Isolation and Identification of Bacterial Burn Wound Infection in Iraqi Patient

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Abstract

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality**.**In current study, 120 samples were collected from 120 patients suffering from contaminated burns. The study was conducted after obtaining ethical approvals from the ethics committee in the Department of Biology, College of Science, University of Baghdad as well after obtaining the patients 'consent. Samples are collected from patients after they have stopped using antibiotics for 48 hours.After the swabs had been cultured on different media, conventional biochemical tests to identify bacterial isolates and antimicrobial sensitivity to the most common antibiotics were performed by vitek 2 compact .The results showed that the highest percentage of bacterial species was Proteus mirabilis (31.1 %). The percentage of isolation of P. aeruginosa was 17.78%. The lowest percentage of bacterial isolates that isolated from infected wound was found in case of Staphylococcus aureus, Pseudomonas fluorescens, Acinetobacterhaemolyticus, Burkholderiacepacia, Salmonella ser. gallinarum, Sphingomonaspaucimobilis, Comamonas testosterone with 2.2 % for each isolate.

Key word: *Burn, wound , Antibiotic , Comamonas testosterone, Sphingomonaspaucimobilis*

Introduction

Burns are one of the foremost common and destroying shapes of injury. Patients with effective thermal injury require prompt specialized care in arrange to reduce morbidity and mortality mortality. Information from the National Center for Injury Avoidance and Control within the Joined together States appear that roughly 2 million fires were investigated each year which result in 1.2 million individuals with burn wounds [1].Direct to extreme burn wounds requiring hospitalization account for roughly hundred thousands of the cases, and around 5 % of patients with bourn wond infection were died on each year from burn-related complications [2].

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The survival rates for burn patients have made strides significantly within the past few decades due to progresses in advanced therapeutic care in specialized burn centers. Improved outcomes for seriously burned patients have been credited to restorative progresses in liquid revival, wholesome back, pneumonic care; burn wound care, and disease control. As a result, burn-related deaths are depending on the degree of damage, have been split inside the past 40 years [3]. In patients with serious burns over more than 40% of the total body surface area (TBSA), 75% of all deaths were related to sepsis from burn wound disease or other contamination complications and/or inhalation injury [4].

Microbes quickly colonize open skin wounds after burn damage. Microorganisms colonizing the burn wound start from the patient's endogenous skin and gastrointestinal and respiratory vegetation. Microorganisms may too be exchanged to a patient's skin surface by means of contact with contaminated external environmental surfaces, water, air and the

dirtied hands of health care persons [5]. Quickly taking after damage, gram-positive bacteria organisms from the patient's endogenous skin vegetation or the outside environment transcendently colonize the burn wound [6]. Endogenous gram-negative microbes from the patient's gastrointestinal greenery too quickly colonize the burn wound surface within the to begin with few days after damage [7].

Staphylococcus aureus got to be the vital etiological agent of burn wound infection. After the discover penicillin G within the early 1950s, which come about within the virtual reducing of Streptococcus pyogenes as a cause of contamination in thermally harmed patients[8]. In spite of the fact that *S. aureus* remains a common cause of early burn wound contamination, *Pseudomonas aeruginosa* and *Proteusmirabilis* from the patient's endogenous gastrointestinal vegetation and/or a natural source is the foremost common cause of burn wound contaminations in numerous centers.The rate of diseases due to less commonly experienced organisms, counting other gram-positive and gram-negative microscopic organisms, fungi, and viruses, has too expanded relentlessly in subsequent decades[9].

Material and Method

Specimen Collection

In current study, 120 samples were collected from 120 patients suffering from contaminated burns. Samples were collected under sterile conditions using sterile swabs. The samples were immediately transported to the laboratory to be implanted in the appropriate media.

The average age of the patients was 42.6 ± 5.8 years. The number of males was 72 and the number of females 48. The study was conducted after obtaining ethical approvals from the ethics committee in the Department of Biology, College of Science, University of Baghdad as well after obtaining the patients 'consent. Samples are collected from patients after they have stopped using antibiotics for 48 hours.

