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Molecular epidemiology of coagulase-negative bloodstream isolates: detection of *Staphylococcus epidermidis* ST2, ST7 and linezolid-resistant ST23

Adrián Martínez-Meléndez[a], *Rayo Morfín-Otero*[b,c], *Licet Villarreal-Treviño*[a], *Adrián Camacho-Ortíz*[d], *Gloria González-González*[e], *Jorge Llaca-Díaz*[f], *Eduardo Rodríguez-Noriega*[b], *Elvira Garza-González*[f,g,*]

[a] *Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Departamento de Microbiología e Inmunología, Monterrey, Mexico*
[b] *Hospital Civil de Guadalajara "Fray Antonio Alcalde", Guadalajara, Mexico*
[c] *Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Instituto de Patología Infecciosa y Experimental, Guadalajara, Mexico*
[d] *Universidad Autónoma de Nuevo León, Hospital Universitario Dr. José Eleuterio González, Servicio de Infectología, Monterrey, Mexico*
[e] *Universidad Autónoma de Nuevo León, Facultad de Medicina, Departamento de Microbiología, México, Monterrey, Mexico*
[f] *Universidad Autónoma de Nuevo León, Hospital Universitario Dr. José Eleuterio González, Departamento de Patología Clínica, Monterrey, Mexico*
[g] *Universidad Autónoma de Nuevo León, Hospital Universitario Dr. José Eleuterio González, Servicio de Gastroenterología, Monterrey, Mexico*

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ABSTRACT

The mechanisms contributing to persistence of coagulase-negative staphylococci are diverse; to better understanding of their dynamics, the characterization of nosocomial isolates is needed. Our aim was to characterize phenotypic and molecular characteristics of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* human blood isolates from two tertiary care hospitals in Mexico, the Hospital Universitario in Monterrey and the Hospital Civil in Guadalajara.

Antimicrobial susceptibility was determined. Biofilm formation was assessed by crystal violet staining. Detection of the *ica* operon and Staphylococcal Cassette Chromosome *mec* typing were performed by PCR. Clonal relatedness was determined by Pulsed-fiel gel electrophoresis and Multi locus sequence typing.

Methicillin-resistance was 85.5% and 93.2% for *S. epidermidis* and *S. haemolyticus*, respectively. Both species showed resistance >70% to norfloxacin, clindamycin, levofloxacin, trimethoprim/sulfamethoxazole, and erythromycin. Three *S. epidermidis* and two *S. haemolyticus* isolates were linezolid-resistant (one isolate of each species was *cfr*+). Most isolates of both species were strong biofilm producers (92.8% of *S. epidermidis* and 72.9% of

* *Corresponding author.*
E-mail address: elvira_garza_gzz@yahoo.com (E. Garza-González).
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S. haemolyticus). The *ica* operon was amplified in 36 (43.4%) *S. epidermidis* isolates. SCC*mec* type IV was found in 47.2% of the *S. epidermidis* isolates and SCC*mec* type V in 14.5% of *S. haemolyticus* isolates. No clonal relatedness was found in either species. Resistance to clindamycin, levofloxacin, erythromycin, oxacillin, and cefoxitin was associated with biofilm production for both species ($p < 0.05$). A G2576T mutation in 23S rRNA gene was detected in an *S. haemolyticus* linezolid-resistant isolate. All linezolid-resistant *S. epidermidis* isolates belonged to ST23; isolate with SCC*mec* type IV belonged to ST7, and isolate with SCC*mec* type III belonged to ST2. This is the first report of ST7 in Mexico.

There was a high genetic diversity in both species, though both species shared characteristics that may contibute to virulence.

