Antimicrobial Susceptibility and Biofilm Production by
Staphylococcus aureus and Staphylococcus epidermidis
Isolated from Elderly Residents of a Nursing Home

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Abstract

Elderly residents of nursing homes are more susceptible to bacterial infections when compared to young adults, mostly by commensal bacteria from microbiota, such as staphylococci. This study aimed to isolate S. aureus and S. epidermidis from the nasal cavity of elderly residents of a nursing home in Presidente Prudente, SP, Brazil, to determine their antimicrobial susceptibility and biofilm production. Swabs were collected from 32 individuals and the S. aureus and S. epidermidis isolates were tested regarding their antimicrobial susceptibility by the disc diffusion method and agar screening with oxacillin. Biofilm production was determined by the Congo Red Agar (CRA) method and adherence to borosilicate test tubes. Forty-one bacterial samples were obtained, corresponding to 87.8% of staphylococci. S. aureus was isolated from 10 individuals and S. epidermidis from 8. The isolates were resistant to oxacillin, cefoxitin, erythromycin, levofloxacin and clindamycin, and 100% susceptible to vancomycin and linezolid. Oxacillin resistance was found in 40% of S. aureus and 62.5% of S. epidermidis isolates. Biofilm production by S. aureus was observed in 90% of strains by CRA and 80% by the borosilicate test tube. By CRA and borosilicate tube test, respectively, 100% and 62.5% of S. epidermidis produced biofilm. Our data emphasize the need for prophylactic and biosecurity measures in order to avoid dissemination of multiresistant and virulent strains in those environments, a potential cause of elderly infections.

Introduction

The old age represents a condition of special needs and particularities, due to physical, metabolic, immunological and nutritional changes. Consequently, elderly are more susceptible to bacterial infections when compared to young adults, especially by microbes that inhabit the human skin and mucosal surfaces [1]. Modifications in skin and mucosal microbiota often lead to colonization by opportunistic microorganisms, such as staphylococci, mostly represented by S. aureus and S. epidermidis [2].

The Staphylococcus species may cause several infections, such as skin infections, endocarditis, osteomyelitis, bacteremia and sepsis [3,4]. Older adults often necessitate using implantable medical devices and prostheses, instruments that are easily colonized by bacteria capable of producing biofilm under suitable physiological conditions. This virulence factor consists of a multilayered polysaccharide which confers excellent protection against immune system and antimicrobial action [5].

Elderly patients in nursing homes may present some particularities that favor the staphyloccocal infection, such as a greater contact and sharing of objects, polymedication, trauma, surgical procedures and chronic diseases [1]. On the other hand, many of the elderly have prior stays in hospitals and prior use of antimicrobials, which raises the selective pressure for the establishment of S. aureus in skin and mucosal microbiota [2]. This study aimed to identify S. aureus and S. epidermidis from nasal cavity of elderly residents of a Nursing Home in Presidente Prudente, SP, Brazil, and to determine their antimicrobial susceptibility and ability to produce biofilm.
Materials and Methods

Ethical Considerations

A cross-sectional prospective study was conducted and approved by the Research Ethics Committee of the Oeste Paulista University, under the protocol CAAE 57069216.0.0000.5515. The participants, elderly residents of a nursing home in Presidente Prudente, São Paulo, Brazil, were randomly selected and invited to be in this study. The inclusion criteria were age above 60 years and a stay of at least four weeks in the nursing home. The exclusion criteria included any sign of upper respiratory tract infections and the use of antimicrobials until one month before the sample collection.

Strains

Bacterial strains were collected using a sterile swab, moistened with saline solution (0.85%), by bearing at the entrance of the nasal fossae. Samples were sent to the Laboratory of Microbiology of the Oeste Paulista University and cultured on Manitol Agar for selection of staphylococci. After incubation, the isolates were submitted to Gram staining, catalase and coagulase tests for identification of S. aureus and Coagulase-Negative Staphylococci (CoNS). CoNS strains were identified by biochemical tests according to Cunha et al. [6].

Disc diffusion method

The disc diffusion test employing oxacillin, cefoxitin, erythromycin, levofloxacin, clindamycin, vancomycin and linezolid was used for phenotypic detection of resistance, according to the Clinical Laboratory Standards Institute-CLSI [7].

Screening test in Mueller Hinton agar

Two screening mediums prepared with Mueller Hinton Agar were used for confirmation of oxacillin resistance: one was added with 6 mg/ml of oxacillin and 4% of NaCl (for S. aureus), and the other one was added with 4 µg/ml of oxacillin and 4% of NaCl (for S. epidermidis). Inoculums were prepared according to Pereira, et al. and oxacillin resistance was determined by growth of at least one colony on the agar surface [8].

