Bacterial isolation from burn wound infections and studying their antimicrobial susceptibility

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Abstract:

The present study was carried out to determine the bacterial isolates and study their antimicrobial susceptibility in case of burned wound infections. 70 burn wound swabs were taken from patients, who presented invasive burn wound infection from both sex and average age of 3-58 years, admitted to teaching medical Al- Kendi hospital from October 2007 to June 2008.

Pseudomonas aeruginosa was found to be the most common isolate (48.9%) followed by *Staphylococcus aureus* (24.4%), *Citrobacter braakii* (13.3%), *Enterobacter spp*. (11.1%), Coagulase-negative *Staphylococci* (11.1%), *Proteus vulgaris* (6.66%), *Corynebacterium spp*. (6.66%), *Micrococcus* (6.66%), *Proteus mirabilis* (4.44%), *Enterococcus faecalis* (4.44%), *E.coli* (4.44%), *Klebsiella spp*. (2.22%), *Bacillus spp*. (2.22%), *Serratia macerscens* (2.22%) and *Serratia rubidia* (2.22%). Antimicrobial susceptibility testing was carried out to the bacterial isolates against 8 antibiotics, in which ciprofloxacin was found to be the most effective drug against most of the Gram-negative and Gram-positive isolates followed by amikacin, while chloramphenicol and gentamicin were less sensitive to few isolates as well as as doxycycline, as compared with the other two, mentioned previously. Oxacillin was the worst at all.

عزل البكتريا من إصابات الجروح الحرقية ودراسة حساسيتها للمضادات الحيوية

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الخلاصة:

أجريت الدراسة الحالية لتحديد الأنواع البكتيرية المعزولة من أخماج الجروح الحرقية، ودراسة حساسيتها للمضادات الحيوية. أخذت 70 مسحة قطنية من المرضى المصابين بالحروق؛ لكلا الجنسين وبمتوسط أعمار 3 -58 سنة، أدخلوا المستشفى التعليمي لكلية طب الكندي للفترة من تشرين الأول 2007 إلى حزيران 2008. وجد أن *Pseudomonas aeruginosa* كانت الأكثر عزلا بنسبة (48.9%) وتلتها *Staphylococcus aureus* بنسبة عزل (24.4%) و *Citrobacter braakii*(13.3%) و *Enterobacter spp*. بنسبة (11.1%) و Coagulase-negative *Staphylococci* (11.1%) ، وكانت نسبة العزل (6.66 %) لكل من *Proteus vulgaris* و *Corynebacterium spp*. و

Micrococcus. بلغت نسبة العزل (4.44 %) لكل من *E.coli* و *Enterococcus faecalis* و *Proteus mirabilis* ، وكانت نسبة العزلات (2.22 %) لكل من *Serratia macerscens* و *Bacillus spp*. و *Klebsiella spp*. و *Serratia rubidia*.

اختبرت حساسية العزلات الجرثومية تجاه ثمان من المضادات الحيوية، ووجدنا أن ciprofloxacin كان الأفضل مقارنة بالمضادات الحيوية الأخرى سواء تجاه البكتريا السالبة أو الموجبة لصبغة الكرام، وكذلك الحال بالنسبة للمضاد الحيوي amikacin الذي كان مؤثرا ضد الكثير من العزلات المدروسة، بينما كان chloramphenicol و gentamicin أقل حساسية لبعض العزلات، فضلا عن doxycycline، مقارنة بالمضادين المذكورين سابقا. كان المضاد الحيوي oxacillin الأسوأ على الإطلاق.

Introduction:

Infection is an important cause of morbidity and mortality in hospitalized burn patients (1), in patients with burn over more than 40% of the total body surface area, 75% of all deaths following thermal injuries are related to infections (2). The rate of nosocomial infections is higher in burn patients due to various factors like nature of burn injury itself, immunocompromised status of the patient (3), age of the patient, extent of injury, and depth of burn in combination with microbial factors such as type and number of organisms, enzyme and toxin production, colonization of the burn wound site, systemic dissemination of the colonizing organisms (4). The denatured protein of the burn eschar provides nutrition for the organisms. Avascularity of the burned tissue places the organisms beyond the reach of host defense mechanisms and systemically administered antibiotics (5).

In addition, cross-infection results between different burn patients due to overcrowding in burn wards (6). Also thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humeral immune responses are pivotal factors contributing to infectious complication in patients with severe burn (7). Burn wound infections are largely hospital-acquired and the infecting pathogens differ from one hospital to another (8).

The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin; thermal injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients (9).

Burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms (10), gram-positive bacteria that survive the thermal insult, such as *S. aureus* located deep within sweat glands and hair follicles, heavily colonize the burn wound surface within first 48 h (10).

