PREVALENCE OF *ICA* AND *ICD* GENES IN *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS* STRAINS ISOLATED FROM PATIENTS AND HOSPITAL STAFF

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SUMMARY

*Staphylococcus* are ubiquitous microorganisms that predominate in normal skin and mucosal flora. *Staphylococcus aureus* and *Staphylococcus epidermidis* have been identified as a major cause of nosocomial infections, especially in patients with predisposing factors such as indwelling or implanted foreign bodies. The ability of both *S. epidermidis* and *S. aureus* to produce biofilm was compared between 116 clinically significant strains (46 from blood cultures of patients with bloodstream infection and 70 isolated from catheters) and 60 strains isolated from nasal swabs of healthy carriers from hospital staff. The presence of the intercellular adhesion genes (*ica* and *icd*) was determined by the Polymerase Chain Reaction method, and slime production was examined using qualitative Congo red agar technique. Among clinical strains, 35.2% (19/54) of *S. aureus* and 48.4% (30/62) of *S. epidermidis* were both positive *ica* and *icd* and they produced slime. Among carrier strains, 22.2% (8/36) of *S. aureus* and 33.3% (8/24) of *S. epidermidis* were positive for slime synthesis and exhibited ica genes. Our results suggest that the virulence factors contributing to the development of infections can be present in patient and hospital staff isolates. Thus, we consider it is important to detect healthy carriers of slime-producing staphylococci and to control the dissemination of these microorganisms especially in a hospital.

Key words: Staphylococcus aureus, Staphylococcus epidermidis, slime production, Congo red Agar, ica genes

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INTRODUCTION

*Staphylococcus aureus* and *Staphylococcus epidermidis* form part of the normal mucosal and skin microflora. Nasal carriers of *staphylococci* are recognized as important reservoir with the risk of developing endogenous infections or of transmitting infections to susceptible persons. *S. aureus* is one of the most frequently isolated bacterial pathogens causing both hospital and community-acquired infections and, together with *S. epidermidis*, is the organism most frequently isolated from medical implant-related infections. Several studies have been done to elucidate the mechanism by which *Staphylococci* are able to cause severe infections associated with biomaterials (1, 2, 3). Certain *S. aureus* and *S. epidermidis* strains have the ability to colonize medical devices such as catheters and to develop a highly consolidated structure: the biofilm, which is believed to make the organisms more resistant to antibiotics and host defenses (4, 5, 6, 7). This is a serious complication, especially when the infection is caused by multiresistant bacteria difficult to eradicate from the prosthetic material.

The ability of bacteria to aggregate and form biofilm is strictly related to the capacity of producing an extracellular mucoid substance: the slime, whose main component is of polysaccharide nature and consists of glycosaminoglycans (8). It has been shown that both *S. aureus* and *S. epidermidis* contain the ica (ADBC) operon responsible for slime production. In the operon, icaA encodes for N-acetylgalcosaminyltransferase, the enzyme for polysaccharide intercellular adhesin (PIA) synthesis (9, 10). However, the coexpression of icaA and icaD leads to significant increase in the activity and is related to the full phenotypic expression of the capsular polysaccharide (11). Several studies subsequently reported that the slime-producing strains of both *S. aureus* and *S. epidermidis* are more virulent and are responsible for intravenous catheters-related bacteremia and severe post-surgical or periprosthetic infections (12, 13, 14, 15).

The comparison between ica genes in *S. epidermidis* associated to either bacteremia or colonized intravenous devices and ica genes in *S. epidermidis* from normal flora of healthy volunteers who were not hospitalized showed that ica genes were twice more frequent in isolates associated to infection (16).

