MICROBIOLOGICAL EVALUATION OF HOSPITALIZED BURNS PATIENTS TREATED WITH NANOCRYSTALLINE SILVER

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ABSTRACT

Background: This study to evaluate the incidence of infection and identify the main etiologic agents present in burn wounds of patients in the healing process in topical treatment with nanocrystalline silver. Casuistic and Method: Sixty-eight burned patients were evaluate, and submitted to the criteria of inclusion. There are children and adults of both sexes, admitted to the burns treatment unit of the hospital study, with burns of second and third degree, transferred or not from other hospitals, aged between two and seventy years, receiving treatment with nanocrystalline silver and who were hospitalized for more than 48 hours. Result: Pseudomonas aeruginosa was the most frequent microorganism (20.6%), for other side Staphylococcus aureus (13.24 %) emerged as significant cause of infection, being the second most frequently isolated agent. The rate Acinetobacter baumannii (10.29%) was higher than the estimated national statistics, which is 0.8%. Although Staphylococcus epidermidis is the causative agent of lower frequency (7.35%), many centers show that this can be a significant pathogen, taking into account the status of burned patients. Conclusion: Approximately 71% of microbiological cultures were positive for any type of bacteria, and the most frequent etiological agents were Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis and Acinetobacter baumannii. The incidence of infection was 70.6 %. The results of this study allow us to suggest that there is still concern about the complications presented by burn patients.

KEYWORDS: Burns; Nanocrystalline silver; Pathogenic microorganisms; Hospital infection.
BACKGROUND
The Burn are highly susceptible to infection due to the impairment of skin integrity and reduction in cell mediated immunity, and is serious problem, representing a major challenge for health care, most often occurring in severe form requiring long periods of hospitalization. Burn skin injuries are classified according depth, surface thickness, with the superficial partial and deep thickness, and the total thickness. Regarding the extent of the injuries, the total body surface area (TBSA) burned is classified as small, when up to 10% TBSA; medium when between 10 and 25% TBSA; and large, when it affects 25% or more TBSA.\(^1\)

The high susceptibility of burned patients to infections is a concern regarding the evolution and success of clinical treatment. It was relate directly to morbidity and mortality of the group.\(^2\)

If there is a skin integrity breach, the wound colonization and bacterial invasion occurs. Infection or sepsis is present in a burn wound when deposition and multiplication of bacteria in the tissue is associated with host reaction or invasion of nearby tissue and a bacterial count of 105 CFU/g of tissue.\(^3\)

Burn injury results in tissue destruction and the presence of avascular burn eschar provides an environment for infection that can progress to septicemia. The exacerbation of infection by immuno-suppression is often associated with the burn injury.\(^4\) The rate of infection depends on the extent of the burn injury, general wound care and various host factors such as nutritional status, age, immune status, and comorbidity conditions.\(^3\)

The diagnosis and the establishment of the etiology of an infection in burn patients present numerous difficulties.\(^4\) The loss of the normal barrier function of the skin causes the most common pathological conditions associated with the infection are burning, heat loss, increased loss of water by evaporation and changes in the major interactive functions such as feel and appearance.\(^5\)

Microorganisms commonly involved in invasive burn wound infection include mainly are not limited to, *Pseudomonas aeruginosa*, *Acinetobacter spp*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter cloacae*, and *Proteus mirabilis*, *Staphylococcus aureus*, including oxacillin - resistant strains - MRSA, *Streptococcus*
pyogenes, Enterococcus spp, including vancomycin-resistant, anaerobic strains, Candida spp, Aspergillus spp, and viral agents such as herpes simplex and cytomegalovirus viruses.\textsuperscript{[6,7]}

Bacterial infection is a common complication in burn patients. The lack of primary barrier and immunosuppression and the presence of multiple foci of infection during the course of treatment may even lead to death.\textsuperscript{[5]}

The frequency and severity of septic complications in severely burned patients impose the need for an accurate diagnosis and appropriate therapy.\textsuperscript{[7]} Although developments in the treatment of major burns, these are still very susceptible to the development of secondary infections, as In patients with more than 40\% of body surface area burned 75\% of deaths are correlated with wound infection in large burns or other infectious complications.\textsuperscript{[8]}

The treatment of burn injuries is a major challenge to health professionals, especially regarding to high potential to develop infections. The systemic or local therapy should target primarily the balance of vital functions as well as the installation of measures to prevent complications, among which are infections of the lesions skins. Dressings containing healing and anti-infectious substances is the option for local therapy.\textsuperscript{[9]}