Topical antimicrobials decrease microbial overgrowth but seldom prevent further colonization with other potentially invasive bacteria and fungi. These are derived from the patient s

gastrointestinal and upper respiratory tract and the hospital environment (11).

Following colonization, these organisms start penetrating the viable tissue depending on their invasive capacity, local wound factors and the degree of the patient s immunosuppression (12). If sub-eschar tissue is invaded, disseminated infection is likely to occur, and the causative infective microorganisms in any burn facility change with time (13). Individual organisms are brought into the burns ward on the wounds of new patients. These organisms then persist in the resident flora of the burn treatment facility for a variable period of time, only to be replaced by newly arriving microorganisms. Introduction of new topical agents and systemic antibiotics influence the flora of the wound (14).

The aim of the present study was to obtain information about the type of isolates, identification and antimicrobial sensitivity of bacterial wound infections in burn patients.

Materials and Methods

Cultural Media:

Media used for bacterial isolation and identification are ordinary media such as Blood agar, Nutrient agar, Triptic Soya agar, and special media such as Kanamycin Aesculin azide agar, pseudomonas agar, Salmonella-Shigella agar. MacConkey agar, Mannitol salt agar and Eosin methylene blue agar.

Sample Collection:

70 burn wound swabs were taken from burned patients, who presented invasive burn wound infection, from both sex, and average age 3-58 year, admitted to burn unit of teaching medical Al-Kendi hospital from October 2007 to June 2008. The most preferred areas were the upper and lower extremities. The specimens were transported in sterile, leak- proof container to zoonotic diseases unit. All specimens were inoculated on 5% blood agar, MacConkey agar and Chocolate agar plates and incubated overnight at 37 ˚C aerobically. The sample was also put into liquid media (Brain Heart Infusion broth) and was subcultured after overnight incubation onto Blood agar and MacConkey agar. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques (13).

Antimicrobial susceptibility was performed on Mueller- Hinton agar by the standard disk diffusion method (15). The antibiotics tested for bacterial isolates were: Ciprofloxacin (Cip5), Amikacin (AK30), Ticarcilin (Tic75), Chloramphenicol (C30), Oxytetracycline (T30), Oxacillin (OX 1), Gentamicin (CN 10) and Doxycycline (Do30).

The zones of inhibition of bacterial isolates for individual antibiotics were measured in mm by applying ordinary ruler.

Results:

The various types of bacteria isolated from burn wound culture of total 70 wound swabs were shown in

table (1). Bacterial isolates were found in 45 (64.3%) wound swabs, and only 25 samples (35.7%) were negative in bacterial growth. The results showed that *P. aeruginosa* was the commonest isolate (22 isolates; 48.9%) followed by *S. aureus* (11 isolates; 24.4%), *Citrobacter braakii* (6 isolates; 13.3%). Both *Enterobacter* spp. and coagulase- negative *Staphylococci* were 5 isolates for each (11.1%). *P. vulgaris, Corynebacterium* spp. and *Micrococcus* spp. were 3 isolates for each (6.66%), P. *mirabilis*, *E. faecalis*, *E. coli* and *Streptococcus* spp. gave (2 isolates for each (4.44%). Each of *Klebsiella* spp., *Bacillus* spp., *S. marcescens* and *S. rubidia*, caused only 2.22% of cases (one isolate for each).

Most of the isolates showed mixed infection as showed in (table 1).

Table 2 showed the following results:

P. aeruginosa isolates were moderately resistant to ciprofloxacin (54.17%), and (45.83%) resistant to amikacin, whereas the resistance was more marked with other antimicrobials like doxycycline (73.3%), oxytetracycline (69.57%), ticarcilin (68.75%) and gentamicin (63.6%).

On the other hand, *S. aureus* was resistant 100% to amikacin, ticarcilin and gentamicin. The resistance was 75%, 81.8 and 85.7% to doxycycline, oxacillin and oxytetracycline, respectively. The less resistance was showed by chloramphenicol (28.57%) followed by ciprofloxacin (41.66%).

Klebsiella spp. were resistant to all of the antibiotics used except ciprofloxacin (the sensitivity was 100%), while *E. faecalis* was sensitive 100% to both ciprofloxacin and chloramphenicol but resistant to the others. *Enterobacter* spp were resistant to most antibiotics, but were moderately sensitive (50%) to ciprofloxacin, oxytetracycline and doxycycline. *P. vulgaris* also was resistant to 4 antibiotics and showed lower resistance (33.3%) to chloramphenicol and gentamicin, but was sensitive to both ciprofloxacin and amikacin.