Introduction

Coagulase-negative staphylococci (CoNS) are among the main causative agents of bacteremia.[1] *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* are the CoNS species most frequently isolated from blood.[2] These species are often associated with infections in immunocompromised patients who have medical device implants.[3] These species persist on medical devices because they can form biofilms, bacterial clusters that attach to materials such as plastics. Biofilm formation has been associated with the presence of the *ica* operon that encodes for the production of a polysaccharide intercellular adhesin (PIA). This operon contains the *icaA*, *icaB*, *icaC*, *icaD*, and *icaR* genes; expression of these genes have been found to be involved in biofilm formation.[4] Furthermore, biofilm production has been associated with an increased resistance to antibiotics.[5] CoNS strains may present a high proportion of resistance to antibiotics,[6,7] particularly methicillin resistance, thus complicating the management of these infections.

Methicillin resistance in *Staphylococcus aureus* was first reported in 1961.[8] Methicillin-resistant *S. aureus* strains produce a penicillin-binding protein, known as PBP2a or PBP2′, that has low binding affinity for β-lactams. PBP2a is encoded by *mecA*, which is contained within the Staphylococcal Cassette Chromosome *mec* (SCC*mec*).[9] Also, methicillin-resistance has been found in CoNS species more often than in *S. aureus* species isolated from clinical samples.[10] To date, 11 types of SCC*mec* have been described in *S. aureus* (http://www.sccmec.org/), and evidence suggests that SCC*mec* structures may be more diverse in CoNS. These various structures may contain combinations of *mec* and *ccr* complexes not described for *S. aureus* or may contain multiple *ccr* complexes.[11] Since methicillin-resistance is more frequent in CoNS, methicillin-resistant CoNS (MR-CoNS) may serve as a large reservoir of SCC*mec* and may contribute to the formation of methicillin-resistant *S. aureus* (MRSA) strains.[10]

Since CoNS are components of the human skin microbiota, often endogenous strains are capable of causing infections in immunocompromised individuals. However, there are reports of persisting strains in hospital wards.[12,13] The genetic relatedness between these strains has been determined by Pulsed-Field Gel Electrophoresis (PFGE); a technique that has been widely used for molecular typing of nosocomial pathogens. Nevertheless, Multilocus Sequence Typing (MLST), based on sequencing of conserved housekeeping genes, is proving to be the most appropriate tool for the study of the global epidemiology, allowing the comparison of isolates from different countries and the naming of international clones. To date, a widely acceptable MLST scheme and database have been developed for *S. epidermidis* only but not for *S. haemolyticus*.[14]

The persistence of strains of these species may be due to their increasing high resistance to antimicrobials, which may enhance their fitness to hospital environments, including linezolid-resistance. At the same time, the production of biofilm has been associated with antibiotic resistance and the persistence of the strains in medical devices. Furthermore, the presence of SCC*mec* worsens the scenario, since horizontal transference of these elements may contribute to antibiotic resistance. Finally, the information of genetic relatedness would allow us to know the dynamics of transmission of these infections. We hypothesized that the presence of strains with traits such as biofilm formation, high resistance to antibiotics and diverse SCC*mec* elements may contribute to the persistence of these strains. Thus, we aimed to characterize phenotypic and molecular characteristics of *S. epidermidis* and *S. haemolyticus* blood isolates from two Mexican tertiary-care hospitals, the Hospital Universitario in Monterrey and the Hospital Civil in Guadalajara, in order to determine distribution of SCC*mec* elements, antibiotic resistance, genetic relatedness, and biofilm formation, as well as to examine its relationship to drug resistance. Both settings are teaching hospitals that offer a variety of services, educational programs, a space for clinical research and health-related community services working as a reference facility for two of the major metropolitan areas in Mexico.

Materials and methods

Hospital setting and identification of clinical isolates

This study was performed at the Hospital Civil de Guadalajara "Fray Antonio Alcalde", a 1000-bed tertiary care teaching hospital, with approximately 30,000 admissions annually, located in Guadalajara, Jalisco, and at the Hospital Universitario "Dr. José Eleuterio González", a 450-bed tertiary-care teaching