The silver has used as treatment for more than 200 years in burned patients. It has antiseptic, antibacterial and anti-inflammatory action. It is biologically active in its soluble ionic form or not (Ag + or Ag\textsuperscript{0}) and form Ag\textsuperscript{0} is present in nanocrystalline compounds. Silver is used against various pathogenic bacteria, acting by inhibiting the enzyme that produces folic acid required for the synthesis of precursors for DNA and RNA within the bacterial cell.\textsuperscript{[7,8,10]}

Motivated by the importance of infections associated with burns, this study aimed to identify the main microorganisms in burn wounds of patients in the healing process after topical treatment with nanocrystalline silver.

CASUISTIC AND METHODS

Bioethics Committee Permission
The study was been submitted and approved by the Ethics Committee of the Irmandade da Santa Casa de Misericordia de Limeira, a Burns Treatment Unit (UTQ) (Protocol 225/2013. The study was been conducted in the burned sector (reference center - high complexity) of a medium-sized hospital in the city of Limeira in São Paulo that serves patients mainly through the Unified Health System (SUS). The data of interest for this study were been collected in...
the information systems of the laboratory and the Committee on Hospital Infection Control (CHIC).

This is an observational clinical “study - cross- cohort” conducted through document analysis of the books of the Microbiology section of the laboratory record, and results of cultures of hospitalized patients.

All patients are hospitalized in the Burns Treatment Unit due to burns of second or third degree, admitted to the unit, or otherwise transferred from other hospitals, aged between two and seventy years were included in the survey, receiving treatment with nanocrystalline silver and hospitalized for more than 48 hours, the period from January to July 2013.

Positive cultures (identification of one or two bacterial species) were included in the statistical survey of incidence of etiologic agents in patients. The negative growth or multiple (more than three identified bacteria) cultures entered only for the total count of results of cultures analyzed.

Microbiological identification of infectious agents was been observed after collecting culture of swabs of the lesion and in aseptic conditions, and does not collect the perianal, genital area and armpits because colonization was performed.

All procedures were been performed by the Santa Casa laboratory staff.. It was used also the databases of the hospital.

In particular protocol, after first cleaning the wound using 0.9% saline (125 ml) with 40x12 needle punched, obtaining biological material was taken from the injured tissue is viable using sterile alginate Swab, a technique proposed by Martins and collaborators\(^{[11]}\) validated by Gardner and all.

The Swab with biological material was inoculated into a tube containing broth Brain Heart Infusion-BHI - Merck) and incubated at 35 ± 2 ° C for 24 or 48 hours. Evidenced the presence of turbidity in the media, will be made on-the ringing surface of MacConkey agar (Bio-RadLaboratories/USA) sheep blood agar and 5% mannitol (Bio-RadLaboratories/USA), which were incubated for 24 or 48 hours at 35 ± 2° C. The grown colonies were been submitted to Gram staining and after identified according to their morphological characteristics and staining.
Control strains when conducting microbial identification (Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 9027) were been used.

The hospital strains were reactivated and purity confirmed by seeding in culture media suitable for the technique of exhaustion. The presumptive identification of Gram-negative and positive was been done using specific means. After this time, the different colonies of bacteria that were been grown in isolated agars were been identified according to standardized by the Microbiology Laboratory of the hospital biochemical tests.

Results above 100,000 CFU/g were been considered as infection. Than it is follow to identify the agent(s) etiological(s), and antibiogram. Results below this value were considered colonization and follow for identification only.

**Statiscal**

The tabulation and quantitative and qualitative data and information resulting from the research analysis were been made using the software (Microsoft Excel) through the systematization of arrays and tables that correlate the data.

**RESULTS**

Sixty-eight cultures of samples from wounds of burn patients hospitalized in the period of January to July 2013, both in adult and pediatric patients were conducted. The final population consisted of 68 patients.

The frequency results for the year 2013 (January to July) is shown in Table 1.

The distribution of the number of cultures performed in each month and their results is available in Table 2.

The incidence in absolute values of each etiologic agent during the study period can be visualized in Table 3.

In 71% of microbiological cultures of samples from wounds of burn patients, was positive. P. aeruginosa was the most frequent etiologic agent (20.6%). Although S. epidermidis is the causative agent of lower frequency (7.3%), many centers are been stressed constantly and this
microorganism could be a significant pathogen in view of the state of severely burned patients.

