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: 2 approved with reservations]

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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 of 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 10 μ g and 464 isolates (90.4%) were multi-drug resistant, 20 isolates (14%) were extensive-

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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 countries¹. Fire leads to skin destruction and simultaneous suppression of both humoral and cellular immune system subsequently resulting burn infection². Complications of burn infection are responsible for more than 70% of death cases among inpatients with burns³. These infections mainly caused by multi-drug resistant gram-negative and gram-positive bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Staphylococcus aureus* (*S. aureus*)^{4,5}. Non-sterile burns 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^{6,7}. Multi-drug resistant (MDR) bacteria is one of the most common pathogens causing burn infection in hospitalized patients worldwide^{8,9}. These pathogens are resisting to at least three different classes of antimicrobials such as, penicillin's, beta-lactams, cepheems, 3rd and 4th generation cephalosporins, aminoglycosides, tetracyclines and quinolones, and is becoming one of the most dangerous health issues in hospitals¹⁰. 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^{11,12}. Burn infection is characterized by difficult healing due to administration of unsuitable treatment, long stays in hospital and the contamination of hospital environments lead to the emergence of new multi-drug resistant bacterial isolates causing dangerous complications such as, bacteremia, septicemia and death^{13,14}. Therefore, we must pay attention to all safety standards in hospitals, especially in burn 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, imvic test, motility test, coagulase test, growth on MacConkey agar (Oxoid™, USA, R061322) and Mannitol salt agar (Oxoid™, USA, CM0085B)¹⁵.

Antimicrobials susceptibility test

Antimicrobials susceptibility testing was performed by disc diffusion method according to Kirby-Bauer method onto Mueller Hinton agar (Oxoid™, USA, PO5007A) surface¹⁶. Fourteen different antimicrobial discs were used in this study provide from Oxoid™, 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 2017¹⁷. 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 resistance to all antimicrobials class considered as pan-drug resistant (PDR)¹⁷.

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)¹⁸.

Phenotypic detection of extended spectrum beta-lactamase-producing bacteria

This test was performed according to modified double disc synergy test (MDDST)¹⁹ 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%)

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.

Pathogenic bacteria	Duration of stay in hospital			Total No. (%)
	5 days	10 days	15 days	
	No. (%)	No. (%)	No. (%)	
<i>P. aeruginosa</i>	24	33	85	142(27.6)
MRSA	10	30	66	106(20.6)
<i>K. pneumoniae</i>	18	30	45	93(18.2)
MSSA	10	12	50	72(14.1)
<i>E. coli</i>	1	10	40	51(10)
<i>A.baumannii</i>	2	8	22	32(6.2)
<i>S.typhi</i>	0	2	15	17(3.3)
Total	65(12.6)	125(24.4)	323(63)	513(100)

Data were presented as numbers (NO.) and percentages (%) of bacterial isolates. MRSA: Methicillin resistance *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

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Discussion

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

Table 2. Numbers and percentages of pathogenic bacteria isolated from hospitalized patients with burn infection according to type of bacterial isolates and duration of stay in hospital during January 2015 to December 2017. N=513.

Type of bacterial isolates	Duration of stay in hospital			Total No. (%)
	5 days No. (%)	10 days No. (%)	15 days No. (%)	
Single isolate	10(1.9)	27(5.3)	85(16.7)	122(23.8)
Mixed isolates	55(10.7)	98(19.1)	238(46.3)	391(76.2)
Total	65(12.6)	125(24.4)	323(63)	513(100)

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

Table 3. Numbers and percentages of pathogenic bacteria isolated from hospitalized patients with burn infection during January 2015 to December 2017 that were resistant to 14 antimicrobials.

AB.	<i>P. aeruginosa</i> 142 No. (100%)	MRSA 106 No. (100%)	<i>K. pneumoniae</i> 93 No. (100%)	MSSA 72 No. (100%)	<i>E. coli</i> 51 No. (100%)	<i>A.baumannii</i> 32 No. (100%)	<i>S.typhi</i> 17 No. (100%)
P	142(100)	106(100)	93(100)	70(97.2)	45(88.2)	30(93.7)	12(70.5)
AX	142(100)	106(100)	85(91.3)	66(91.6)	40(78.4)	29(90.6)	13(76.4)
AMC	140(98.5)	99(93.3)	80(86)	65(90.2)	39(76.4)	28(87.5)	10(58.8)
CRO	130 (91.5)	90(84.9)	80(86)	33(45.8)	35(68.6)	28(87.5)	11(64.7)
CTX	128(90.1)	96(90.5)	81(87)	36(50)	38(74.5)	27(84.3)	11(64.7)
CAZ	132(92.5)	100(94.3)	86(92.4)	39(54.1)	39(76.4)	30(93.7)	10(58.8)
GM	102(71.8)	80(75.4)	75(80.6)	35(48.6)	40(78.4)	18(65.2)	8(47)
TM	112(78.8)	75(70.7)	74(79.5)	40(55.5)	25(49)	19(59.3)	7(41.1)
NA	110(77.4)	81(76.4)	40(43)	42(58.3)	20(39.2)	15(46.8)	7(41.1)
CIP	125(88)	88(83)	70(75.2)	60(83.3)	18(35.2)	24(75)	9(52.9)
IMP	23(16.1)	6(5.6)	3(3.2)	0(0.0)	0(0.0)	1(3.1)	0(0.0)
S	124(87.3)	63(59.4)	90(96.7)	49(68)	48(94.1)	29(90.6)	10(58.8)
E	122(85.9)	61(57.5)	88(94.6)	50(69.4)	47(92.1)	28(87.5)	12(70.5)
TE	129(90.8)	60(56.6)	84(90.3)	52(72.2)	44(86.2)	28(87.5)	14(82.3)

