β-lactamase-producing bacteria isolated from inpatients with burn infection in Al-Najaf central hospital in Al-Najaf City, Iraq over three years, from January 2015 to December 2017 to increase our understanding of the most prominent bacteria and their resistance to different antimicrobials to prevent the emergence of these isolates in the future.

Methods

**Ethical considerations**

We confirm that we received approval for this study including: patient’s swabs and consent from the participants. All swabs were taken by physician and consent by the hospital treatment and care team responsible and then handed the samples over to us. All swabs were provided from the participants physician in Al-Najaf central hospital in Al-Najaf City, (Burns Department). All swabs were immediately transported to the Laboratory of Microbiology in Faculty of Science, University of Kufa to process. Note: Each swab was labeled with the following items: age, sex, duration of stay in the hospital after burning. Al Najaf Central Hospital is part of the University of Kufa and therefore written approval was not sought as there is a pre-existing agreement between the university and hospital regarding clinical sample collection sample collection. Oral consent to take swabs was taken from each patient.

Eligibility criteria for patients

Patients will be considered eligible for registration into this study if they fulfill all the inclusion criteria and none of the exclusion criteria as defined below.

1. Patients (Male or female) at least more than 18 years old.
2. Patients should have sufficient capacity for informed consent.
3. Patients should don’t have any other infections.

Study design, burns swabs collection and bacterial identification

This is a cross-sectional descriptive study performed in Al-Najaf central hospital in Al-Najaf City, Iraq. From January 2015 to December 2017. A total of 295 swabs (emulsion with normal saline) were collected from the burned area of hospitalized patient with burn infections (2nd degree, shown the signs of infection during the change of dressings), ages ranges 18-45 years old (males and females), 3 swabs were taken from each patient at 5, 10 and 15 days of stay. Immediately, all collected swabs were incubated with brain heart infusion broth (Oxoid™, USA, CM1135R) for 24h at 37°C to encourage bacterial growth and then streaked onto blood agar (Oxoid™, USA, CM0055B) using a swab (Himedia, India, PW1210G) and chocolate agar (Oxoid™, USA, R01293) surface and incubated aerobically at 37°C for 24-48 h. All emerged bacterial isolates were identified according to colony morphology and standard microbiological tests such as; colony morphology, blood hemolysis onto blood agar surface (Oxoid™, USA, CM0055B), gram stain, oxidase test, catalase test, invic test, motility test, coagulase test, growth on MacConkey agar (Oxoid™, USA, R061322) and Mannitol salt agar (Oxoid™, USA, CM0085B). Any.

Antimicrobials susceptibility test

Antimicrobials susceptibility testing was performed by disc diffusion method according to Kirby-Bauer method onto Mueller Hinton agar (Oxoid™, USA, PO5007A) surface. Fourteen different antimicrobial discs were used in this study provide from OxoidTM, USA as follow: penicillin 10IU (P) (CT0043B), amoxicillin 25µg (AX) (CT0161B), Amoxiclav 30µg (AMC) (CT0538B), ceftriaxone 30µg (CRO) (CT0417B), cefotaxime 30µg (CTX) (CT0166B), ceftazidime 30µg (CAZ) (CT0412B), gentamicin 10µg (GM) (CT0024B), tobramycin 10µg (TM) (CT0056B), amikacin 30µg (NA) (CT0107B), ciprofloxacin 5µg (CIP) (CT0425B), imipenem 10µg (IMP) (CT0455B), Streptomycin 10µg (S) (CT0047B), erythromycin 30µg (E) (CT0021B), tetracycline 30 µg (TE) (CT0054B). The diameters of inhibition zones (mm) were measured using a caliper measure each zone with the unaided eye, and compared with clinical and laboratory standards institute (CLSI) guideline 2017. Any bacterial isolate was resistance to at least three different antimicrobials classes considered as MDR, if any bacterial isolate was
resistance to all antimicrobial classes except two or three antimicrobial classes considered as extensive-drug resistant (XDR) and when any bacterial isolate was resistant to all antimicrobials class considered as pan-drug resistant (PDR).

**Identification of methicillin resistance S. aureus**

All *S. aureus* isolates growth was adjusted according to turbidity of standard McFarland tube 0.5 (measured by Vis-Nir spectrophotometer, Biobase, UK, bk-S410). All isolates were streaked onto Mueller Hinton agar (Oxoid™, USA, PO5007A) surface supplemented with 4% NaCl. Five µg of methicillin disc (Oxoid™, USA, CT0159B) was placed at the surface of Mueller Hinton agar and incubated aerobically at 37°C for 24h. All *S. aureus* isolates that were resistant to methicillin with diameter of inhibition zone < 17 mm were considered as methicillin resistant *S. aureus* (MRSA), while those isolates with diameters of inhibition zone ≥ 17 mm considered methicillin sensitive *S. aureus* (MSSA).