The aim of this study was to associate carriage and disease to the presence of icaA and icaD genes and slime production in both carrier and clinically significant strains. The present study was achieved by studying bacterial isolates collected from blood cultures, intravenous catheters and nasal mucosa of healthy volunteers from a regional hospital provided with 290 beds, in San Luis, Argentina.
MATERIALS AND METHODS

**Bacterial strains:** Forty-six strains of *Staphylococcus* were obtained from blood of patients with non catheter-related bloodstream infections, 70 strains from intravenous catheters and 60 strains were isolated from nasal swabs of healthy care workers. The catheters from a localized infection revealed by signs of infection at the exit site, tunnel tract or pocket were analyzed. All isolates were grown in blood agar plates and mannitol salt agar for 24–48 h at 37 °C.

**Species identification.** Isolates were identified as *S. aureus* using Gram stain characteristics, traditional biochemical test, including catalase, coagulase, slide agglutination and acid production from glucose, D-maltose, D-trehalose, D-mannitol, sucrose. *S. epidermidis* was identified by a simplified method proposed by De Paulis et al. (17). Briefly, this five-test simple scheme combines the novobiocin susceptibility test with tests for urease, pyrrolidonyl arylamidase, ornithine decarboxylase, and aerobic fermentation at the exit site, tunnel tract or pocket were analyzed. All isolates were grown in blood agar plates and mannitol salt agar for 24–48 h at 37 °C.

**DNA extraction method.** The DNA extraction method has been previously described (19). A single colony of each bacterial strain was grown at 37 °C for 24 h in trypticase soya broth. After incubation, the bacteria were harvested by centrifuging 100 µl and resuspended in 50 µl of lysostaphin solution (100 µg/ml), and samples were incubated at 37 °C. After 10 min, 50 µl of proteinase K solution (100 µg/ml) and 150 µl of 0.5 M Tris-HCl (pH 8) were added and incubated for a further 10 min. Samples were then heated for 5 min at 100 °C and were centrifuged for 4 min at 5,000 x g. The DNA precipitate was resuspended in bidistilled water and used as a template for Polymerase Chain Reaction (PCR) method.

**PCR method for the amplification of the icaA and icaD sequences:** Two pairs of primers were designed for the amplification of the icaA and icaD genes with previously published sequences (19). For the detection of icaA, the following primers were used: 5’-ACAGTCGCTACGAAAAGAAA as the forward primer and 5’-GGAAATGCCCATAATGACAC as the reverse primer, yielding a PCR product of 103 bp. For the detection of icaD the following primers were used: 5’-ATGGTCAAGGCCC AGACAGAG and 5’-CGTGTTTTCCAACATTTAATGC AA as forward and reverse primer respectively, yielding a PCR product of 198 bp. PCR reactions were performed using the method described by Arciola et al. (19) with some modifications. The reaction mixture consisted of a reaction buffer (50 mM KCl, 10 mM Tris-HCl [pH 9], 2,5 mM MgCl2) in a total volume of 25 µl containing 5 µl of template DNA (150 ng), 0,2 mM of each deoxyribonucleotide triphosphate (dATP, dTTP, dGTP, dCTP), 1 U Taq DNA polymerase and the above-mentioned primers (0,4 µM each for icaA and 1µM each for icaD). DNA amplification was carried out in a thermocycler with the following thermal cycling profile: initial denaturation at 94 °C for 5 min, followed by 50 cycles of amplification (denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 30 s) ending with a final extension at 72 °C for 1 min. After the first 30 cycles, a further 1U of Taq DNA polymerase was added.

Eight microliters of PCR products were analyzed by electrophoresis in 2% agarose gel during 50 min at 80V. The bands were stained with ethidium bromide (0.5 µg/ml) and observed under UV light. Each run included a DNA marker and the ATCC 12228 and ATCC 35984 *S. epidermidis* strains as controls.

**RESULTS**

The slime-production of all strains under study was performed by cultures on CRA. Slime-producing strains appear as black and bordeaux almost black colonies, and non slime-producing strains as bordeaux and red colonies (Fig. 1).

Among all the clinical isolates, 19 out of 54 *S. aureus* (9 from blood cultures and 10 from catheters) and 29 out of 62 *S. epidermidis* (6 from blood cultures and 23 from catheters) were found to be biofilm positive by CRA technique (Table 1).