These pathogens were responsible for approximately 80.9% of infections in burn. The incidence of infection during the study period was 70.6%. The peaks of infection in burn patients from January to July 2013 were the months of January, February, May and June, with an average of 71% of positive microbiological cultures. This may be because these patients were more likely to contract some kind of infection while in hospital, being a population with large open wounds and difficult to treat. Burn patients with systemic inflammatory reaction that modifies the metabolism and may have a higher probability of infection of the burned area. For patients with burns of medium and large, the various organic amendments caused interfere and hinder treatment.

Table 1: Frequency of culture results from patient samples of burnt industry conducted between January to July 2013, Limeira, 2013.

<table>
<thead>
<tr>
<th>Results</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>48</td>
<td>71%</td>
</tr>
<tr>
<td>Negative</td>
<td>08</td>
<td>11%</td>
</tr>
<tr>
<td>Multiple growths</td>
<td>12</td>
<td>18%</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2: Distribution of the results of patient samples from the burned industry conducted between January to July 2013, Limeira, 2013.

<table>
<thead>
<tr>
<th>Cultures performed</th>
<th>Positives</th>
<th>Negatives</th>
<th>Various species</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>12</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>February</td>
<td>10</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>March</td>
<td>08</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>April</td>
<td>08</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>May</td>
<td>10</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>June</td>
<td>11</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>July</td>
<td>09</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 3: Percentage Incidence of absolute values of etiologic agents identified in cultures from patient samples of burnt industry conducted between January to July 2013, Limeira, 2013.

<table>
<thead>
<tr>
<th></th>
<th>PSEUDOMONAS AERUGINOSA/PROTEUS MIRABILIS</th>
<th>ACINETOBACTER BAUMANNI/KLEBSIELLA</th>
<th>BACILIO GRAM POSITIVO</th>
<th>CITROBACTER</th>
<th>ENTEROCOCCUS SP</th>
<th>NEGATIVO/STAPHYLOCCUS EPIDERMIDS</th>
<th>NEGATIVO/STAPHYLOCCUS AUREUS</th>
<th>NEGATIVO/ACINETOBACTER RESISTANCE SUPERIOR A 3 BACTERIAS</th>
<th>NEGATIVO</th>
<th>PROTEUS MIRABILIS</th>
<th>PSEUDOMONAS AERUGINOSA</th>
<th>STAPHYLOCCUS AUREUS</th>
<th>STAPHYLOCCUS EPIDERMIDS</th>
<th>ACINETOBACTER BAUMANNI</th>
<th>ESCHERICHIA COLI/ENTEROCOCCUS SP</th>
<th>PSEUDOMONAS AERUGINOSA / KLEBSIELLA PNEUMONIAE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAN/13</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>FEB/13</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<td>2</td>
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<td>MAR/13</td>
<td>1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
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<td>0</td>
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<td>1</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MAY/13</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>JUN/13</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>JUL/13</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL:</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>2</td>
<td>14</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL %</td>
<td>2,94</td>
<td>2,94</td>
<td>4,41</td>
<td>2,94</td>
<td>2,94</td>
<td>1,47</td>
<td>1,47</td>
<td>1,47</td>
<td>11,76</td>
<td>2,94</td>
<td>20,59</td>
<td>13,24</td>
<td>7,35</td>
<td>10,29</td>
<td>1,47</td>
<td>1,47</td>
<td>2,94</td>
</tr>
</tbody>
</table>

Source: Beretta et al. World Journal of Pharmacy and Pharmaceutical Sciences
DISCUSSION
From the findings reported, the study revealed that in the period of seven months, the incidence of bacterial infections in wounds of burn patients admitted to the unit was 71%.

This is due to the fact that these patients have a high propensity to contract some kind of infection while still hospitalized, being a population with large open wounds and difficult to treat this in a potentially infectious environment, the hospital environment.\(^\text{[12]}\)

The average rate of positive cultures was 71 %, with peaks of high incidence in January (21%), February (15%), May (17%) and June (17%). These peaks may be associated with infection risk factors are the patient's own, such as size and depth of burn, age, preexisting disease, temperature and humidity of the lesion, occurrence of shock and acidosis, or related to the microorganism, such as number, virulence, motility, extracellular products, ie enzymes or bacterial exotoxins and resistance. There is also generalized immunosuppression in burns over 30% area, compromising the humoral and cellular defense.

