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Colonization of Burn Wounds and Susceptibility of Bacterial Isolates

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Abstract

The aim of the present study was to assess the time-related changes in the microflora of burn wounds in our setting and to determine the susceptibility pattern to commonly used antibiotics. In a period of 8 months a total of 90 burn wound samples (swabs) from 23 hospitalized patients at the Burn Unit were sent for microbiological examination. Swabs were taken weekly in a period of four weeks or until the patients were dismissed from the hospital. Isolation, identification of bacteria and determination of the antimicrobial susceptibility were according to standard microbiological techniques. In the first and second week of hospitalization, the predominant organism was *Acinetobacter* spp. By the end of the third week, *Pseudomonas aeruginosa* had become more predominant. In a period of four and more weeks of hospitalization, 7 samples from two patients were positive (yielding double and triple isolates) with further prevalence of *Acinetobacter* spp. Most of Gram-negative isolates were multidrug-resistant. *Acinetobacter* spp isolates were resistant as follows: 100% to amoxicillin-clavulanic acid and to cephalosporins, 86.4% to cefepime, 91% to both amynoglycosides, 88.6% to ciprofloxacin and co-trimoxazole, and 84% to piperacillin/tazobactam and carbapenems. *P. aeruginosa* isolates were resistant as follows: 100% were resistant to AMC, cefuroxime and cefixime, 95% to co-trimoxazole, from 9% to 40% to cephalosporins, 13.6% to gentamicin,9% to ciprofloxacin, and 4.5% to amikacin and carbapenems. In conclusion, knowledge of the responsible bacterial flora of burn wounds, its prevalence and bacterial resistance, is of crucial importance for fast and reliable therapeutic decisions.

Keywords: burn wound, colonization, wound swabs, *Acinetobacter* spp, *Pseudomonas aeruginosa*

Резюме

Целта на настоящото проучване е да се оценят промените във времето в микрофлората на рани от изгаряне и да се определи чувствителността на микроорганизмите към най-често използваните антибиотици. За период от 8 месеца в Отделението за изгаряния са изпратени за микробиологично изследване общо 90 проби (тампони) от рани, взети от 23 хоспитализирани пациенти. Тампоните се събират ежеседмично в продължение на 4 седмици или до изписване на пациентите. Изолирането, идентифицирането на бактериите и определянето на антимикробната чувствителност са извършени съгласно стандартните микробиологични техники. През първата и втората седмица от хоспитализацията, преобладаващият микроорганизъм е *Acinetobacter* spp., но в края на третата седмица доминиращ е *Pseudomonas aeruginosa*. При пациенти, хоспитализирани за 4 и повече седмици се установи, че 7 проби от двама пациенти са положителни (дават двойни и тройни изолати) с по-нататъшно разпространение на *Acinetobacter* spp. Повечето от грам-отрицателни изолати проявяват множествена лекарствена резистентност. Изолатите от *Acinetobacter* spp са устойчиви, както следва: 100% към амоксицилин-клавуланова киселина и към цефалоспорини, 86.4% към цефепим, 91% към аминогликозиди, 88.6% към ципрофлоксацин и ко-тримоксазол и 84% към пиперацилин/тазобактам и карбапенеми. Изолатите на *P. aeruginosa* са устойчиви, както следва: 100% са резистентни на AMC, цефуроксим и цефиксим, 95% към ко-тримоксазол, от 9% до 40% към цефалоспорини, 13.6% към гентамицин, 9% към ципрофлоксацин и 4.5% към амикацин и карбапенеми. В заключение, знанията за бактериалната флора, отговорна за раните от изгаряне, нейното разпространение и бактериална

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резистентност е от решаващо значение за бързите и надеждни терапевтични решения.

