

BACTERIOLOGICAL EVALUATION OF WOUNDS IN SERIOUSLY BURNED HOSPITALIZED PATIENTS.

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During the period between May and December 1988, 21 patients were studied bacteriologically at Hospital João XXIII's burn's unit which belongs to "Fundação Hospitalar do Estado de Minas Gerais" in Belo Horizonte, Brazil. A qualitative and quantitative evaluation of aerobic and facultative bacteria from burn wounds was carried out by the standard filter paper disc technique, including antibiotic susceptibility. At the same time an evaluation of those bacteria isolated from the environmental unit was performed. The most common organisms recovered from wounds of patients were: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. *P. pseudomallei* was the most frequent strain recovered from environmental specimens. In nearly all patients specimens (16 in total) from whom *P. aeruginosa* was isolated, the rate of CFU/cm² of skin was above 10². In nine of these, it reached 10⁵, which is equivalent to 10⁷ CFU/g of burned tissue.

Key-words: Nosocomial infection. Burn wounds infection.

According to Sucena²⁴ in 1982, in the United States of America, two million cases of burns are registered per year, 200,000 of which require hospitalization, with a mortality of 12,000. In Brazil, (where statistics are very poor), Russo²² registered a total of 871,692 labor accidents in São Paulo (in 1975), 27,894 of which were burned patients. Twenty percent of these (6.189) had to be hospitalized. It was estimated that hospitalization of these burned patients led to 836,820 lost working days. Nosocomial infections have probably been more prevalent following major burns than is any other condition in medicine. According to the information available, infection has been the leading cause of death in

this type of injury^{6 11 14}. As burn wounds are invariably contaminated, it's not easy to determine the presence and degree of infection. On the other hand, multiple organisms are often involved in burn wound infections, and it is not difficult to find two or more bacteria associated with septicemia or other types of invasive infection. It therefore becomes essential to control infection in high-risk patients^{9 10}.

It is estimated that 80 percent of wounds are colonized by microorganisms from the patient's own gastrointestinal and respiratory tracts. The remaining 20 percent are colonized by cross-contamination. In both groups, half of the cases result in sepsis¹³.

In the 50's and 60's the increasing amount of antibiotic-resistant bacteria became an additional problem to burned patients. Thus, resistance of Gram negative and Gram positive bacteria in one patient might cause serious consequences for other patients in a same burns unit. This should therefore influence the selection of antibiotics for the treatment of suspected sepsis in these other patients⁹.

In the pre-antibiotic era (1920-1950),

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streptococci and staphylococci were becoming prominent microorganisms in burn wound infection, along with Gram negative aerobic rods. After penicillin was discovered, during the 1950's, *S. aureus* become predominant. By the early 1960's, *P. aeruginosa* outnumbered *S. aureus*, followed by other Gram negative and facultative rods. Since the 1960's *Candida albicans*, *Candida* sp and other fungi have appeared with increasing frequency. Further the oportunistic viruses (CMV, Herpes) have become noteworthy in the 1980's^{14,19}. While resistant Gram negative organisms clearly became clinically important in the 1970's, the resistant Gram positive cocci became more frequent in the 1980's. This also occurred in the burns unit^{9,25}.

Thus, in the antibiotic era, bacterial resistance has become a factor. The changing bacterial patterns in burn wounds infections are secondary to the recurrent use of both topical and systemic antimicrobial agents. In other words, changing bacterial patterns often reflect changing methods of therapy, more than any other factor. This is specially affected by the method of drug utilization^{8,14,25}.

The purpose of this paper is to present microbiological data which help develop both effective prophylactic and active infection control programmes for hospitalized burned patients.

MATERIAL AND METHODS

Patients with serious burns were selected according to extent of area injury and depth^{21,24}. The age range consisted of adults and children of both sexes. The majority of burns was due to accidents with alcohol, combustible and fireworks, or suicide and homicide.

During the period between May and December 1988, 21 patients were studied bacteriologically at Hospital João XXIII's burn's unit which belongs to "Fundação Hospitalar do Estado de Minas Gerais" in Belo Horizonte, Brazil. Environment specimens of six different sources in the same unit were also studied.

The CFU/cm² of skin was evaluated according to Williams et al²⁶ in 1984, by inoculation of one standard filter paper disc, which was used to collect specimens, in a 10 ml

thioglycollate broth. Successive dilutions up to 10⁻¹⁰, after homogenization in mechanic shaker (Vortex), were then performed. From previously established dilution, 0.03 ml doubled volumes were subcultured in blood agar plates. After 24h of incubation at 30°C and 37°C, the colony count was made²⁶.

