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Bacterial and fungal colonization of burn wounds

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A prospective study of fungal and bacterial flora of burn wounds was carried out from February 2004 to February 2005 at the Burns Unit of Hospital Regional da Asa Norte, Brasília, Brazil. During the period of the study, 203 patients were treated at the Burns Unit. Wound swab cultures were assessed at weekly intervals for four weeks. Three hundred and fifty four sampling procedures (surface swabs) were performed from the burn wounds. The study revealed that bacterial colonization reached 86.6% within the first week. Although the gram-negative organisms, as a group, were more predominant, Staphylococcus aureus *(28.4%) was the most prevalent organism in the first week. It was however surpassed by* Pseudomonas aeruginosa *form third week onwards. For* S. aureus *and* P. aeruginosa *vancomycin and polymyxin were found to be the most effective drugs. Most of the isolates showed high level resistance to antimicrobial agents. Fungi were found to colonize the burn wound late during the second week postburn, with a peak incidence during the third and fourth weeks. Species identification of fungi revealed that* Candida tropicalis *was the most predominant, followed by* Candida parapsilosis*. It is crucial for every burn institution to determine the specific pattern of burn wound microbial colonization, the time-related changes in the dominant flora, and the antimicrobial sensitivity profiles. This would enable early treatment of imminent septic episodes with proper empirical systemic antibiotics, without waiting for culture results, thus improving the overall infection-related morbidity and mortality.*

Key words: burn wound - colonization - infection

Despite advances in the use of topical and parenteral antimicrobial therapy, and the practice of early tangencial excision, bacterial infection remains a major problem in the management of burn victims today. Few patients are as susceptible to the development of infections as burn patients. Severe dysfunction of the immune system, a large cutaneous colonization, the possibility of gastrointestinal translocation, a prolonged hospitalization and invasive diagnostic and therapeutic procedures, all contribute to infections (Barreto et al. 1998, Macedo et al. 2000, Macedo 2003).

The experience accumulated over the past three decades, in the early interventional treatment of burns patients has dramatically changed the cause of death; it is now estimated that about 75% of the mortality following burn injuries is related to infections, rather than osmotic shock and hypovolaemia. 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.

Following colonization, the organisms on the surface start to penetrate the burn eschar to a variable extent, depending on their invasive capacity, local wound factors, and the degree of patient's immunosuppression. If viable subeschar tissue becomes invaded, disseminated infection is likely to occur.

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Streptococcus pyogenes was the most frequently recognized cause of burn wound sepsis in the early part of the last century. Over the years, however, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have become the most frequently isolated organisms in most burn units (Lawrence 1992, Nasser et al. 2003, Agnihotri et al. 2004).

It is generally known that the spectrum of infective agents varies from time to time and from place to place. It is, therefore, desirable to carry out periodic reviews of the bacterial flora of burn wounds so that preventive strategies could be modified as necessary. The aim of this prospective study is therefore to assess the current fungal and bacterial profile of burn wounds in Brasília, Brazil.

PATIENTS AND METHODS

Our analysis is based on a prospective study of bacterial and fungal isolates from wound swabs taken from patients admitted to the Burn Unit of the Hospital Regional da Asa Norte, Brasília, Brazil, from February 2004 to February 2005.

Since 1980, patients having partial skin thickness burns covering less than 25% of the body surface area, were not generally admitted to the Burns Unit if they were adults, or less than 10% if they were children. Patients with full skin thickness burns of small extent (≤ 5% of the body surface area) were also treated as outpatients until the wound was ready for excision and grafting by members of plastic surgery team. Commonly admission to the Burns Unit only occurred with severely burned patients (> 25-30% of the body surface area).

All patients who were admitted to the Burns Unit were resuscitated based on the Parkland formulae guidelines using crystalloids, except in few cases where there was either delayed or difficult resuscitation and the colloids were not used earlier than 24 h postburn.