Introduction

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 (Church *et al*., 2016). Bacterial infection remains a major problem in the management of burn victims today. Severe dysfunction of the immune system, a large cutaneous colonization, the possibility of gastrointestinal translocation, prolonged hospitalization and invasive diagnostic and therapeutic procedures, all contribute to infections (Macedo *et al*., 2003). In patients with severe burns over more than 40% of the total body surface area (TBSA), 75% of all deaths are currently related to sepsis from burn wound infection or other infection complications and/or inhalation injury (Church *et al*., 2006; Norbury *et al*., 2016; Sabetha *et al*., 2017). *Streptococcus pyogenes* was the most frequently recognized cause of burn wound sepsis in the early part of the last century. Contrary to those findings, the isolation of beta-haemolytic streptococci from burn wounds has now become rare. Over the years, however, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have become the most frequently isolated organisms in most burn units (Lawrence *et al*., 1992; Nasser *et al*., 2003; Agnihotri *et al*., 2004). In various countries, including India, the importance of *Acinetobacter* spp as a rapidly emerging nosocomial pathogen, has been documented as an important cause of nosocomial infection in burn units (Mehta *et al*., 2007). It is therefore essential for every burn institution to determine the time-related changes in the predominant flora and the antimicrobial susceptibility profile. Rational antibiotic therapy according to the prevalent strains of organisms should help in reducing the mortality and morbidity associated with burns (Ulku *et al*., 2004).

The aim of this prospective study is to assess the time-related microflora changes in burn wounds in our setting and to determine the susceptibility pattern to commonly used antibiotics.

Material and Methods

This prospective study was conducted over a period of 8 months (January-August 2016). A total of 90 burn wound samples (swabs) collected from 23 patients admitted to the Burn Unit were sent for microbiological examination. Out of these 23 patients, 13 patients were male (56.5%) and 10 patients were female (43.5%). In terms of age, 17 patients were adults (mean age of 43.5 years) and 6 were children (mean age of 4.8 years). The mean total body surface area (TBSA) burn was 23% (range 5 to 60%).

Swabs from the burn wounds were collected weekly in a period of four weeks or until the patients were dismissed from the hospital. A different number of samples was taken from each patient. One, two, three, four and multiple samples were taken from 5, 7, 3, 3 and 4 patients, respectively. Pus was collected using sterile cotton tipped swabs. The sampling procedure included collection of swab from the burn wound site prior to any cleansing. In each sampling procedure, the bandages were removed, the remnants of topical antimicrobial agents were scraped away and the wounds were swabbed before washing and applying new topical antimicrobial agents. Specimens were immediately transferred to a sterile test tube. After collection, tubes were plugged properly, labeled and carried promptly to the microbiology laboratory. The specimens were subjected to direct Gram staining and culture. They were inoculated on Blood agar, Schaedler agar and Sabouraud agar (Oxoid). The sample was also put in liquid media (glycose broth) and was subcultured after overnight incubation onto Blood agar. After incubation of 18-48h at 37°C, bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques. Antimicrobial susceptibility was performed on Mueller-Hinton agar by the standard disk diffusion method recommended by the CLSI. The following antibiotics were tested for Gram-positive cocci (concentration was in μg): penicillin 10IE, ampicillin (10), oxacillin (1), amoxicillin-clavulanic acid - AMC (20+10), vancomycin (30), cefadroxil (30), cefpodoxime (10), ceftriaxone (30), amikacin (30), clindamycin (2), erithromycin (15), ciprofloxacin (5) and cotrimoxazol (1.25+23.75), and for Gram-negative bacilli: amoxicillin-clavulanic acid (20+10), piperacillin-tazobactam (100+10), imipenem (10), meropenem (10), cefuroxime (30), ceftriaxone (30), ceftazidime (30), cefixime (5), cefepime (30), gentamicin (10), amikacin (30), ciprofloxacin (5), cotrimoxazol (1.25+23.75), colistin (10).

The confirmation of precision and accuracy of the procedures to evaluate the antimicrobial susceptibility was made using standard strains of *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

Results

The predominant cause of burn injuries among patients was flame - 9, then explosion of gas - 7, scald - 5 and electricity – 2 (Fig. 1).

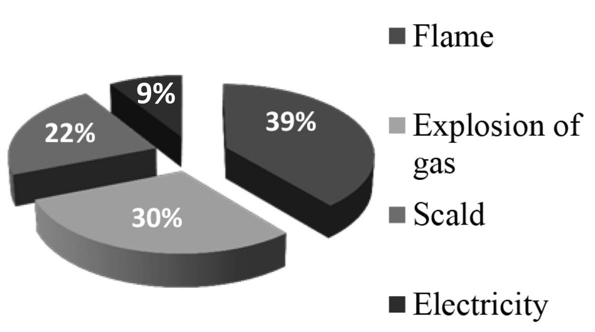

Fig. 1. Most common causes of burn injuries

Periodic wound swabs were collected at the 1st, 2nd, 3rd, and 4th weeks of hospital stay. From all 23 patients samples were taken in the first week of hospitalization, from 10 in the second week, from 6 in the third week and from 2 patients in the fourth and fifth weeks. Out of 90 samples, 26 (29%) were sterile and 64 (71%) were positive (with one or more isolates). In the first week of sampling, a total of 60 samples were taken, of which 35 (58.3%) samples were positive and 25 (41.6%) were sterile. In the second week of sampling, out of 14 samples, 13 (92.9%) were positive and only 1 (7.1%) was sterile. In the third week, 9 samples were collected and they were all positive (100%). In the last week of sampling, out of a total of 7 samples every single one was positive (100%) (Fig. 2).