Bacterial isolation

The collected samples were cultured on MacConkey agar, Blood agar under aerobic and sterile conditions. To diagnose the isolated bacteria the select colonies were re-cultured on mannitol salt agar, SS agar, nutrient agar, XLD agar and EMB agar. For further identification of isolated bacteria catalase test, oxidase test and Gram stain were used to identify the pure isolated bacteria [10].

Microscopic Examination

The morphological identification of the isolates as bacilliwas confirmed microscopically by performing Gram staining, for which single colony of each isolate was picked up and stained as per the standard protocol and viewed under oil immersion for similar type of cells.

Catalase test

The collected samples were cultured on MacConkey agar, Blood agar under aerobic condition and sterile conditions**,** usea loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide then Place a drop of 3% H2O2 in the glass slide the result observed for the evolution of oxygen bubbles [10].

Oxidase test

The collected samples were cultured on MacConkey agar, Blood agar under aerobic condition and sterile conditions**,** strip of Whatman's No. 1 filter paper are soaked in a freshly prepared 1% solution of tertramethyl-p-phenylene-diaminedihydrochloride, After draining for about 30 seconds, the strips are freeze dried and stored in a dark bottle tightly sealed with a screw cap, for use, a strip is removed, laid in a petri dish and moistened with distilled water. The colony to be tested is picked up with a platinum loop and smeared over the moist area. A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds, a "delayed positive" reaction by colouration in 10-60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds[10].

Identification using the VITEK 2 fluorescent system (ID-GNB card)

The VITEK 2 DensiCheck instrument, fluorescence system (bioMe´rieux) (ID-GBB card and ID- GNB card) includes 43 non enterobacterial gram-negative taxa and gram positive. Testing was performed according to the instructions of the manufacturer. Briefly, strains were cultured on nutrient agar for 18 to 24 h at 37°C before the isolate was subjected to analysis. A bacterial suspension was adjusted to a McFarland standard of 0.50 to 0.63 in a solution of 0.45% sodium chloride using the VITEK 2 DensiCheck instrument (bioMe´rieux). The time between preparation of the solution and filling of the card was always less than 1 h. Analysis was done using the identification card for gram-negative and gram positive bacteria (ID-GNB card) and (ID-GBB) containing 41 fluorescent biochemical tests. Cards are automatically read every 15 min. Data were analyzed using the VITEK 2 software version VT2- R03.1 [11].

Antibiotic susceptibility

The standard method of Mazzariol*et al.* (2008) was followed to test the susceptibility of identified bacteria to the several antibiotics (Cefotaxime, Ampicillin, amoxicillin/Clavulanic acid, ampicillin/Sulbactam, Piperacillin/ Tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, imipenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole, tricarcillin, amikacin). VITEK 2 DensiCheck instrument (bioMe´rieux) was used to check the supportability of isolated and identified bacteria [12].

Results and Discussion

Isolation and identification of bacterial species

In present study, 120 swabs were collected from infected burns. The samples were collected from 120 patients. The swabs were inoculated onto number of culture media (Blood agar, MacConkey agar, Manitol salt agar, SS agar and XLD agar) for growing and isolating and then for pre-identification. Most of isolates were grown on blood agar with different shape according to the genera of isolates. The suspected staphylococcus isolates were cultured onto mannitol salt agar to identify the staphylococcus species. Some of isolates were grown on MacConkey agar with pale or pink color. The bacteria that grown onto MacConkey agar with pale color were cultured onto SS agar and XLD to identify whether they were Salmonella or Shigella isolates [13]. Total pre-identified species of bacteria was 50 species but when further identification was done by VITIK 2 technology, only 45 species was identified and 5 was specified as unidentified organism. Thus the further study was done only on the 45 species that isolated from infected wound and identified by VITIK 2 technology (**Table 1**). The results showed that the highest percentage of bacterial species was *Proteus mirabilis* (31.1 %). The percentage of isolation of *P. aeruginosa* was 17.78%. The lowest percentage of bacterial isolates that isolated from infected wound was found in case of *Staphylococcus aureus*, *Pseudomonas fluorescens, Acinetobacterhaemolyticus, Burkholderiacepacia, Salmonella ser. gallinarum, Sphingomonaspaucimobilis, Comamonas testosterone* with 2.2 % for each isolate .

