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Detection of *Staphylococcus aureus* Clinical Isolates Harboring the *ica* Gene Cluster Needed for Biofilm Establishment

The incidence of chronic nosocomial infections by gram-positive bacteria has drastically increased during the last years in association with the more frequent use of in-dwelling medical devices (1, 2). Infections derived from the use of invasive methods, e.g., catheters, are mainly due to staphylococci, especially those strains which produce an extracellular slime and constitute a biofilm, making clinical treatment extremely difficult (4, 5, 7, 9–12). The biofilm development process requires polysaccharidic intercellular adhesin, which is synthesized by the enzymes encoded by the intercellular adhesion cluster (*ica*) (6, 13, 14).

A great variety of *Staphylococcus aureus* and *Staphylococcus epidermidis* strains carry the *ica* cluster, and some of them constitute biofilm. Loss of the *ica* locus results in an incapacity to produce polysaccharidic intercellular adhesin and to develop biofilms (1). Staphylococcal infections produced by *ica* carriers can be more problematic due to the presence of methicillin and mupirocin resistance genes (5, 9, 12). The rapid detection of the *ica* locus in hospital staphylococcal isolates, together with the simultaneous detection of antibiotic resistance genes, will allow the development of prevention methods to reduce the bacterial capacity to invade the in-dwelling medical devices.

We have analyzed 65 clinical isolates, 7 from catheter samples and 58 clinical isolates randomly selected. The catheter isolates were recovered from the Oncology Medical Service, which follows a specific protocol to avoid catheter colonization, which explains the small number of isolates. The isolates, including *S. aureus* (60, 2 of them from catheter samples) and *S. epidermidis* (5, all from catheter samples), recovered during a one-year period, were analyzed by PCR to determine the presence or absence of the genes that confer constitutive methicillin resistance (*mecA*) and high mupirocin resistance (*ileS*-2) and a fragment of a gene that identifies *S. aureus* at the species level (*femB*) and to detect the presence of the intercellular adhesion gene cluster (*ica*). Detection of *femB*, *mecA*, and *ileS*-2 genes was performed by applying a triplex PCR method that has been previously described (8). PCR detection of the *ica* cluster was performed by amplification of a DNA region partially covering the *icaA* and *icaB* genes. For *S. epidermidis*, we used the previously described primers icaAB-F and icaAB-R (3), which yielded a 546-bp fragment, while for *S. aureus* we designed a pair of primers from the sequence available from the National Center for Biotechnology Information gene bank (locus AF086783): icaA-S (5′ AAA CTT GGT GCG GTT ACA GG 3′) and icaA-E (5′ TCT GGG CTT GAC GTT G 3′) (Roche Diagnostics, Mannheim, Germany). Amplification with this pair of primers generated a 750-bp fragment.

From the 58 clinical isolates randomly selected, 5 (8.6%) methicillin-resistant *S. aureus* isolates (MRSA) and 1 (1.7%) methicillin-resistant and highly mupirocin-resistant *S. aureus* isolate (MMRSA) were negative for *icaAB*, while the other 52 (89.7%) isolates were positive for *icaAB* (Table 1). Fifty-six of these 58 isolates showed *mecA* (96.5%), 52 presented *icaAB* (89.7%), and 40 isolates (61.5%) were characterized by harboring both *icaAB* and *mecA*. Eleven of the 58 isolates (16.9%) carry both resistance genes *mecA* and *ileS*-2 in addition to the *ica* cluster, and only 2 of 58 (3.5%) lacked the *femB* marker. Regarding the seven catheter isolates, two *S. aureus* isolates showed the *icaAB* marker but were negative for both resistance markers, i.e., *mecA* and *ileS*-2, while only one *S. epidermidis* isolate presented the *icaAB* cluster (Table 1).

Our results showed a very high percentage of the *ica* cluster in nosocomial MRSA and MMRSA isolates. The proportion of *ica* carriers was slightly lower (42.9%) in the case of catheter isolates, but it reached 89.6% in the case of isolates recovered from mucous membranes and skin.

Frebourg et al. (3) have reported that a high proportion of clinical isolates harboring the *ica* locus also carry the *mecA* gene. Here, we observed that 68.9% of the MRSA isolates harbor both loci, *ica* and *mecA*. Of these isolates, 18.6% showed a higher virulence potential, since they also presented high mupirocin resistance encoded by the *ileS*-2 gene.

TABLE 1. Frequency of detection of *femB, mecA, ileS-2*, and *icaAB* loci

Group of *Staphylococcus* isolates	Total no. of isolates	No. of isolates[a] carrying:							
		femB	*mecA*	*ileS*-2	*icaAB*	*mecA*, and *icaAB*	*mecA*, and *ileS*-2	*mecA*, *ileS*-2, and *icaAB*	*mecA*$^-$, *ileS*-2$^-$, and *icaAB*
Randomly selected	58[b]	56 (96.5), 91.7–100	56 (96.5), 91.7–100	12 (20.7), 9.7–31.4	52 (89.6), 81.5–97.7	40 (69), 56.7–81.2	1 (1.7), <6	11 (19), 8.6–29.4	2 (3.4), <9
Recovered from catheters	7[c]	2 (28.6), 10.1–47	2 (28.6), 10.1–47	2 (28.6), 10.1–47	3 (42.9), 22.7–63.1		2 (28.6), 10.1–47		3 (42.9), 22.7–63.1

[a] Values in parentheses are percentages. For each entry, the second value given is the 95% confidence interval.
[b] All isolates were *S. aureus*.
[c] Five isolates were *S. epidermidis*, and two isolates were *S. aureus*.

Therefore, we suggest simultaneous PCR detection of the *ica* locus and antibiotic resistance genes as a rapid and effective method to be used for discrimination between potentially virulent and nonvirulent isolates, which would be especially relevant for detection of isolates with high capacity to invade in-dwelling medical devices.

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