Silver sulphadiazine 1% was the topical antimicrobial of choice. The wound was inspected during change of dressing and swabs for culture from the burn wounds were taken weekly for four weeks or until all burn wounds were healed. Early excision of the burn eschar and grafting were practiced as soon as the patients were stabilized following the initial resuscitation.

At direct patient contact a protective gown and disposable gloves were used. Hands were washed with conventional soap when necessary, and disinfected with 70% ethanol/glycerol before and after patient contact.

The bacteriological isolation was carried out in the microbiology laboratory of the Hospital Regional da Asa Norte, Brasília. The swabs were dipped in Stuart´s transport medium then plated on blood agar, chocolate agar, MacConkey, and Sabouraud's dextrose agar media (Difco). After incubation for 18-48 h at 37°C, the isolates were identified using convencional protocol. Afterwards, the sensitivity to the antibiotics was accomplished by automated method Vitek-bioMérieux. The confirmation of precision and accuracy of the procedures to evaluate the antimicrobial susceptibility was made using ATCC (American Type Culture Collection) standard strains. When isolated *S. aureus* oxacillin resistant, *Acinetobacter* sp. and *P. aeruginosa* multirresistants were confirmed by disc, by agar diffusion method according to the rules established by the National Committee for Clinical Laboratory Standards (NCCLS 1999). Fungal cultures were obtained on Sabouraud dextrose agar (Difco) and on "mycogel" agar (Oxoid) at 37°C and observed daily for 20 days. The characterization of fungi was done by the germ tube test, morphological examination and automated method Vitek YBC yeast identification system (bioMérieux Vitek, Inc., MI, US) (Freydiere et al. 2001). However, antibiotic sensitivity of fungi can not be done due to technical problems.

RESULTS

Two hundred and three cases were treated as in-patients at the Burns Unit of Hospital Regional da Asa Norte during the period of study. One hundred and twenty (59.1%) were males, 83 (40.9%) females, with a mean age of 24 years (range 1 to 82 years) and mean total body surface area burn of 15% (range 1 to 88%).

The flame burn was the predominant cause of burn among patients; 117 had flame burns, 58 a scald, and 19 had a electrical burn.

A total of 354 samples were processed from as many patients admitted to the burn unit. Single isolates were found in 316 (89.3%) samples, while 36 (10.2%) and 2 (0.5%) samples yielded double and triple isolates, respectively.

Tables I and II show the positive and sterile results of the wound swabs culture at weekly intervals postburn.

Table III shows the bacteriological isolates from the burn wounds at weekly intervals postburn. The predominant organism was *S. aureus* which formed 28.4% of all isolates at the end of the first week after admisson. However, by the end of the third week, *P. aeruginosa* had become more predominant (26.9%), while *S. aureus* formed only 12.2% of all isolates.

Burn wound sampling performed revealed further prevalence of gram-negative bacilli (51.2%) over gram-positive cocci (36%). Most of the gram negative isolates obtained were found to be multi drug resistant. All strains of staphylococci were susceptible to vancomycin.

However, the most common isolate overall was *S. aureus* (20.5%) followed by coagulase-nagative staphylococci (15.2%), *P. aeruginosa* (11.4%), *Klebsiella* sp. (11.2%), and *Enterobacter* sp. (10.4%).

The only kind of fungi recovered on culture from swabs of burn wounds were *Candida* species. The frequency of fungal isolates increased steadily during the stay in hospital, peaking at third and fourth weeks (Table IV).

Table V presents the antimicrobial susceptibility of the gram-positive bacteria isolated from wound culture of burned patients. The incidence of oxacillin resistance among coagulase-negative staphylococci were high (44.6%) and among *S. aureus* were low (4.7%). However all staphylococci were susceptible to vancomycin. *S. aureus* showed high susceptibility to a wide range of antibiotics. Coagulase-negative *Staphylococcus* showed low to moderate susceptibility to amoxicillin/clavulanic acid, cephalothin, oxacillin, gentamicin, clindamycin, ciprofloxacin, ampicillin/sulbactan, and co-trimazole.

