American Journal of Clinical Microbiology and Antimicrobials

Research Article
Published: 05 Apr, 2018

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

Luiza Pinheiro[1,2]*, Isabella Carolina Rodrigues Dos Santos Goes[3], Kesia Priscila Isquerdo Dias[3], Lana Aparecida De Oliveira Pereira[3], Caroline Rodrigues Costa[3] and Valéria Cataneli Pereira[3]

[1]*Department of Microbiology and Immunology, Institute of Biosciences, UNESP - Univ. State of São Paulo, Brazil*

[2]*Department of Pathology, Lauro de Souza Lima Institute, Brazil*

[3]*UNOESTE-Universidade do Oeste Paulista, Brazil*

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.

OPEN ACCESS

Correspondence:
Luiza Pinheiro, Department of Microbiology and Immunology, Institute of Biosciences of Biosciences, UNESP-UNESP - Univ. State of São Paulo, Botucatu, Brazil,
E-mail: luizapinheiro@ibb.unesp.br

Received Date: *23 Feb 2018*
Accepted Date: *29 Mar 2018*
Published Date: *05 Apr 2018*

Citation:
Pinheiro L, Dos Santos Goes ICR, Dias KPI, De Oliveira Pereira LA, Costa CR, Pereira VC. Antimicrobial Susceptibility and Biofilm Production by Staphylococcus aureus and Staphylococcus epidermidis Isolated from Elderly Residents of a Nursing Home. Am J Clin Microbiol Antimicrob. 2018; 1(3): 1011.

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 staphylococcal 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.

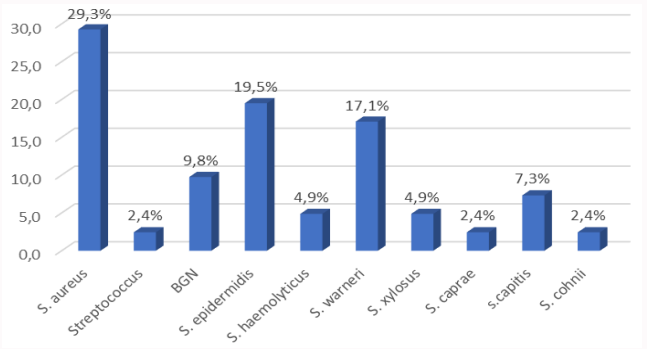

Figure 1: Distribution of species isolated from elderly residents of the nursing home. BGN = Gram-negative bacilli.

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, levofloxacyn, 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 grown 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), erytromycin, 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

Table 1: Susceptibility of *S. epidermidis* and *S.aureus*.

	S. aureus		*S. epidermids*	
	Resistant	**Susceptible**	**Resistant**	**Susceptible**
Erytromycin	5 (50%)	5 (50%)	3 (37,5%)	5 (62,5%)
Cefoxitin	1 (10%)	9 (90%)	2 (25%)	6 (75%)
Clindamycin	2 (20%)	8 (80%)	1 (12,5%)	7 (87,5%)
Oxacillin	3 (30%)	7 (70%)	2 (25%)	6 (75%)
Vancomycin	-	10 (100%)	-	8 (100%)
Levofloxacin	-	10 (100%)	2 (25%)	8 (100%)
Linezolid	-	10 (100%)	-	8 (100%)

Table 2: Determination of MRSA and MRSE by the screening method and disc diffusion test with oxacillin and cefoxitin.

Method	MRSA n (%)	MRSE n (%)
Oxacillin	2 (50%)	-
Cefoxitin	-	1 (20%)
Screening	1 (25%)	2 (40%)
Oxacillin + Cefoxitin	1 (25%)	-
Oxacillin + Screening	-	1 (20%)
Cefoxitin + Screening	0%	-
Oxacillin + Cefoxitin + Screening	-	1 (20%)
TOTAL	4 (100%)	5 (100%)

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, depending on the observer experience, what may lead to diverse interpretation [15]. Surprisingly, biofilm production by the borosilicate tube test was higher in *S. aureus*. Biofilm is considered the main virulence factor of *S. epidermidis* 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

1. Araújo RA de. Estudo dos factores de risco associados a infecções do tracto urinário em idosos institucionalizados. 2011;50.
2. Silveira M da. Prevalência e fatores de risco para carreamento de *Staphylococcus aureus* resistente à meticilina em idosos institucionalizados na cidade de Bauru-SP. Universidade Estadual Paulista "Júlio de Mesquita Filho". 2013.
3. DeLeo F, Diep BA, Otto M. Host Defense and Pathogenesis in *Staphylococcus aureus* Infections. Infect Dis Clin North Am. 2009;23(1):17-34.
4. Otto M. *Staphylococcus epidermidis*--the "accidental" pathogen. Nat Rev Microbiol. 2009;7(8):555-67.
5. Dai L, Yang L, Parsons C, Findlay VJ, Molin S, Qin Z. *Staphylococcus epidermidis* recovered from indwelling catheters exhibit enhanced biofilm dispersal and "self-renewal" through downregulation of agr. BMC Microbiol. 2012;12:102.
6. Cunha MD, Sinzato YK, Silveira LV. Comparison of methods for the identification of coagulase-negative staphylococci. Mem Inst Oswaldo Cruz. 2004;99(8):855-60.
7. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. Approv Stand M100-S19. 2009;29(3).
8. Pereira VC, Martins A, de Souza Rugolo LMS, de Lourdes Ribeiro de Souza da Cunha M. Detection of Oxacillin Resistance in *Staphylococcus aureus* Isolated from the Neonatal and Pediatric Units of a Brazilian Teaching Hospital. Clin Med Pediatr. 2009;3:23-31.
9. Freeman D, Falkiner F, Keane C. New method for detecting slime production by coagulase-negative staphylococci. J Clin Pathol. 1989;42(8):872-4.
10. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immun. 1982;37(1):318-26.
11. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol. 2011;9(4):244-53.
12. Kloos WE, Schleifer KH. Isolation and Characterization of Staphylococci from Human Skin II. Descriptions of Four New Species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus simulans*. Int J Syst Bacteriol. 1975;25(6):62-79.
13. Denis O, Jans B, Deplano A, Nonhoff C, De Ryck R, Suetens C, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) among residents of nursing homes in Belgium. J Antimicrob Chemother. 2009;64(6):1299-306.
14. Becker K, Heilmann C, Peters G. Coagulase-Negative Staphylococci. Clinical Microbiology Reviews. 2014;27(4):870-926.
15. Sanches P. Detecção da Produção de Biofilme em *Staphylococcus aureus* e Estafilococos Coagulase- Negativa isolados de Recém-Nascidos. 2008;36.
16. Schoenfelder SMK, Lange C, Eckart M, Hennig S, Kozytska S, Ziebuhr W. Success through diversity - How *Staphylococcus epidermidis* establishes as a nosocomial pathogen. Int J Med Microbiol. 2010;300(6):380-6.