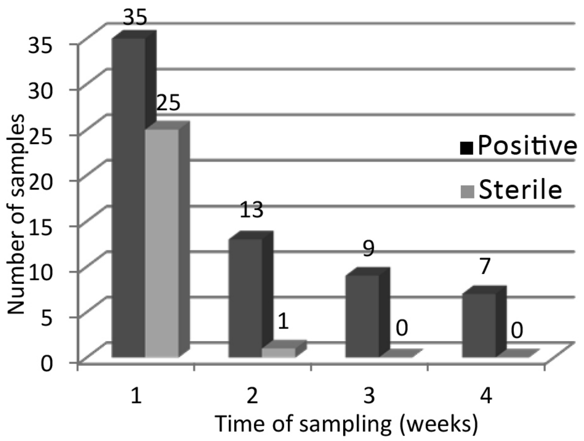

Fig. 2. Number of positive and sterile samples from burn wounds in a period of four weeks

In the first week of hospitalization, out of 35 positive samples a single isolate was found in 27 (77%) samples, while 8 (22.8%) samples yielded double isolates. The predominant organism was *Acinetobacter* spp, which formed 53.5% of all isolates at the end of the first week after admission, while *Staphylococcus* spp. and *P. aeruginosa* formed 21% and 16% of all isolates, respectively.

In the second week of hospitalization, out of 13 positive samples a single isolate was found in 4 (31%) samples and in 9 (69%) samples two bacteria were found. The predominant organism was *Acinetobacter* spp., which formed 50% of all isolates at the end of the second week after admission, followed by *P. aeruginosa* - 27%, while *Staphylococcus* spp formed 14 % of all isolates.

In the third week of hospitalization, out of 9 positive samples a single isolate was found in 5 (56%) samples and in 4 (44%) samples two bacteria were found. However, by the end of the third week, *P. aeruginosa* had become more predominant (54%), while *Acinetobacter* spp. formed 31% of all isolates. There were no *Staphylococcus* spp. isolates in the third week of hospitalization.

In a period of four and more weeks of hospitalization, 7 samples from 2 patients were positive (yielding double and triple isolates). Burn wound sampling performed revealed further prevalence of Gram-negative bacilli (*Acinetobacter* spp. - 50% and *P. aeruginosa* - 17%) over Gram-positive bacteria (*Enterococcus* - 17% and *Corynebacterium* spp. - 17%). There were no *Staphylococcus* spp. isolates in the period of four and more weeks of hospitalization. Table 1 shows the bacterial isolates from the burn that appeared separately throughout the weeks.

Table 1. Number and percentage of bacteria isolated from wound swabs in different sampling times

Bacteria	Time of sampling (weeks)			
	I	II	III	IV
Acinetobacter spp.	23 (54%)	11 (50%)	4 (31%)	6 (50%)
P. aeruginosa	7 (16%)	6 (27%)	7 (54%)	2 (17%)
Proteus	/	1 (5%)	/	/
Enterobacter spp.	/	1 (5%)	/	/
Aeromonas	2 (5%)	/	/	/
Staphylococcus spp. (CNS+MRSA)	9 (21%)	3 (14%)	/	/
Corynebacterium gr. JK	1 (2%)	/	1 (7,7%)	2 (17%)
Enterococcus	/	/	/	2 (17%)
Anaerobes	1 (2%)	/	1 (7,7%)	/
Total number of bacteria	43 (100%)	22 (100%)	13 (100%)	12 (100%)

Figure 3 shows the total number of bacterial isolates that appeared during all four weeks of hospital stay. Most of the Gram-negative isolates