Table VI presents the antimicrobial susceptibility of the gram-negative bacteria isolated from wound culture of burned patients. More than 80% of *A. baumannii* isolated were susceptible only to ticarcillin/clavulanic acid and imipenem. More than 90% of *E. cloacae* and *K. pneumoniae* were sensitive to imipenem, amikacin, and ciprofloxacin. All strains of *P. aeruginosa* showed low susceptibility to a wide range of antibiotics, except only to polymyxin and amikacin.

TABLE I

Percentage of bacteriological cultures from wound of patients treated at the Burns Unit of Hospital Regional da Asa Norte, Brasília, from February 2004 to February 2005

	Time of sampling (weeks)			
Result	1st n = 194	2nd n = 120	3rd n = 41	4th n = 21
Sterile	13.4	15	9.8	-
Positive	86.6	85	90.2	100

TABLE II

Percentage of fungal cultures from wound of patients treated at the Burns Unit of Hospital Regional da Asa Norte, Brasília, from February 2004 to February 2005

	Time of sampling (postburn weeks)			
Result	1st n = 147	2nd n = 97	3rd n = 27	4th n = 11
Sterile	87.6	77.4	62.9	45.5
Positive	12.4	22.6	37.1	54.5

DISCUSSION

The results of this study have further confirmed that contamination of the burn wound is almost the rule rather

TABLE III

Percentage of bacteriological organisms isolated from wound cultures of patients treated at the Burns Unit of Hospital Regional da Asa Norte, Brasília, from February 2004 to February 2005

	Time of sampling (weeks)				
Isolate	1st n = 194	2nd n = 120	3rd n = 41	4th n = 21	Total
Staphylococcus aureus	28.4	13.3	12.2	4.7	20.5
Coagulase-negative staphylococci	15.5	15	14.6	14.4	15.2
Pseudomonas aeruginosa	6.7	10.8	26.9	28.7	11.4
Klebsiella sp.	9.8	14.3	4.9	19.1	11.2
Enterobacter sp.	9.8	10	14.6	9.5	10.4
Acinetobacter baumannii	2.1	4.2	9.8	9.5	3.9
Serratia sp.	2.1	5	-	-	2.7
Aeromonas hydrophila	4.1	1.7	-	-	2.7
Escherichia coli	1.5	3.3	2.4	4.7	2.3
Enterococcus faecalis	3.6	0.8	-	-	2.1
Proteus mirabilis	0.5	2.5	2.4	-	1.3
Streptococcus pyogenes	0.5	-	-	-	0.3
Others	2.0	4.2	2.4	9.5	3.2
Total number of isolates					376
Total number of sampling procedures					354
Total number of patients studied					203

TABLE IV

Percentage of fungal organisms isolated from wound cultures of patients treated at the Burns Unit of Hospital Regional da Asa Norte, Brasília, from February 2004 to February 2005

	Time of sampling (weeks)				
Isolate	1st n = 147	2nd n = 97	3rd n = 27	4th n = 11	Total
Negative	87.8	77.4	62.9	45.5	80.1
Candida tropicalis	4	12.4	25.9	36.5	10.3
Candida parapsilosis	4	6.2	-	-	4.2
Candida humiloca	1.4	1	7.5	9	2.1
Candida famata	0.7	1	3.7	9	1.4
Candida albicans	1.4	1	-	-	1.1
Candida guilliermondii	0.7	-	-	-	0.4
Candida curvata	-	1	-	-	0.4

than an exception in major burns. In spite of the fact that all burned patients were routinely cleaned with an antiseptic solution (chlorhexidine) and had 1% silver sulphadiazine cream applied to their wounds, 86.6% of the patients studied had microorganisms isolated from their burn wounds at the end of the first week after admission.