Data were presented as numbers (No.) and percentages (100%) of pathogenic bacteria that were resistant to antimicrobials.

AB: Antimicrobials, P: Penicillin 10IU, AX: amoxicillin 25µg, AMC: Amoxiclav 30µg, CRO: Ceftriaxone 30µg, CTX: Cefotaxime 30µg, CAZ: Ceftazidime 30µg, GM: Gentamicin 10µg, TM: Tobramycin 10µg, NA: Amikacin 30µg, CIP: Ciprofloxacin 5µg, IMP: Imipenem 10µg, S: Streptomycin 10µg, E: Erythromycin 30µg, TE: Tetracycline 30µg, MRSA: Methicillin resistance *S.aureus*, MSSA: Methicillin sensitive *S.aureus*.

Figure 2. Numbers and percentages of overall resistance to 14 antimicrobials of pathogenic bacteria isolated from hospitalized patients with burn infection during January 2015 to December 2017. N=513.

Table 4. Numbers and percentages of multi-drug resistant, extended-drug resistant and pan-drug resistant pathogenic bacteria isolated from hospitalized patients with burn infection during January 2015 to December 2017. N=513.

Pathogenic bacteria	MDR No. (%)	XDR No. (%)	PDR No. (%)
<i>P. aeruginosa</i> 142	130(91.5)	4(2.8)	8(5.6)
MRSA 106	95(89.6)	6(5.6)	5(4.7)
<i>K. pneumoniae</i> 93	84(90.3)	6(6.4)	3(3.2)
MSSA 72	70(97.2)	2(2.7)	0(0.0)
<i>E. coli</i> 51	42(82.3)	0(0.0)	0(0.0)
<i>A.baumannii</i> 32	29(90.6)	2(6.2)	1(3.1)
<i>S.typhi</i> 17	14(82.3)	0(0.0)	0(0.0)
Total 513	464(90.4)	20(14)	17(3.3)

Data were presented as numbers (No.) and percentages (%) of pathogenic bacteria. MDR: multi-drug resistant, XDR: extensive-drug resistant, PDR: pan-drug resistant, MRSA: Methicillin resistance *S.aureus*, MSSA: Methicillin sensitive *S.aureus*.

Figure 3. Modified double disc synergy test show positive result of extended spectrum beta-lactamase-producing *Pseudomonas aeruginosa* on Muller-Hinton agar.

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 studies²⁰⁻²³. *Pseudomonas aeruginosa* is one of the most important pathogens causing

different infections such as bacteremia and burn infections²⁴. 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 treatment²⁵. *Pseudomonas aeruginosa* has become responsible for more than 70% of mortality in burn patients^{26,27}. The results of this study showed that MRSA was the second most common bacteria isolated from patients with burn infection, 106 isolates (20.6%)

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.

of total bacterial isolates, while, MSSA was the most 4th common pathogen with 72 isolates (14.1%). These results are similar with many previous studies^{28,29}. *Staphylococcus aureus* is the most common bacteria causing both hospital and community associated infections, including bacteremia, pneumonia and burn infection³⁰. Hospital-associated MRSA accounts for a high proportion of hospitalized infected with *S. aureus*³¹. As compare with different bacteria that cause burn infection in hospitalized patients, MRSA-infections is associated with higher morbidity and mortality³². *Klebsiella pneumoniae* was the most 3rd common bacteria isolated from burn infection in this study with, 93 isolates (18.2%). This result is similar to past studies^{33,34}. *Klebsiella pneumoniae* is an opportunistic pathogen which causes serious infections like, urinary tract infection, pneumonia, burn infection, and soft tissue infections in compromised and hospitalized patients. It has number of virulence factors such as a capsule that enable this pathogen to colonize and provides phagocytosis resistance^{35,36}. 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^{37–39}. 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 burning 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 areas⁴⁰. 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^{41,42}. Some studies suggested that burn infection is the most common type of infection, while, others studies reports show it to be bacteremia and pneumonia^{43,44}. 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^{5,45–47}. 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 material⁴⁸. Biofilms are regarding 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^{49,50}. 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 control⁴⁷. 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^{1,51,52}. Infections caused by ESBL-producing gram negative bacteria are associated with an increase of health care costs, morbidity and mortality^{53,54}. Extended spectrum beta-lactamases (ESBLs) have been reported as one of the most important hospital-acquired infections such as burn infection and bacteremia^{9,55}. Most bacteria harboring ESBLs are usually resistant to beta-lactam antibiotics and other classes of antimicrobials⁵⁶. These enzymes are carried

in and transferred from bacteria to bacteria by plasmids^{57,58}. 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 tests⁵⁹.