The PCR technique was applied to the 176 staphylococcal strains (Table 1 and Fig. 2). All strains that were positive for icaA were also positive for icaD.

![Fig. 1. Slime-production by cultures on CRA. a) Black colonies of slime producing *S. epidermidis* strain ATCC 35984. b) Bordeaux colonies of a non slime-producing strain. c) Red colonies of the non slime-producing *S. epidermidis* strain ATCC 12228.](image)
et al. (23) and Arciola et al. (19) reported respectively, of saprophytic strains from skin and mucosa were non slime-producing strains as virulence markers for clinically significant other authors who have indicated the important role of healthy volunteers was 37.5%, but differ from those reported by who found that the rate of strains carrying the These results are in agreement with those of Frebourg et al. (16), not exclusive characteristic of clinical midis was detected in 22.2% and 33.3% of strains isolated from hospital staff respectively. Thus, it appears that the presence of ica genes and slime production are not exclusive characteristics of clinical Staphylococcus strains. These results are in agreement with those of Frebourg et al. (16), who found that the rate of strains carrying the ica locus among healthy volunteers was 37.5%, but differ from those reported by other authors who have indicated the important role of ica genes as virulence markers for clinically significant Staphylococcus isolates (11, 23).

Arciola et al. (11) found that all saprophytic S. epidermidis strains from skin and mucosa were non slime-producing strains and ica genes were negative. However, in other studies, Ziebuhr et al. (23) and Arciola et al. (19) reported a 6% and 11.5%, respectively, of saprophytic S. epidermidis strains isolated from hospital staff, which formed slime and exhibited positive ica genes. In our study, a higher percentage of these strains was detected. This leads us to think that the ica genes might be more prevalent in Staphylococcus strains isolated from the hospital staff than in those obtained from healthy individuals of a non-hospital community. Hospital staff is often colonized with nosocomial staphylococcal strains that could be the reason for increased proportion of ica positive strains in our study.

In agreement with other authors (11, 16, 23), in the present study, the presence of ica genes was found to be more common in Staphylococcus strains associated with disease than in strains isolated from healthy carriers (Table 1).

In addition, a good correspondence between the phenotypic characterization by Congo red agar plate technique and ica genes presence was detected (Table 1). These data are in agreement with some authors (11, 23). However, Silva et al. (24), from blood culture isolates, reported that of the 49 S. epidermidis strains found positive for the ica operon, only 29 (59%) were found to be biofilm producers by the CRA technique.

Using PCR we detected icaA and icaD genes in 19 (35.2%) clinical S. aureus strains isolated from catheters. Previous reports (11) detected these genes in 14 of 23 (61%) isolates. On the other hand, Crampton et al. (9) and Fowlie et al. (25) found all S. aureus isolates were icaADBC positive. A possible explanation for this discrepancy is that the primers used in our study were based on the icaAD sequence of RP62A S. epidermidis (GenBank accession nº U43366) in which icaA and icaD display only 76% and 72% identity to the sequence of ATCC 35556 S. aureus respectively (9).

### DISCUSSION

*S. epidermidis*, together *S. aureus*, represents the most frequent cause of nosocomial sepsis, and they are the most common agents of infections associated with catheters and other indwelling medical devices. It is known that the capacity of staphylococci to adhere and grow on plastic surfaces is a crucial step in the pathogenesis of polymer-associated infections (20, 21). Moreover, biofilm production has also been shown to be associated with virulence in the absence of prosthetic material in an animal model (22).

In this study, the presence of ica genes and slime production was detected in 22.2% and 33.3% of *S. aureus* and *S. epidermidis* strains isolated from hospital staff respectively. Thus, it appears that the presence of ica genes and slime production are not exclusive characteristics of clinical *Staphylococcus* strains. These results are in agreement with those of Frebourg et al. (16), who found that the rate of strains carrying the ica locus among healthy volunteers was 37.5%, but differ from those reported by other authors who have indicated the important role of ica genes as virulence markers for clinically significant *Staphylococcus* isolates (11, 23).