Table (1): The scientific names and frequency of the isolated bacteria:

Name of isolated bacteria	Number & frequency	Total No.	Percentage
Pseudomonas aeruginosa	8 single isolates & 14 mixed with other Bacteria	22	48.9
Staphylococcus aureus	4 single isolates &7 mixed with other Bacteria	11	24.4
Citrobacter braakii	1 single isolate & 5 mixed with other Bacteria	6	13.3
Enterobacter spp	5 single isolates	5	11.1
Coagulase- negative *Staphylococci*	3 single isolates & 2 isolates mixed With other bacteria	5	11.1
Proteus vulgaris	1 single isolate & 2 mixed isolates with other bacteria	3	6.66
Corynebacterium spp.	3 isolates mixed with other bacteria	3	6.66
Micrococcus spp.	2 single isolates & 1 mixed with other bacteria	3	6.66
Proteus vulgaris	1 single isolate & 2 isolates mixed with other bacteria	3	6.66
Streptococci spp.	2 isolates mixed with other bacteria	2	4.44
Proteus mirabilis	2 isolates mixed with other bacteria	2	4.44
E .coli	1 single isolate & 1 isolate mixed with one other	2	4.44
Enterococcus faecalis	2 isolates mixed with other bacteria	2	4.44
Serratia marcescens	1 single isolate	1	2.22
Serratia rubidia	1 isolate mixed with one other bacteria	1	2.22
Klebsiella spp.	1 isolate mixed with 2 other bacteria	1	2.22

Table (2): the percentage (%) of resistance showed by the bacterial isolates to different antibiotics

Bacterial isolate	CIP5	AK30	TIC75	C30	T30	OX1	DO30	CN10
Pseudomonas aeruginosa	54.17	45.83	68.75	56.25	69.57	100	73.3	63.6
Staphylococcus aureus	41.66	100	100	28.57	85.7	81.8	75	100
Citrobacter braakii	16.6	0	16.6	0	50	100	0	0
Enterobacter spp	50	100	100	100	50	100	50	100
Coagulase-negative *Staphylococci*	0	0	0	0	0	0	0	0
Proteus vulgaris	0	0	100	33.3	100	100	100	33.3
Klebsiella spp.	0	100	100	100	100	100	100	100
Enterococcus faecalis	0	100	100	0	100	100	100	100
Streptococcus spp.	0	0	100	0	100	100	0	0
Corynebacterium spp.	0	0	0	100	0	100	0	0
Serratia marcescens	0	0	100	0	0	100	0	0
Serratia rubidia	0	0	100	0	100	100	100	0
E.coli	0	0	100	0	0	100	0	0

Cip5: Ciprofloxacin; AK30: Amikacin; Tic75: Ticarcilin; C30: Chloramphenicol; T30: Oxytetracycline; OX 1: Oxacillin; Do30: Doxycycline and CN 10: Gentamicin.

Discussion:

Bacteria isolated from only 45 burn wound swabs from the total 70 swab indicated that 64.29% of examined burn patients had invasive burn wound infections, this idea supported the investigation of Moonery *et al.* (9) who explained that the burn wound infections are one of the most important and potentially serious complications that occur in the acute period following injury, also Raja and Singha (16) demonstrated that the infectious complications are considered a major causes of morbidity and mortality and the type and amount of microorganisms on and in the injured tissues influence wound healing.

Most of the isolates in our research had mixed with other bacterial species and some of these have shown to be resistant to many antimicrobials, and this indicates the high contamination of burn wounds in our hospitals .

In the present study, the most commonly isolated organisms from burned patients were *P. aeruginosa* followed by *S. aureus*, *C. braakii* and *Enterobacter* spp. The reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients

with recurrent or long-term hospitalization, complicating illnesses, prior administration of antimicrobial agents, or the immunosuppressive effects of burn trauma. This evidence was consistent with previous observation mentioned by some workers. Initially, the immunologic response to severe burn injury is proinflammatory but later becomes predominately anti-inflammatory responses in an effort to maintain homeostasis and restore normal physiology; cytokines and cellular response mediate both of these phases (17).