Previous study of Forson*et al.* (2017) mentioned that *P. aeruginosa* represented the highest percentage of bacterial species that isolated from burn wound, while Church *et al.* (2006) reported that the highest percentage of bacterial species that isolated from infected burn wound was *P. aeruginosa* followed by *E. coli* and the lowest percentage was found in case of *Acinetobacterspp and Bacteroides* spp. Similar finding was reported by other investigators[16].

Table 1:- Number and percentage of bacterial species that isolated from 120 clinical samples.

	Bacteria	Number	Percentage (%)
1	Proteus mirabilis	14 (Pm1, Pm2, Pm3, Pm4, Pm5, Pm6, Pm7, Pm8, Pm9, Pm10, Pm11, Pm12, Pm13, Pm 14).	31.1
2	Escherichia coli	10 (Ec1, Ec2, Ec3, Ec4, Ec5, Ec6, Ec7, Ec8, Ec9, Ec10)	22.2
3	Pseudomonas aeruginosa	8 (Pa1, Pa2, Pa3, Pa4, Pa5, Pa6, Pa7, Pa8).	17.78
4	KlebsiellaPneumoniae	2 (Kp1,Kp2).	4.4
5	Serratiaficaria	2 (Sf1, Sf2).	4.4
6	Burkholderia mallei	2 (Bm1, Bm2).	4.4
7	Staphylococcus aureus	1 (Sa1)	2.2
8	Pseudomonas fluorescens	1 (Pf1)	2.2
9	Acinetobacterhaemolyticus	1 (Ah1)	2.2
10	Burkholderiacepacia	1 (Bc1)	2.2
11	Salmonella ser. Gallinarum	1 (Sg1)	2.2
12	Sphingomonaspaucimobilis	1 (Sp1)	2.2
13	Comamonas testosterone	1 (Ct1)	2.2

The diversity of bacteria species isolated from infected wounds was one of the features that distinguished the present study. Bacteria rapidly colonize open skin wounds after burn injury. Microorganisms colonizing the burn wound originate from the patient's endogenous skin and gastrointestinal and respiratory flora [17]. Microorganisms may also be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, water, fomites, air, and the soiled hands of health care workers [15]. Immediately following injury, gram-positive bacteria from the patient's endogenous skin flora or the external environment predominantly colonize the burn wound [18]. Endogenous gram-negative bacteria from the patient's gastrointestinal flora also rapidly colonize the burn wound surface in the first few days after injury [15]. Microorganisms transmitted from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora [19].

Previous study, 185 (61.87%) bacteria were isolated from the wounds of burnt patients. Among the culture positive samples, 112 (60.54%) were from female patients and 73 (39.46%) were from male patients. The most commonly isolated organisms were Pseudomonas species (43%). *K. pneumoniae* and *A. baumannii* were second and third predominant bacterial pathogen with a prevalence of 28% and 14.83% respectively. Similar finding with *P. aeruginosa* a predominant isolate followed by *K. pneumoniae* and *A. baumanniii*natertiary care hospital in India were also reported [20]. High

prevalence of these pathogens is associated with their ability to flourish well in a moist environment and persistence in hospital environment [20]. In present study, *P. aeruginosa* was reported as one of domain species that isolated from burn wound infection.

Antibiotic susceptibility

The susceptibility of 45 isolates to different antibiotics was done by VITIK 2 DensiCheck instrument. The antibiotics that used were different according to the group of species of bacteria because the routinely antibiotic that used clinically was different according to the clinical cases and species [21] that covered in the study.