The isolated colonies were subcultured in brain heart infusion broth to get pure culture. The tests for presumptive identification were made according to what is precognized for each group of bacteria^{2,5,12,18}.

Susceptibility profiles of isolates were determined following Bauer - Kirby disk agar diffusion method³, using properly selected antibiotic¹², including those used in the hospital. - Strains of reference were used as control: *Klebsiella pneumoniae* ATCC 27736 and *Staphylococcus aureus* ATCC 25923.

RESULTS

The most common organisms recovered from wounds of patients were: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (Table 1). *P. pseudomallei* was the most frequent strain recovered from environmental specimens (Table 2). In nearly all patients specimens (16 in total) from whom *P. aeruginosa* was isolated, the rate of CFU/cm² of skin was above 10². In nine of these, it reached 10⁵.

Table 1 - Bacterial organisms isolated from burn wounds of child and adult patients during the period studied.

Organisms	Nº of patients
Gram negative aerobic an facultative bacteria	
<i>Pseudomonas aeruginosa</i>	16
<i>Pseudomonas</i> sp	1
<i>Klebsiella</i> sp	2
<i>Proteus</i> sp	2
<i>Citrobacter</i> sp	1
<i>Escherichia coli</i>	2
<i>Acinetobacter calcoaceticus</i> subsp. <i>anitratus</i>	1
Gram positive aerobic and facultative bacteria	
<i>Staphylococcus aureus</i>	5
<i>Staphylococcus epidermidis</i>	4
<i>Enterococcus faecalis</i>	2

Table 2 - Bacterial organisms isolated from 3 of 6 distinct environment specimens (soap, floor, shower hose).

Organisms	N ^o of isolations
<i>Pseudomonas pseudomallei</i>	3
<i>Acinetobacter calcoaceticus</i> subsp <i>lwoffii</i>	1
<i>Enterobacter</i> sp	1

Antimicrobial susceptibility tests showed multiple drug resistance (MDR= resistant to at least two aminoglycosides, ampicillin and first generation cephalosporins⁹) in 87 percent of *P. aeruginosa* strains isolated from burn - wounds (Table 3). *P. pseudomallei* strains recovered from environmental specimens (soap, floor and shower hose) were sensitive to aminoglycosides; moderately sensitive to cefotaxime and resistant to all other tested antibiotics.

DISCUSSION

It is accepted that the number of 10³ bacteria/cm² of skin in burned patients, equivalent to 10⁵ bacteria/g of burned tissue, is indicative of impending sepsis for aerobic and facultative bacteria²⁶. Other reports suggest that there is *critical number* of bacteria, between 10⁴ and 10⁶ organisms/g of burned tissue¹⁰. This figure is compatible with the existence of category intermediate between colonization and invasion¹¹ which would correspond to 10² - 10⁴ microorganisms/cm² of skin, with the disc technique used in this work. Reports of 10³ CFU/cm² of skin, (Witch would be equivalent to 10⁵ organisms per gram of tissue), are compatible with, but not diagnostic of, burn wound infection^{10, 20}. Though less diagnostic, surface cultures do give information which is useful for surveillance and epidemiologic control¹.

In nearly all patient specimens from which *P. aeruginosa* (16 patients; Table 1) was isolated, the rate of CFU/cm² of skin was above 10². In 9 of these, it reached 10³, which is equivalent to 10⁷

Table 3 - In vitro antimicrobial susceptibility of *P. aeruginosa* strains isolated from burn - wounds as determined by Kirby - Bauer disc diffusion.

Antimicrobial drugs	Patient strains															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Amikacin	S	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R
Amoxicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Carbenicillin	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R
Cefotaxime	R	MS	MS	R	R	MS	R	MS	MS	MS	R	MS	R	R	MS	R
Cephaloridine	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Cephatothin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Chloramphenicol	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Gentamicin	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R
Kanamycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Penicillin G	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Tetracycline	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Trimethoprin-sulfamethoxazole	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

S = susceptible; MS = moderately susceptible; R = resistant

CFU/g of burned tissue. The high CFU/cm² rates found in nearly half of the patients examined are also important when one takes into consideration the high level of susceptibility of burned patients, and the potential source of cross - contamination (as observed by MacMillan et al¹⁴ in 1986).