Systemic responses to burn occur by proinflammatory cytokines (18), but the anti-inflammatory responses and the subsequent immunosuppression following burn injury are characterized by a set opposing cells and cytokines, the production and release of monocytes/ macrophages are decreased following burn injury and sepsis (19), also Embile *et al*. (20) mentioned that the nosocomial transmission of microorganisms to the burn wound occurred by transfer from the hands of health care personnel and through immersion hydrotherapy treatment. Our results of bacterial isolation from burn wound were in accordance with other previous studies. Manjula *et al.* (21) reported that *Pseudomonas* species was the commonest pathogen isolated (51.5%) from burn wound followed by *Acinetobacter* species (14.28%), *S. aureus* (11.15%), *Klebsiella* species (9.23%) and *Proteus* species (2.3%). Also Agnihotri *et al.* (22) demonstrated that *P. aeruginosa* form the most common isolate (59%), followed by *S. aureus* (17.5%), *Acinetobacter* spp. (7.2%), Klebsiella spp (3.9%), *Enterobacter* (3.9%), Proteus spp. (3.3%) and others (4.8%). Arslan *et al.* (23) reported that *P. aeruginosa* is the main isolate (53%) from burn wound Adana, Turkey followed by *P. mirabilis* (10%), *Acinetobacter* spp. (7%), *K. pneumonia* (7%) and *E. coli* (3%).

Microbial infection is one of the major serious complications in wound patients, the results of the present study showed that 22 (48.9%) burn wound swabs revealed *P. aeruginosa*, this goes to confirm that *P. aeruginosa* is a major factor in the etiology of wound infection. Previously, Al-hadithi (24) and Mahmoud (25) had reported isolation of *P. aeruginosa* from 10% and 13% burn wound infection, respectively.

Our results showed that the rate of isolation of gram-negative organism was more than gram-positive, these results are consistent with those reported by Kehinde *et al.* (26), who reported that the rate of gram-negative bacterial isolation from burn wound was more than twice that gram- positive and they noticed that *Klebsiella* spp. was the pathogen most commonly isolated constituting 34.4% followed by *P. aeruginosa* (29%) and *S. aureus* (26.8%).

The change in the pattern of bacterial resistance in the burn unit is important both for clinical settings and epidemiological purposes. The results of antimicrobial sensitivity

showed that *S. aureus* was highly resistant for most of the antibiotics tested, while it had less resistance to ciprofloxacin. The adaptation of *S. aureus* to the modern hospital environment has been marked by the acquisition of drug resistance genes soon after antibiotic introduction (27). Also the present study showed that *P. aeruginosa* and all other bacterial isolates were highly sensitive to ciprofloxacin while *P. aeruginosa*, *Enterobacter* spp. and *E. faecalis* were found to be highly resistant to gentamicin, oxacillin and ticarcilin, these results were consistent with investigation of Kehinde *et al.* (26) who reported that more than 75% of the Gram-negative isolates of burn wound were resistant to gentamicin, a commonly used antibiotic for Gram-positive infections. Increasing resistance to various anti-Pseudomonas agents has been reported worldwide and this poses a serious problem in therapeutic management of *P. aeruginosa* infections (28).

Also our results explained that most of the isolates were resistant to many antibiotics.

Antimicrobial resistance among nosocomial pathogens is a significant problem in clinical settings that may be added to the cost of medical care and the morbidity and mortality of patients (29). Gram-negative bacteria produce large quantities of type 1 cephalosporinase when exposed to first- generation cephalosporins, ampicillin, and penicillin G, these antimicrobials are readily hydrolysed by this enzyme, and inducible organisms are intrinsically resistant to these agents (29).

Our results showed that 45.83% of *P. aeruginosa* and 58.34% of *S. aureus* isolates were sensitive to ciprofloxacin. Similar reduced resistance of *P. aeruginosa* to ciprofloxacin has been reported in Jamaica (19.6%) (30), Latin America (28.6%) (31), Ilorin Nigeria (24.7) (32) and in Iraq (86%) (24).

Ciprofloxacin is a bactericidal, rapidly acting antimicrobial agent with a wide spectrum and is very effective against many gram-negative bacterial pathogens, including *P. aeruginosa* (33).

In another study that has been done on 2067 clinical isolates of *P. aeruginosa* in United Kingdom, the resistance amount of isolates to ciprofloxacin was 7.3% (34). In another survey that has been done on *P. aeruginosa* isolated from burn patients at two hospitals of Tehran, Iran in 2003, the resistance amount of isolates to ciprofloxacin was 86.7% (35).

In United States, Van Eldere (36) reported that the overall incidence of ciprofloxacin resistance among *P. aeruginosa* isolates ranged between 30 and 40%. However, in our study *P. aeruginosa* and *S. aureus*, as the predominant organisms causing invasive burn wound infections, were multi-drug resistant. A similar report of multi-drug resistant gram-negative bacilli was also reported by Singh *et al* (37). Such high antimicrobial resistance is probably due to

excessive and indiscriminate use of broad-spectrum antibiotics. These multi-drug resistant strains establish themselves in the hospital environment in areas like sinks, taps, railing, mattress, toilets and thereby spread from one patient to another (22). According to this evidence we suggest that at present time ciprofloxacin is the most effective antibiotic against *P. aeruginosa* and other bacterial burn wound infections.

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