The susceptibility of burn wound to such opportunistic colonization by bacteria and fungi results from several factors including the presence of coagulated proteins, the absence of blood-borne immune factors, and the avascularity of the burn wound.

In this study, we evaluated the pattern of burn wound microbial colonization, as well as the time-related changes in the predominant flora throughout the patients' hospital stay.

There is no doubt that efforts at combating infection in burns must remain a continuing preoccupation, *S. aureus* was the most prevalent single organism (28.4%) colonizing the burn wounds in the first week following burn injuries. *P. aeruginosa* which came fourth in the second week surpassed staphylococci in all subsequent weeks.

The study has also shown that the flora of individual burn wounds changes over time. Gram-positive organisms are initially prevalent, then gradually become superceded by the gram-negative organisms that appear to have a greater propensity to invade.

However, *S. aureus* (20.5%) was the most predominant organism in the burn wounds of patients in this study, followed by coagulase-negative staphylococci and *P. aeruginosa* (15.2 and 11.4%, respectively).

TABLE V
Percentage of antimicrobial susceptibility of gram-positive bacteria isoladed from wound culture of patients treated at the Burns Unit of Hospital Regional da Asa Norte, Brasília, from February 2004 to February 2005

Antibiotic	*Staphylococcus aureus* n = 77	Coagulase-negative staphylococci n = 57
Amoxicillin/ Clavulanic acid	92.9	50.8
Cephalothin	90.6	50.8
Oxacillin	95.3	55.4
Gentamicin	94.1	80
Amikacin	100	92.3
Ciprofloxacin	96.5	80
Clindamycin	89.4	72.3
Co-trimoxazole	91.8	52.3
Vancomycin	100	100
Ampicillin/ Sulbactam	90.6	50.8

Our finding that *S. aureus* was the most common isolate coincides with previous reports (Taylor et al. 1992, Vindenes & Bjerknes 1995, Lesseva & Hadjiiski 1996, Komolafe et al. 2003) but is in contrast to other studies which report *P. aeruginos*a as predominant organism (Atoyebi et al. 1992, Revathi et al. 1998, Singh et al. 2003, Nasser et al. 2003, Agnihotri et al. 2004).

Compared to several earlier reports on burn wound colonization and invasive infection, one of the most striking differences is the high frequency of coagulase-negative staphylococci throughout the hospital stay in this study. Even though the pathogenicity of these microorganisms in burn patients has been questioned, it should be noted that these patients are immunocompromised. Several studies have consistently suggested that coagulase-negative staphylococci should be considered a significant pathogen in both burn patients and critically ill surgical patients (Vindenes & Bjerknes 1995). As coagulase-negative staphylococci are also a bacteria frequently isolated from blood cultures in our ward (Macedo et al. 2003), it is of considerable concern that 44.6% of these isolates were resistant to oxacillin.

Contrary to findings in the beginning of last century before, the isolation of β-haemolytic streptococci from burn wounds has now become rare. It was also confirmed in this study that which the isolation rate of this bacteria was 0.3%.

History indicates that the relative importance and the cyclic pathogenicity of various microorganisms have changed and may be expected to continue changing as systemic and topical antibacterial treatment develops. The pattern of bacterial resistance is important for epidemiological and clinical purposes.

Even though some reports indicate a decrease in *P. aeruginosa* colonization of burn wounds, this microorganism has, since the mid-twentieth century, been held responsible for the majority of invasive burn wound infections in many burn centers (Vindenes & Bjerknes 1995). In our series, *P. aeruginosa* accounted for 11.4% of all burn-wound isolates, and only 60% were sensitive to imipenem.

In addtion, the nosocomial pathogen *A. baumannii* was demonstrated in 3.9% of all isolates in this study. *Acinetobacter* can cause infections in patients with burns, and these bacteria have been of much concern because of a rapid increase of resistance to a variety of antibacterial drugs. In our series 53.3% of these bacteria were resistant to ampicillin/sulbactam.