Analyzing the effect of each agent in the cited months, we can observe that *P. aeruginosa* (20.6%), *S. aureus* (13.2%), and *Acinetobacter baumannii* (10.3%) were the cause of much these infections and showed greater frequency in cultures analyzed.

*P. aeruginosa* is the major causative pathogen colonization of the burn injury and serious wound infections, sepsis is an important, high morbidity and mortality in burns patients cause\(^\text{[12, 13]}\), and its action in burned patients ranging from pneumonia to bacteremia. Studies point to possible sources of infection to water, flowers, grids of hospital beds, desks and the mattress surface of the patient. Transmission may also occur through personal contact, as some people have *P. aeruginosa* as resident microbiota.\(^\text{[13]}\)

Incidence in infections caused by *A. baumannii* (10.3%) proved to be quite high compared to literature data, since national statistics point estimate of 0,8% of infections caused by this bacterium and international studies estimate 15.7 %.\(^\text{[14,15,16]}\)

The long hospitalization time is an important risk factor for multidrug resistance of *A. baumannii* in a unit of burn care.\(^\text{[17]}\) The microorganism are obtain or endogenous, the microflora also can be transmitted from exogenous sources, and subsequently may become endogen.\(^\text{[18]}\)
Recently *A. baumannii*, *K. pneumoniae* replaced as the second most common pathogen causing infection in burn patients. This is due mainly to its ability to remain viable in a hospital environment, thanks to its status of multidrug-resistant microorganism and several other factors.\[13\]

The colonization by *S. aureus* is often associated with delayed wound healing, an increase in the need for surgical interventions and prolonged internation.\[18\]

The thermal injury prevents the skin from performing its normal barrier function, allowing microbial colonization of burns. The type and amount of microorganisms colonizing the wound influenced the frequency of invasive infections, septicemia and dead.\[18\]

As these microorganisms are the major clinical and epidemiological problem in hospitals, especially in ICUs, which are easily transferred between hospital personnel and patients, great importance should be given to this effect in a unit burned.\[19,20\]

Beyond the extent of body surface area burned, which causes no skin coverage with large load of bacterial colonization, other factors such as immunosuppression resulting from thermal injury, the possibility of gastrointestinal bacterial translocation and prolonged hospitalization, favoring infectious complications in burn.\[20\] Also, the use of catheters and tubes, or invasive diagnostic and therapeutic procedures that end altering the natural defenses of the host, favor the occurrence of infection.\[21,22\]

Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality. Significant thermal injuries induce a state of immunosuppression that predisposes burn patients to infectious complications.\[23, 24\]

Today, despite medical advances in the treatment of burns, development of potent topical and systemic antimicrobial agents, new techniques of silver-based dressings, advances in nutritional support and use of surgical techniques for excision of devitalized tissue and early grafting burned area, infectious complications still represent a major challenge, leaving serious consequences and leading to high mortality.\[24-25;26\]

**CONCLUSION**

Despite increasing research process and upgrading as regards to the treatment of burn patients, infections remain as an obstacle to be overcame completely, and represents one of
the major causes of death of burned patients. Therefore, it is vitally important to understand the different manifestations in infectious processes, as non-intact skin is the main gateway of microorganisms.

Although studies suggest that the toppings containing nanocrystalline silver are an excellent option to combat microbial topic exuding wounds that present in abundance, the results of this study allow us to suggest that there is still concern about the complications presented by burn patients.

The relentless rise in antibiotic resistance among pathogenic bacteria and fungi, coupled with the high susceptibility of burn wounds to infection, and the difficulty of systemically administered antibiotics to reach damaged tissue, taken together have made the development of novel topical antimicrobials for burn infections a fertile area of innovation for researchers.

COMPETING INTERESTS
All the authors declare that do not have any competing interest (s) which can interfere in their judgement of analysis and interpretation of results of this study.

AUTHORS CONTRIBUTIONS
1. Daniela Scheuer Bom - Participated in the design of the study, execution of the research and writing of the article.

2. Armindo Antonio Alves - Participated in the design of the study and performed the statistical analysis.

3. Ana Laura Remédio Zeni Beretta - Participated in the conception, design, and performed the statistical analysis, implementation of research, writing the article and final approval of the version to be published.

All authors read and approved the final manuscript.

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REFERENCES