**Phenotypic detection of extended spectrum beta-lactamase-producing bacteria**

This test was performed according to modified double disc synergy test (MDDST) as follows: all bacterial isolates (turbidity was adjusted according to McFarland tube 0.5) were streaked by sterile swab (Himedia, India) onto Mueller Hinton agar (Oxoid™, USA) surface, AMC disc 30µg was placed in the center of agar plate, CRO 30µg, CTX 30µg and CAZ 30µg were placed around AMC disc 30µg (15 mm from center to center). All plates were incubated aerobically at 37 °C for 24h. Any increase in the inhibition zone towards AMC disc 30µg was considered as positive for the extended spectrum beta-lactamase.

**Statistical analysis**

Percentages were used in this study to compare between the prevalence of pathogenic bacteria and their resistant to antimicrobials using Graphpad-prism V.10 computer software.

**Results**

Of the 295 burn swabs, 513 different bacterial strains were isolated, 335 isolates (65.3%) were gram negative bacteria and 178 isolates (34.7%) were gram positive bacteria (Figure 1). *Pseudomonas aeruginosa* was one of the most common bacteria causing burn infection, 142 isolates (27.6%), followed by methicillin resistant *S. aureus* 106 isolates (20.6%), *K. pneumoniae* 93 isolates (18.2%), methicillin sensitive *S. aureus* 72 isolates (14.1%), *E. coli* 51 isolates (10%), *A. baumannii* 32 isolates (6.2%) and *S. typhi* 17 isolates (3.3%). 323 different bacterial strains (63%) were isolated from patients with burn infection who stayed in hospital for 15 days (Table 1). Out of total 513 bacterial isolates, 122 (23.8%) were isolated as single growth, while 391(76.2%) were isolated as multiple growth.

**Figure 1.** Numbers and percentages of total bacterial isolates from hospitalized patients with burn infection during January 2015 to December 2017. N=513.

**Table 1.** Numbers and percentages of pathogenic bacteria isolated from hospitalized patients with burn infection during January 2015 to December 2017. N=513.

<table>
<thead>
<tr>
<th>Pathogenic bacteria</th>
<th>Duration of stay in hospital</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 days</td>
<td>10 days</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>MRSA</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>MSSA</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>65(12.6)</td>
<td>125(24.4)</td>
</tr>
</tbody>
</table>

Data were presented as numbers (NO.) and percentages (%) of bacterial isolates. MRSA: Methicillin resistant *S. aureus*, MSSA: Methicillin sensitive *S. aureus*. 
were isolated as mixed growth (Table 2). According to the results of antimicrobial susceptibility tests, most bacterial isolates were resistant to most antimicrobials with high percentages. Out of the 513 bacterial isolates, only 33 isolates (6.40%) were resistant to imipenem 10µg. The results of antimicrobials susceptibility test and overall resistant of 513 bacterial strains to 14 antimicrobials are shown in Table 3 and Figure 2. Of the total 513 bacterial isolates, 464 isolates (90.4%) were MDR, 20 isolates (14%) were XDR and 17 isolates (3.3%) were PDR (Table 4). Pseudomonas aeruginosa was the most common MDR-bacteria, 130 strains (91.5%), while 4 strains (2.8%) were XDR and 8 strains (5.6%) were PDR. All resistant types of all bacterial isolates are shown in Table 4. According to MDDST, Pseudomonas aeruginosa was the most common ESBL-producing bacteria 51 isolates (35.9%) (Figure 3) followed by K.pneumoniae 21 isolates (2.6%), while, no strain of S.typhi was ESBL. All ESBL-producing gram negative bacteria are shown in Figure 4.

### Dataset 1. Test results for patient swabs

http://dx.doi.org/10.5256/f1000research.15088.d21154

### Discussion

Burn infection is one of the most serious problems in hospitals caused by different pathogens that infect most patients who stay

<table>
<thead>
<tr>
<th>Type of bacterial isolates</th>
<th>Duration of stay in hospital</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 days No. (%)</td>
<td>10 days No. (%)</td>
</tr>
<tr>
<td>Single isolate</td>
<td>10(1.9)</td>
<td>27(5.3)</td>
</tr>
<tr>
<td>Mixed isolates</td>
<td>55(10.7)</td>
<td>98(19.1)</td>
</tr>
<tr>
<td>Total</td>
<td>65(12.6)</td>
<td>125(24.4)</td>
</tr>
</tbody>
</table>

Data were presented as numbers (No.) and percentages (%) of bacterial isolates.