### Table 1. Properties of blood culture and intravenous catheter-isolated and hospital staff staphylococci

<table>
<thead>
<tr>
<th>Type of isolates</th>
<th>No. (%) of isolates</th>
<th>Presence of ica genes</th>
<th>Colony morphology on CRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture (46)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em> (46)</td>
<td>30/62 (49)</td>
<td>10/24 (41.7)</td>
<td>Black</td>
</tr>
<tr>
<td><em>S. epidermidis</em> (16)</td>
<td>4/5 (80)</td>
<td>2/5 (40)</td>
<td>Bordeaux almost black</td>
</tr>
<tr>
<td>Catheter (70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em> (24)</td>
<td>10/41.6 (52.1)</td>
<td>7/29 (29)</td>
<td>Bordeaux</td>
</tr>
<tr>
<td><em>S. epidermidis</em> (46)</td>
<td>24/52.1 (15)</td>
<td>3/12.5 (25)</td>
<td>Bordeaux</td>
</tr>
<tr>
<td>Hospital staff (60)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em> (36)</td>
<td>8/22.2 (24)</td>
<td>6/16.6 (25)</td>
<td>Bordeaux</td>
</tr>
<tr>
<td><em>S. epidermidis</em> (24)</td>
<td>8/33.3 (33)</td>
<td>7/29.2 (25)</td>
<td>Bordeaux</td>
</tr>
</tbody>
</table>

*One strain developed bordeaux colony and presented ica genes
CRA: Congo red agar

#### Properties of blood culture and intravenous catheter-isolated and hospital staff staphylococci

- **Blood culture (46)**: 30/62 (49) of *S. aureus* and 4/5 (80) of *S. epidermidis* isolates were icaA positive. A 14/41.6% of *S. aureus* and 24/52.1% of *S. epidermidis* isolates were icaD positive. Only one strain of *S. epidermidis* was found to be non slime-producing by the CRA technique (Table 1).

- **Catheter (70)**: 10/41.6% and 24/52.1% of *S. aureus* and *S. epidermidis* isolates were icaA positive. Of all strains isolated from catheters, 19/54 and 30/62 respectively were positive for the icaA and icaD genes. Only one strain of *S. epidermidis* was found to be non slime-producing by the CRA technique (Table 1).

- **Hospital staff (60)**: 8/22.2% and 8/33.3% of *S. aureus* and *S. epidermidis* isolates were icaA positive. Among the isolates from nasal mucosal of healthy volunteers, 8 out of 36 and 8 out of 24 *S. aureus* and *S. epidermidis* strains respectively were found to be slime-producing, and the ica genes were present.

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In relation to *S. epidermidis*, 48.4% (30/62) of clinical strains present ica genes and only one of these strains was non-slime producing. Some authors suggest that the inactivation of ica operon occurs due to the insertion of the IS256 insertion sequence element ([3, 23, 26]). The percentage of clinical slime-producing *S. epidermidis* strains found in the present study, is very close to that found by Arciola et al. ([11]) from catheters-associated infections whereas Zieburg et al. ([23]) reported that an 87% of *S. epidermidis* strains isolates from blood culture infections exhibits this characteristic.

The results reported here indicate that ica genes and slime production were not exclusive property of clinical *Staphylococcus* strains. This suggests that virulence factors can somehow be present in hospital staff strains and are not specific to clinical isolates only. We, in a previous study, detected slime-producing coagulase-negative staphylococci environmental strains that were also resistant to oxacillin, from diverse surface and materials of the hospital rooms ([27]). For this reason we consider important the early and precise detection of slime producing *Staphylococcus* strains both in hospital environments and healthy carriers to control the dissemination of these microorganisms in a hospital community, especially among immunocompromised patients.

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REFERENCES


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