The current results showed that the effect of antibiotics varies greatly according to the species of bacterial used and the type of antibiotics. Where, many types of antibiotics were used in present study. Through an overview of the results, it can be confirmed that there are no bacterial species sensitive to all antibiotics used, and no bacterial isolate that resists to all antibiotics . Figure (1) shows that the *P. mirabilis* gave the highest percentage of resistance to different kinds of antibiotic, followed by the *P. aeruginosa*. While, the lowest percentage of sensitivity to different kind of antibiotics was shared among *S. paucimobilis: C. testosterone and B. mallei.* The present study showed that the highest percentage of intermediate response of bacteria to antibiotics was seen in case of *S. paucimobilis* followed by *P.fluorescens*.

Nosocomial infection in the burnt patients is major challenge for a clinician. It has been estimated that 75% of all deaths in burnt patients were associated with infections. Prolonged use of antibiotic leads to the development as well as selection of multidrug resistant (MDR) bacteria which results in treatment failure and intensifies the complications. Thus, the information of microbial flora and the current antibiotic susceptibility patterns are important for the clinician treating burn sepsis [21].

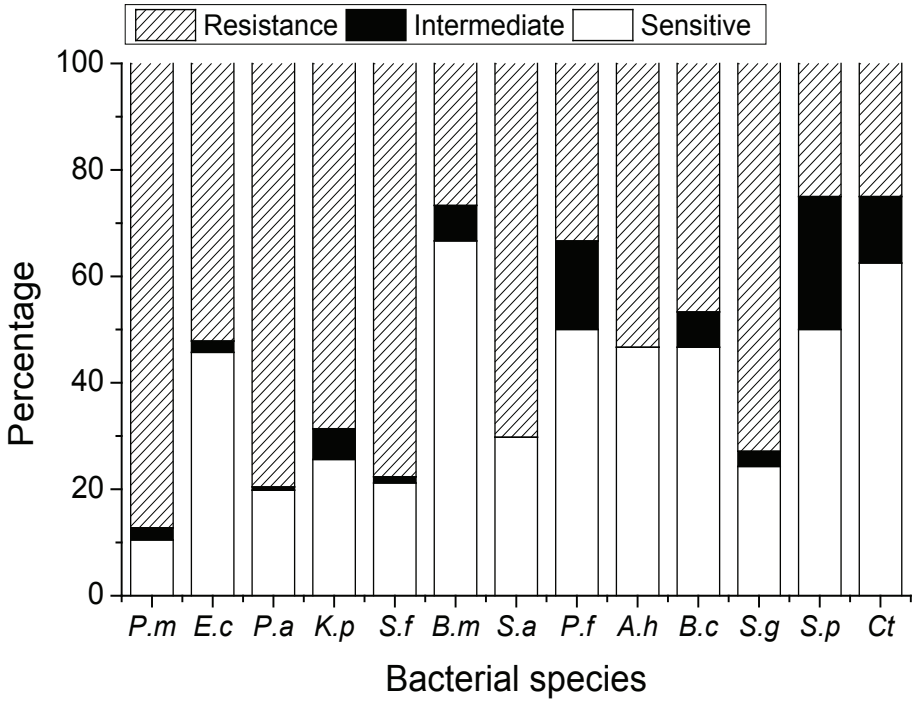

Figure 1:- The percentages of susceptibility of different species of bacteria to different kinds of antibiotics (*P.m: P. mirabilis, E.c: E. coli, P.a: P. aeruginosa, K.p: K. pneumonia, S.f: S. ficaria; B.m: B. mallei, S.a: S. aureus, P.f: P.fluorescens, A.h:A. haemolyticus, B.c: B. cepacia, S.g:S. gallinarum, S.p: S. paucimobilis, C.t: C. testosterone.*

Conflict of Interest: The authors declare that there is no conflict of interest regarding this study.

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