It's thought that from the time of burn injury the infection of wound follows a set sequence, with respect to aetiological agent and the duration of infection. Firstly, it is invaded by *Streptococcus* sp and *Staphylococcus aureus*; followed a few days later by Gram-negative rods, which are future pathogens. At end of this process, after the patient has been furnished with broadspectrum antibiotics, the fungi appear¹⁷. According to information available, in the majority of burn infections one strain of Gram negative bacteria (*Pseudomonas* sp) predominates. However, a great number of other organisms have been replacing *P. aeruginosa* in importance^{13,15}. The present results show that in Brazil, *P. aeruginosa* still requires special care for its control, for it is still the most frequent organism, occurring in approximately 75 per cent of patients.

The great majority of these microorganisms were shown to be resistant to all antibiotics tested. According to Muir et al¹⁶ in 1987, 30 per cent of burned patients develop *P. aeruginosa* infection, but, in recent years, only 5 per cent of patients have been given anti-pseudomona antibiotics (aminoglycosides). A rational and firmly enforced antibiotic policy is essential, both for the management of the individual patient and to prevent the development of antibiotic - resistant strains of organisms in the ward environment.

Studies about epidemiology and control of multiresistant strains, have shown that aminoglycoside resistance has occurred with higher frequency in isolates of wounds and sputum, which usually have poor penetration. It's thought therefore, that the presence of sub inhibitory levels of drugs in these situations contributes to the production of multiresistant strains. The resistance of the isolates to aminoglycosides has been also related to inadequate dosage²⁵. However, there is some controversy as to the relation between amikacin use and resistance, as some hospitals

have used amikacin extensively, without noticing any increase in resistance. Therefore, the incidence depends, in any situation, on the prevalence of the various inactivating amikacin enzymes in organisms of the hospital, as well as on adequate aminoglycosides dosing^{7,25}.

Pseudomonas pseudomallei isolated from environment specimens showed a different susceptibility from those of *P. aeruginosa* isolated from patients. These *P. pseudomallei* invariably shown to be sensitive to amikacin, gentamicin, and kanamycin; moderately sensitive to cefotaxime and resistant to all other tested antibiotics.

The sepsis prevention in wound burns depend on maintenance of a low concentration of bacteria in the injury^{4,21}. A wide variety of topical agents are available to inhibit the bacterial growth in burns but no one has fulfilled all the requirements for an ideal topical agent^{19,16,23}. With the extended use of any of these agents (silver nitrate, 0.5% silversulfadiazine, mafemide of sodium, povidone-iodine), resistant organisms inevitably appear.

The use of topic antibiotic agents has decreased mortality among patients with less than 40% of corporal area burned. In most of the burned patients centers, topic agents are now used in association with debridement or surgical excision for serious injury^{1,13,14}.

A fundamental aspect to be considered is the situation of the *Antibiotic* subject in medical courses, at the moment included as a small topic. The little time devoted to this matter is a consequence of the subject not being considered as a speciality. Thus, no other group of drugs has such a remarkable effect in the community as a result of its misuse in terms of both cost and increased bacterial resistance⁸.

Antibiotic proliferation, the availability of other drugs and the existence of several different sources of information about drugs, has made it difficult to secure rational orientation and controlled prescription⁸. However efforts must be made in this direction, mainly by Infection Control Comissions.

Additional studies, correctly structured, in the field of microbiology, in different centers for burned patients are therefore necessary in Brazil. With the microbiologic data from the data

obtained, it will be possible to provide better infection control to the burned patient.

RESUMO

No período de maio a dezembro de 1988, foram avaliados bacteriologicamente 21 pacientes da Unidade de Queimados do Hospital João XXIII pertencente à Fundação Hospitalar do Estado de Minas Gerais, em Belo Horizonte, Brasil. A avaliação qualitativa e quantitativa de bactérias aeróbicas e facultativas de queimaduras foi realizada através da técnica do disco de papel de filtro padronizado, incluindo a susceptibilidade a antimicrobianos. Ao mesmo tempo, foi realizada uma avaliação das bactérias isoladas do ambiente da Unidade. Os microrganismos mais frequentemente recuperados das feridas dos pacientes foram: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*. A espécie *P. pseudomallei* foi a mais frequente nos espécimes ambientais. Em quase todos os espécimes de pacientes dos quais *P. aeruginosa* (16 pacientes) foi isolada, a taxa CFU/cm² de pele estava acima de 10². Em nove destes, alcançou 10⁵, o que equivale a 10⁷ CFU/g de tecido queimado.

Palavras-chaves: Infecção hospitalar. Infecção de queimaduras.

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