<table>
<thead>
<tr>
<th>AB.</th>
<th>P. aeruginosa 142 No. (100%)</th>
<th>MRSA 106 No. (100%)</th>
<th>K. pneumoniae 93 No. (100%)</th>
<th>MSSA 72 No. (100%)</th>
<th>E. coli 51 No. (100%)</th>
<th>A.baumannii 32 No. (100%)</th>
<th>S.typhi 17 No. (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>142(100)</td>
<td>106(100)</td>
<td>93(100)</td>
<td>70(97.2)</td>
<td>45(88.2)</td>
<td>30(93.7)</td>
<td>12(70.5)</td>
</tr>
<tr>
<td>AX</td>
<td>142(100)</td>
<td>106(100)</td>
<td>85(91.3)</td>
<td>66(91.6)</td>
<td>40(78.4)</td>
<td>29(90.6)</td>
<td>13(76.4)</td>
</tr>
<tr>
<td>AMC</td>
<td>140(98.5)</td>
<td>99(93.5)</td>
<td>80(86)</td>
<td>65(90.2)</td>
<td>39(76.4)</td>
<td>28(87.5)</td>
<td>10(58.8)</td>
</tr>
<tr>
<td>CRO</td>
<td>130 (91.5)</td>
<td>90(84.9)</td>
<td>80(86)</td>
<td>33(45.8)</td>
<td>35(68.6)</td>
<td>28(87.5)</td>
<td>11(64.7)</td>
</tr>
<tr>
<td>CTX</td>
<td>128(90.1)</td>
<td>96(90.5)</td>
<td>81(87)</td>
<td>36(50)</td>
<td>38(74.5)</td>
<td>27(84.3)</td>
<td>11(64.7)</td>
</tr>
<tr>
<td>CAZ</td>
<td>132(92.5)</td>
<td>100(94.3)</td>
<td>86(92.4)</td>
<td>39(54.1)</td>
<td>39(76.4)</td>
<td>30(93.7)</td>
<td>10(58.8)</td>
</tr>
<tr>
<td>GM</td>
<td>102(71.8)</td>
<td>80(75.4)</td>
<td>75(80.6)</td>
<td>35(48.6)</td>
<td>40(78.4)</td>
<td>18(65.2)</td>
<td>8(47)</td>
</tr>
<tr>
<td>TM</td>
<td>112(78.8)</td>
<td>75(70.7)</td>
<td>74(79.5)</td>
<td>40(55.5)</td>
<td>25(49)</td>
<td>19(59.3)</td>
<td>7(41.1)</td>
</tr>
<tr>
<td>NA</td>
<td>110(77.4)</td>
<td>81(76.4)</td>
<td>40(43)</td>
<td>42(58.3)</td>
<td>20(39.2)</td>
<td>15(46.8)</td>
<td>7(41.1)</td>
</tr>
<tr>
<td>CIP</td>
<td>125(88)</td>
<td>88(83)</td>
<td>70(75.2)</td>
<td>60(83.3)</td>
<td>18(35.2)</td>
<td>24(75)</td>
<td>9(52.9)</td>
</tr>
<tr>
<td>IMP</td>
<td>23(16.1)</td>
<td>6(5.6)</td>
<td>3(3.2)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>1(3.1)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>S</td>
<td>124(87.3)</td>
<td>63(59.4)</td>
<td>90(96.7)</td>
<td>49(68)</td>
<td>48(94.1)</td>
<td>29(90.6)</td>
<td>10(58.8)</td>
</tr>
<tr>
<td>E</td>
<td>122(85.9)</td>
<td>61(57.5)</td>
<td>88(94.6)</td>
<td>50(69.4)</td>
<td>47(92.1)</td>
<td>28(87.5)</td>
<td>12(70.5)</td>
</tr>
<tr>
<td>TE</td>
<td>129(90.8)</td>
<td>60(56.6)</td>
<td>84(90.3)</td>
<td>52(72.2)</td>
<td>44(86.2)</td>
<td>28(87.5)</td>
<td>14(82.3)</td>
</tr>
</tbody>
</table>

Data were presented as numbers (No.) and percentages (%) of pathogenic bacteria that were resistant to 14 antimicrobials.

in hospitals for prolonged periods. In this study, 513 different bacterial strains were isolated from 295 swabs of hospitalized patients with burn infections over the three years. Gram negative bacteria were responsible for more than half of infections while gram positive bacteria accounted for 34.7% of overall bacterial isolates.

Pseudomonas aeruginosa was the most common bacteria, accounting for 27.6% of total isolates. These results are in agreement with previous studies. 

This pathogen is well adapted to the hospital environments due to biofilm formation that provides long survival advantages for the pathogen, and effectively prevent eradication by the host immune system or antimicrobial drug treatment. 