Detection of Biofilm production by Congo Red Agar (CRA)

Strains were cultured on Congo Red Agar and incubated at 37°C for 24 hours to 48 hours. Biofilm producer colonies presented a black aspect. The isolates presenting a color from red to maroon were considered as non-biofilm producers [9].

Detection of Biofilm production by the borosilicate tube test

Determination of biofilm production was also performed by adherence to the borosilicate test tube. A culture of Staphylococcus on TSB broth was incubated at 37°C for 48 hours in the borosilicate tubes. After the discard of the broth, 1mL of Tripan blue 0.4% was added. The presence of a layer of stained material adhered to the inner wall of the tube was defined as a positive result [10].

Results and Discussion

A total of 41 strains were isolated from 32 elderly individuals, of which 87.8% corresponded to staphylococci isolates. Such prevalence was expected, since human microbiota is mostly composed by Staphylococcus [11]. The biochemical tests identified 24 (58.5%) CoNS, and 8 (33.3%) were identified as S. epidermidis (Figure 1). According to Kloos et al. S. epidermidis is the leading colonizer of skin and mucosa, mostly found on surfaces of armpits, head and nostrils [12].

S. aureus were identified in 10 (29.3%) samples. Silveira, et al. has studied 8 Brazilian nursing homes and found a prevalence of 18% of S. aureus [2]. On the other hand, Denis et al. showed a colonization rate of 51% by S. aureus in a work performed in Belgium [13]. Such difference may be due to the fact that, in the present study, only one nursing home was included, with a consequent low number of strains. Besides, those rates might be related to the peculiarities of each nursing home, considering that hygiene measures are different between institutions and countries.

The analysis of the antimicrobial susceptibility profile in S. aureus and S. epidermidis revealed resistance to oxacillin, cefoxitin (which relates to oxacillin resistance), erythromycin, levofloxacin and clindamycin. All strains were susceptible to vancomycin and linezolid (Table 1). Comparing S. aureus and S. epidermidis, surprisingly the first presented higher rates of resistance to clindamycin and erythromycin, and the second for oxacillin and levofloxacin. In general, CoNS presents higher rates of resistance than S. aureus [14]. Notwithstanding, the low number of resistant strains limits such comparison.

The detection of Methicillin-Resistant S. aureus (MRSA) and Methicillin-Resistant S. epidermidis (MRSE) was performed by the disc diffusion test with the oxacillin and cefoxitin discs, and confirmed by the screening method. Four (40%) MRSA and 5 (62.5%) MRSE were detected (Table 2). The frequency of MRSA and MRSE in the elderly population living in nursing homes reflects the indiscriminate use of antimicrobials, probably due to prophylactic measures and treatment of infections in elderly. The condition of S. epidermidis as a human commensal turns it into an ideal carrier and reservoir of resistance genes [4].

Regarding the biofilm formation by S. aureus, the CRA method

![Figure 1: Distribution of species isolated from elderly residents of the nursing home. BGN = Gram-negative bacilli.](image)

<table>
<thead>
<tr>
<th>S. aureus</th>
<th>S. epidermidis</th>
</tr>
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<tbody>
<tr>
<td>Susceptible</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>1(10%)</td>
</tr>
</tbody>
</table>

Table 1: Susceptibility of S. aureus and S. epidermidis.
determined 9 (90%) biofilm producers, and the borosilicate tube test found 8 (80%) biofilm producers. On the other hand, by the CRA and the borosilicate tube test, respectively, 8 (100%) and 5 (62.5%) of S. epidermidis produced biofilm.

The adherence by the borosilicate tube test was performed for confirmation of the results, since the CRA method is subjective, and contributes to antimicrobial resistance, since the biofilm matrix interferes with the diffusion of the drug, acting almost like an impermeable barrier [16]. Furthermore, biofilm environment enables the exchange of genetic elements, such as plasmids, which contains resistance genes and virulence factors, enhancing the pathogenic potential of bacteria in that community [4]. The high prevalence of biofilm production in our study is concerning due to the frequent use of medical devices by elderly individuals.

The characterization of S. aureus and S. epidermidis from elderly individuals living in a nursing home has demonstrated resistance to common antimicrobials and total susceptibility to vancomycin and linezolid, as well as biofilm production by more than 90% of the strains. We emphasize the need for prophylactic control of certain strains in nursing institutions, especially for S. aureus.

References