TABLE VI
Percentage of antimicrobial susceptibility of gram-negative bacteria isoladed from wound culture of patients treated at the Burns Unit of Hospital Regional da Asa Norte, Brasília, from February 2004 to February 2005

Antibiotic	*Acinetobacter baumannii* n = 15	*Enterobacter cloacae* n = 39	*Klebsiella pneumoniae* n = 42	*Pseudomonas aeruginosa* n = 43
Ampicillin/ sulbactam	46.7	14.6	56.1	13.3
Aztreonan	13.3	65.9	70.7	24.4
Cephalothin	6.7	2.4	65.9	4.4
Cefoxithin	6.7	9.8	87.8	6.7
Ceftriaxone	6.7	63.4	73.2	15.6
Ceftazidime	20	68.3	75.6	51.1
Ceftaxima	13.3	65.9	73.2	8.9
Ticarcillin/ clavulanic acid	80	56.1	68.3	28.9
Piperacillin/tazobactam	66.7	63.4	70.7	53.3
Imipenem	93.3	95.1	100	60
Gentamicin	26.7	85.4	78	48.9
Amikacin	33.3	95.1	92.7	86.7
Ciprofloxacin	40	90.2	95.1	53.3
Co-trimoxazole	20	24.4	53.7	8.9
Cefepime	26.7	78.0	75.6	48.9
Polymyxin	ND	ND	ND	97.8

ND: not done

Colonization of the burn wound with fungi is not a surprising phenomenon in view of the changes in microbial flora induced by systemic and topical chemotherapy. The origin of the fungi in these patients does not appear to be the gastrointestinal tract as suggested in other studies of diseases complicated by fungemia (Colombo & Guimarães 2003). An epidemiological study demonstrated recovery of *Candida* from the wounds of 8 to 10% of severely burned patients studied and the absence of fungi in the stool or nasofharynx of these patients (Bruck et al. 1972). Colonization of fungi was found more commonly after third and fourth week postburn.

Species identification revealed that postburn patients harbored various species of *Candida*. These fungi species are the most common fungal organisms in burn wounds (Bruck et al. 1972, Vindenes & Bjerknes 1995) and in this study no other fungi were isolated. The most predominant species obtained was *C. tropicalis* (10.3%), followed by *C. parapsilosis* (4.2%). This high incidence of *C. tropicalis* observed in our study is specially alarming. As it is now well known that unlike *C. albicans*, which can be found as a commensal, *C. tropicalis* when present, is not a commensal and is almost always associated with the development of deep fungal infections.

C. albicans has always been considered as the most frequent pathogenic species causing nosocomial fungal infections in burn patients, with mortalility rates due to deep-seated infections raging from 38 to 50% (Macedo et al. 2003). However, recently other species of *Candida*, as *C. tropicalis*, has emerged to be equally important in immunocompromised patients (Mathews et al. 2001, Leung et al. 2002, Gupta et al. 2004).

The colonization of the wounds with *Candida* species does not validate the start of antifungal therapy in burned patients. However, if the appearance of the wound is suggestive of invasive fungal infection, or if the patient has received intravenous antibiotics for bacterial infections (specially older patients with burn larger than 40% total body surface area), or the patient is in a critical phase suggestive of a generalized breakdown on his/her host defense mechanisms, systemic antifungal therapy should be considered.

The high percentage of multi drug resistant isolates is probably due to empirical use of broad-spectrum antibiotics. However, in the instances of imminent clinical burn wound sepsis, the success of treatment greatly depends on prompt administration of empirical i/v antimicrobial therapy.

Burns provide a suitable site for bacterial multiplication and infection, mainly because of the larger area involved and longer duration of patient stay in the hospital. To ensure early and appropriate therapy in burn patients, a frequent evaluation of the wound is necessary. Therefore, a continuous surveillance of microorganisms and a regular update of their antibiotic resistance pattern is essencial to maintain good infection control programmes in the burn unit, thus improving the overall infection-related morbidity and mortality.

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