Pseudomonas aeruginosa has become responsible for more than 70% of mortality in burn patients. The results of this study showed that MRSA was the second most common bacteria isolated from patients with burn infection, 106 isolates (20.6%)
was the most opportunistic pathogen which causes burn infection during January 2015 to December 2017. The results of this study showed that Escherichia coli, A.baumannii and S.typhi were prevalent in different percentages, 10%, 6.2% 3.3%, respectively. Our results are similar with some previous studies. On the other hand, the results of the current study proved that there was a positive relationship between a longer stay in hospital and the high prevalence of pathogenic bacteria causing burn infections. Contaminated burned wards and duration of patients stay in hospital, in addition to the size of surface area of burned skin are the most important reasons to increase of persistent and multiplication of pathogenic bacteria in the burned areas. There are a number of factors that influence the emergence of infection in burns patient including; prolonged hospital stays, contamination of burns wards, nature of burn injury itself, as well as intensive diagnostic and therapeutic procedures. Some studies suggested that burn infection is the most common type of infection, while, others studies reports show it to be bacteremia and pneumonia. In this study, most pathogenic bacteria isolated from burn infection were highly resistant to most antimicrobials, especially against beta-lactams and 3rd generation cephalosporins. All pathogenic bacteria were MDR with high percentages and most of them were XDR. P. aeruginosa was the most common PDR- bacteria followed by MRSA and A.baumannii. These results are similar with many previous studies. Biofilm formation by microorganisms is one of the most important mechanisms in antimicrobials resistant, consisting of the irreversible assemblage of bacterial cells associated with a surface and enclosed in matrix of polysaccharides. Biofilms are regarded as a major factor contribution to many chronic inflammatory diseases such as burn infection due to enabling bacteria to colonize the burned skin, altering growth rate and allowing genes to be transcribed that provide these pathogens to high resistance to antimicrobials and host immune system. The overuse and unsuitability of different antimicrobials to treat burn infections has led to the emergence of new MDR, XDR and PDR-bacterial strains that are able to resistant a wide range of many antimicrobials such as aminoglycosides, beta-lactams, cephalosporins, streptomycin and tetracycline. Burn infection in hospitalized patients caused by MDR, XDR and PDR-gram negative and gram positive bacteria such as; P. aeruginosa, K. pneumoniae, MRSA, MSSA and A.baumannii may lead to delays in burn healing, graft lose, as well as development of sepsis and death; therefore, determination of the risk factors for these pathogens infections is essential for infection control. The results of this study showed that P. aeruginosa and K. pneumoniae were the most common ESBL-producing gram negative bacteria followed by A.baumannii and E.coli while there was no any strain of ESBL- S.typhi. These results are similar to previous studies. Infections caused by ESBL-producing gram negative bacteria are associated with an increase of health care costs, morbidity and mortality. Extended spectrum beta-lactamases (ESBLs) have been reported as one of the most important hospital-acquired infections such as burn infection and bacteremia. Most bacteria harboring ESBLs are usually resistant to beta-lactam antibiotics and other classes of antimicrobials. These enzymes are carried

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>ESBL</th>
<th>Non-ESBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhi</td>
<td>0 (0.00%)</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>4 (12.50%)</td>
<td>28 (87.50%)</td>
</tr>
<tr>
<td>E. coli</td>
<td>3 (5.90%)</td>
<td>48 (94.10%)</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>21 (22.60%)</td>
<td>72 (77.40%)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>51 (35.90%)</td>
<td>91 (64.10%)</td>
</tr>
</tbody>
</table>

Figure 4. Numbers and percentages of extended spectrum beta-lactamase-producing bacteria isolated from hospitalized patients with burn infection during January 2015 to December 2017. ESBL: extended spectrum beta-lactamase-producing bacteria.
in and transferred from bacteria to bacteria by plasmids. The most important steps to ensure the safety of patients with burn infections are: to control the spread of ESBL-producing bacteria, isolation of colonized patients in sterile wards, and continuously performing antimicrobial sensitivity tests.

Conclusions

There was a high incidence of MDR-bacteria causing burn infections in Al-Najaf hospital in Al-Najaf City, Iraq. Pseudomonas aeruginosa was the most common MDR, XDR and PDR-bacteria, and the most common of ESBL-producing bacteria causing burn infection over three years followed by MRSA. Imipenem 10µg had good antibacterial activity against more than 93% of bacterial isolates. There was positive correlation between a long stay in hospital and high prevalence of pathogenic bacteria causing burn infection.

Limitation of the study

In this study, some gram negative and gram positive bacterial isolates are excluded because of the small number of isolates (less than seven isolates over the three years) such as proteus spp (5 isolates), citrobacter spp (4 isolates), enterobacter spp (6 isolates) and enterococcus spp (5 isolates). We think this small number of bacterial isolates don’t has any significant effect on the results of this study.

Data availability

Dataset 1: Test results for patient swabs 10.5256/f1000research.15088.d211541

Competing interests

No competing interests were disclosed.

Grant information

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References


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