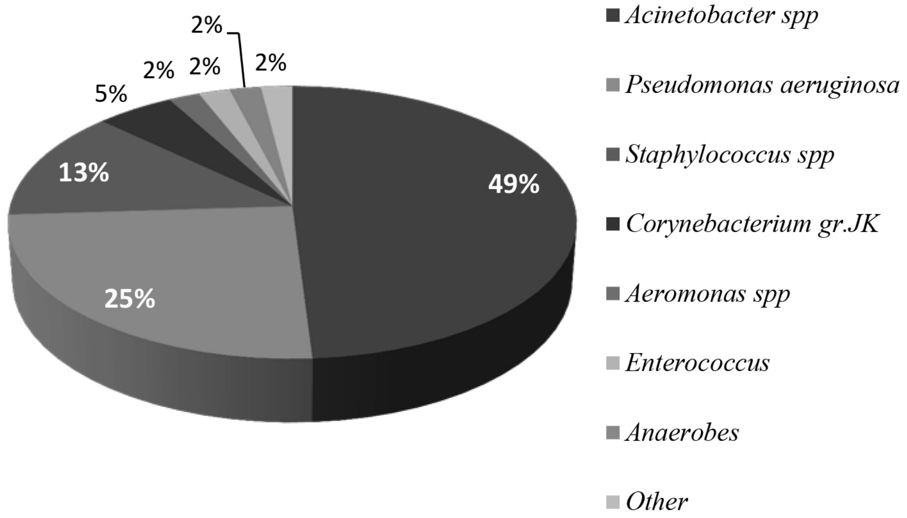

Fig. 3. Bacterial isolates from the burn wounds

were multidrug-resistant. The percentages of resistance of *Acinetobacter* spp. isolates were as follows: 100% were resistant to amoxicillin-clavulanic acid and to cephalosporins, 86.4% to cefepime, 91% to both amynoglycosides, 88.6% to ciprofloxacin and co-trimoxazole and 84% were resistant to piperacillin/tazobactam and carbapenems.

The percentages of resistance of *P. aeruginosa* isolates were as follows: 100 isolates were resistant to AMC, cefuroxime and cefixime, 95% to co-trimoxazole, from 9% to 40% to cephalosporins, 13.6% to gentamicin, 9% to ciprofloxacin and 4,5% isolates were resistant to amikacin and carbapenems.

Not a single strain of *Acinetobacter* spp. and *P. aeruginosa* was found to be resistant to colistin.

Coagulase-negative *Staphylococcus* showed resistance to a wide range of antibiotics, except to amikacin and vancomycin. *S. aureus* (2 isolates) showed high susceptibility to a few antibiotics: AMC, amikacin, erythromycin, ciprofloxacin and co-trimoxazole. However, all *Staphylococci* were susceptible to vankomycin (Table 2).

Discussion

Contamination of the burn wound is almost the rule in major burns. Coagulase-negative *Staphylococci* and *S. aureus* are the most prevalent

Table 2. Resistance/susceptibility of Gram-negative and Gram-positive bacteria isolated from wound samples

Antibiotics	*A. baumanii* (n=44)		*P. aeruginosa* (n=22)		Antibiotics	*Staphylococcus* spp (n=12)	
	R (%)	I/S (%)	R (%)	I/ S (%)		R (%)	I/S (%)
AMC	100	0	100	0	Penicillin	100	0
PIP/TAZ	84	16	0	100	Ampicillin	100	0
Imipenem	84	16	5	95	Oxacillin	83	17
Meropenem	84	16	5	95	AMC	83	17
Cefuroxime	10	0	100	0	Vancomycin	0	100
Ceftriaxone	100	0	41	59	Cefadroxil	100	0
Cefotaxime	100	0	41	59	Cefpodoxime	100	0
Ceftazidime	100	0	18	82	Ceftriaxone	83	17
Cefixime	100	0	100	0	Cefotaxime	83	17
Cefepime	86	14	9	91	Amikacin	1	83
Gentamicin	91	8	14	86	Clindamycin	100	0
Amikacin	91	8	5	95	Erithromycin	83	17
Ciprofloxacin	89	11	9	91	Ciprofloxacin	83	17
Cotrimoxazol	89	11	95	5	Cotrimoxazol	83	17
Colistin	0	100	0	100			