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](https://doi.org/10.5256/f1000research.15088.d211541)⁶⁰

Competing interests

No competing interests were disclosed.

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Dana M. Walsh

Rebiotix, Roseville, MN, USA

This paper provides an observational report of the number of drug resistant bacteria isolated from infected burn wounds in patients in a hospital in Iraq. Increasing numbers of drug resistant organisms are identified with increasing length of hospital stay. The most commonly identified organism was *Pseudomonas aeruginosa*, a bacteria that commonly identified in burn wound infections.

Overall, the science in this article is sound. Appropriate MDR testing is used and compared to accepted standards.

Methods section: It is unclear if an institutional review board approved this study; this needs to be directly mentioned in the methods section. No statistics were performed on this study; however, a simple Student's T test could be performed to determine statistical significance between two groups or analysis of variance (ANOVA) could be performed to show statistically significant differences between the number of isolates depending on length of hospital stay. This could also be done for number and type of drug resistant organisms to show which antibiotic has the least resistance. Including statistics would strengthen this work.

Discussion section: Why are biofilms mentioned at all? This seems out of place, especially since no testing was done to determine if these microbes are capable of making biofilms. This either needs to be removed or more appropriately addressed in the context of the study.

Conclusions section: This could be strengthened. For example, it seems that a majority of the organisms were not resistant to imipenem. This is an important finding for this hospital and should be mentioned as an antibiotic that could help this facility control MDR infections. It would be helpful if the authors addressed the following questions in this section: What new information does this study provide? Is this important for the hospital's burn ward? Will this increase awareness of the prevalence of MDR organisms and encourage physicians to use imipenem for treatment of infections?

Other comments: Metagenomic sequencing and biofilm testing would both be interesting additions to this study. Metagenomics could be used to determine what other bacteria were present in the infections that were not culturable.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Toxicology, microbiology, metagenomics, bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 02 July 2019

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Vikrant Chitnis

Department of Pathology, Choithram Hospital and Research Center, Manik Bagh, Indore, India

1. Abstract needs rewriting with focus on role of hospital infection control committee (HICC), if existing in hospital. How HICC has helped or not or will help in near future in preventing such infections in burn unit.
2. Name of organism in abstract: staphylococcus typhi? No such organism exists either it is *staphylococcus aureus* or *salmonella typhi*.

3. There are many grammatical errors throughout the manuscript and very long sentences needs revision.
4. Study is not useful if you do not involve hospital infection practices and corrective action.
5. Is there an antibiotic policy in the hospital? If yes, are they drawing data to take care of antibiotic resistance? Are microbiologist active? Could be mentioned as corrective action in discussion or as it is suitable.
6. Role of hospital infection control committee?
7. What disinfectants were used?
8. Why they did not go for anaerobic culture?
9. Inclusion criteria of study needs redefining. Why patients above 60 were not chosen - justification needed, is it due to weak immunity or no such patient was admitted during study time?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biomedical waste disposal, hospital infection control, bacteriology, molecular biology, disinfectants.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Jul 2019

ahmed abduljabbar jaloob aljanaby, University of Kufa, Najaf, Iraq

Greetings

- We think the abstract does not need rewriting with focus on role of hospital infection

control committee (HICC), because the our work focused on types of bacteria and antimicrobials.

- The name of organism in abstract: *Staphylococcus typhi*. Yes, this is a print mistake, we are so sorry the right name of organism in abstract must be *Salmonella typhi*. We can correct this mistake.
- There was an antibiotic policy in the hospital according to antimicrobial sensitivity test protocol.
- The role of hospital infection control committee is not in our study, because the only goal of our study is the type of aerobic bacteria and antimicrobials.
- About your question: what disinfectants were used in hospitals? We don't know, this is hospital work only and we can not know about these security things.
- About your question: why they did not go for anaerobic culture? This is another different study, our study focused on aerobic bacteria only.
- About your question: why patients above 60 were not chosen. Because we did not find patients above 60 in our study.

Competing Interests: There was not found competing interests

Author Response 09 Jul 2019

ahmed abduljabbar jaloob aljanaby, University of Kufa, Najaf, Iraq

Greetings,

- We think the abstract does not need rewriting with focus on role of hospital infection control committee (HICC), because our work focused on types of bacteria and antimicrobials.
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- About your question, Why patients above 60 were not chosen? Because we did not find patients above 60 in our study.

Competing Interests: No competing interests were disclosed.

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