organisms colonizing burn wounds in the first week following burn injuries (Altoparlak *et al*., 2004; Erol *et al*., 2004; Macedo *et al*., 2005; Guggenheim *et al*., 2009; Norbury *et al*., 2016). These bacteria were not predominant in our study. In fact, in the first week, only 9 (21%) isolates of *Staphylococcus* spp (2 were MRSA strains and 7 were *Staphylococcus* coagulase negative) were isolated at the end of the first week, and 3 (14%) (2 were MRSA strains and 1 was *Staphylococcus* coagulase negative) in the second week after admission to the Burns unit. Our findings coincide with many previous studies regarding the fact that the growth of Gram-negatives was predominant during the time of longer hospitalization with a greater propensity to invade. *P. aeruginosa* was reported as the predominant organism (Agnihotri *et al*., 2004; Norbury *et al*., 2016) or *Klebsiella* as the most common isolate, followed by *Proteus* spp. and *Pseudomonas* spp. (Ghai *et al*., 2015). In other study, *P. aeruginosa* was the most common isolate, followed by *Acinetobacter* spp. and *S. aureus* (Rajbahak *et al*., 2014). In our study, *P. aeruginosa*, which was third in the first week, became second in the second week, first in the third week and second again in the fourth week. However, *Acinetobacter* spp was the most predominant organism in the burn wounds of patients in this study. As stated by Sengypta *et al*. (2001), *Acinetobacter* spp. are emerging as an important cause of nosocomial infections in burn units. There are many factors which may contribute to this increase like its presence as a normal skin commensal and its easy spread due to multi drug resistance in a hospital setting. Perhaps, the time of admission to burn units after the injury and the time of sampling influenced the flora of individual burn wounds. That is to say, four patients, after one or two weeks after the injury and admission to other hospitals, were transferred to a burn unit. And, maybe, we missed the time when Gram-positive organisms were prevalent. Considering the fact that we have no data on antibiotic treatment in the period before hospitalization, there is a possibility that empirical antibiotic treatment has affected gram-positive bacteria as a normal skin flora. Therefore, they are present in a small number in the first week of hospitalization.

The reason for the smaller number of samples collected in the third week of hospitalization was due to the fact that patients were discharged at the end of first or second week, because most of the patients had smaller percent of TBSA of burn. The mortality rate was low (2 patients - 8.7%). These patients died at the end of second week of hospitalization. Only 8 patients were hospitalized for three to five weeks. These data are in agreement with other studies (Sabetha *et al*., 2017). Antibiotic-resistant organisms such as MRSA and multiply-resistant Gram-negative rods, including *P. aeruginosa*, *Acinetobacter* spp. and various members of the *Enterobactreriaceae* family have been associated with infections of the burn wound and other anatomic sites in patients with major thermal injury, occasionally in the form of nosocomial outbreaks. Risk factors for acquisition of an antibiotic-resistant organism include receipt of antibiotics prior to the development of infection, extended duration of hospitalization, previous hospitalization, invasive procedures, comatose state, and advancing age (Church *et al*., 2006; Norbury *et al*., 2016; Sabetha *et al*., 2017).

Monitoring of bacterial resistance in the burn unit is important both for clinical settings and epidemiological purposes. *Acinetobacter* can cause infections in patients with burns, and it has been of much concern because of a rapid increase of resistance to a variety of antibacterial drugs. A high percentage of resistance among *Acinetobacter* spp. to amoxicillin-clavulanic acid, cephalosporins, amynoglycosides, ciprofloxacin, co-trimoxazole, piperacillin/tazobactam and carbapenems was detected, which coincides with previous reports in which almost all isolates of *Acinetobacter* spp. were completely resistant to most antibiotics used at the 4th week of culture (Rajbahak *et al*., 2014).

Compared to *Acinetobacter*, *Pseudomonas* spp. were highly resistant to AMC, cefuroxime, cefixime, cotrimoxazole, moderately resistant to cephalosporins, gentamicin, ciprofloxacin, and lowly resistant to amikacin and carbapenems. Not a single strain of *Acinetobacter* spp. and *P. aeruginosa* was found to be resistant to colistin. Susceptibility to colistin could be due to the fact that this drug is used as the last option for multi-drug resistant bacteria in our hospital setting. Considering *Staphylococci*, vancomycin was shown to be highly effective. This is in accordance with other studies (Ghai *et al*., 2015).

To ensure early and appropriate therapy in burn patients, frequent evaluation of the wound is necessary. Therefore, knowledge of the responsible bacterial flora of burn wounds, its prevalence and bacterial resistance, is of crucial importance for fast and reliable therapeutic decisions. Liaison between plastic surgeons, infectious disease physicians, and clinical microbiologists is essential to facilitate the development of treatment of multi-drug resistant pathogens in burn infections.

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