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Correlation of *Staphylococcus aureus icaADBC* Genotype and Biofilm Expression Phenotype

We read with interest the article of Arciola et al. (1) on the presence of *icaA* and *icaD* genes in *Staphylococcus aureus* and *S. epidermidis* isolates from catheter-associated infections and its correlation with a slime-positive phenotype detected with Congo red agar. These are the organisms most frequently the cause of foreign-body-related infections. The polysaccharide intercellular adhesin (PIA), synthesized by *icaADBC*-encoded proteins, is essentially involved in *S. epidermidis* biofilm accumulation (10). PIA has been shown to be a virulence factor of *S. epidermidis* (15). Recently, the *icaADBC* genes and PIA/poly-*N*-succinyl β-1-6-glucosamine (PNSG) were also detected in *S. aureus* (4, 13), together with evidence for a role of PIA/PNSG in *S. aureus* infections (13).

Using PCR, Arciola et al. (1) detected *icaA* and *icaD* in only 14 (61%) of 23 *S. aureus* isolates. These results are in contrast to data reported by others, who found all *S. aureus* isolates examined to be *icaADBC* positive (4, 7). Our own data on the prevalence of *icaADBC* in a collection of clinical *S. aureus* isolates confirm these latter observations, as all of 80 *S. aureus* isolates were *icaADBC* positive by PCR with oligonucleotides specific for *icaA* of *S. aureus* (M. A. Horstkotte, J. K.-M. Knobloch, H. Rohde, and D. Mack, unpublished data). A reasonable explanation for this discrepancy is that the primers used by Arciola et al. (1) were based on the *icaADBC* sequence of *S. epidermidis* RP62A (GenBank accession no. U43366), in which *icaA* and *icaD* display only 76 and 72% identity to the sequence of *S. aureus* ATCC 35556, respectively (4). Essentially, this leads to mismatches of 4 to 5 bases within three of the four primers used (Fig. 1).

icaA primer 1

S. epidermidis RP62A (1337-1356)	TCTCTTGCAGGAGCAATCAA
S. aureus ATCC 35556 (2906-2925)	ACACTTGCTGGCGCAGTCAA
S. aureus MU50 (65890-65909)	ACACTTGCTGGCGCAGTCAA
S. aureus N315 (73198-73217)	ACACTTGCTGGCGCAGTCAA

icaA primer 2

S. epidermidis RP62A (1505-1524)	TGCTGGATGTTAGTGCCTGA
S. aureus ATCC 35556 (3074-3093)	TGTTGGATGTTGGTTCCAGA
S. aureus MU50 (66058-66077)	TGTTGGATGTTGGTTCCAGA
S. aureus N315 (73366-73385)	TGTTGGATGTTGGTTCCAGA

icaD primer 1

S. epidermidis RP62A (1963-1982)	ATGGTCAAGCCCAGACAGAG
S. aureus ATCC 35556 (3532-3551)	ATGGTCAAGCCCAGACAGAG
S. aureus MU50 (66516-66535)	ATGGTCAAGCCCAGACAGAG
S. aureus N315 (73824-73843)	ATGGTCAAGCCCAGACAGAG

icaD primer 2

S. epidermidis RP62A (2138-2160)	TTGCATTAAATGTTGAAAACACG
S. aureus ATCC 35556 (3707-3729)	TTGCTTTAAACATTGAAAATACT
S. aureus MU50 (66691-66713)	TTGCTTTAAACATTGAAAATACT
S. aureus N315 (73999-74021)	TTGCTTTAAACATTGAAAATACT

FIG. 1. Comparison of oligonucleotides specific for *S. epidermidis* as used by Arciola et al. (1) for *icaA* and *icaD* detection with homologous sequences of different *S. aureus* strains. The primers were derived from the *icaADBC* sequence data of *S. epidermidis* RP62A (GenBank accession no. U43366). The homologous sequences of the *icaADBC* locus of *S. aureus* ATCC 35556 (GenBank accession no. AF086783), *S. aureus* Mu50 (localized in section 9/9; DDBJ accession no. AP003366), and *S. aureus* N315 (localized in section 10/10; DDBJ accession no. AP003138) are aligned. Base mismatches are in boldface.

Arciola et al. (1) also described a close association between *icaADBC* detection and slime formation as detected with Congo red agar in 14 (61%) of 23 *S. aureus* strains. Congo red agar was used earlier to detect biofilm (slime) production of *S. epidermidis* (6, 8), which correlated well with a biofilm-positive phenotype observed in vitro (8, 16). However, in a standard biofilm assay with Trypticase soy broth (Becton Dickinson, Cockeysville, Md.) as the growth medium (2, 3, 11), most *icaADBC*-positive *S. aureus* isolates in our collection (78 of 80 isolates) were biofilm negative (Horstkotte et al., unpublished data), which is in accordance with previous reports (4, 7, 13). It does not seem reasonable to propose that Congo red agar be used as a means of screening clinical *S. aureus* isolates for a biofilm (slime)-positive phenotype and a *icaADBC*-positive genotype while the correlation of these properties is still uncertain. Moreover, studies that evaluate whether there exists a correlation between a black colony-forming phenotype on Congo red agar and a biofilm-positive phenotype of *S. aureus* are necessary. This should be explored using several different growth media, as expression of *icaADBC* depends significantly on environmental factors and regulatory mechanisms apparently differ between *S. epidermidis* and *S. aureus* (5, 9, 12, 14).

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Authors' Reply

We thank Rohde et al. for their comments on our previously published work (2). Their main observation is on the percentage of slime-forming, *ica*-positive *Staphylococcus aureus* isolates, which in our work was 61% but in their opinion should reach the totality of the isolates. This opinion is based on their unpublished data and on the work of Cramton et al. (4). It would be of interest to know whether the data of Horstkotte et al. are drawn from catheter-associated infections, from prosthesis-associated infections, or from infections not related to indwelling devices. In the Cramton's work, only 10 *S. aureus* strains were examined, most of them coming from a national strain collection of clinical isolates, picked for their exemplariness, so it is not surprising to find that all 10 of them were *ica* positive. The finding in the pioneer study of Cramton et al. of the presence of an *ica* locus in *S. aureus* was far from representing an extensive study of molecular epidemiology. Rohde attributes the discrepancy between the number of *ica*-positive clinical isolates from catheter-associated infections that we reported (61%) and that reported by Cramton et al. to the presence of mismatches in our primers. It is well known that some degree of mismatch is allowed and does not impair annealing, unless high stringency conditions are used, which was not the case in our study. Moreover, none of the mismatches of the reverse primers was in the critical 3′ position, which would have hampered amplification.

After the delivery of our paper (2), we continued and extended our work to a collection of clinical isolates from orthopedic prosthesis-associated infections, designing new primers for *icaA*, in order to obtain, in a duplex PCR, a simultaneous 134-bp amplification product for *icaA*, together with the 200-bp amplification product for *icaD* (C. R. Arciola and L. Montanaro, unpublished data). By means of this improved PCR method, we have reinvestigated our collection of staphylococci. All data for catheter-associated infections that were reported in our previous published work (2) were confirmed by the use of this new PCR procedure, and, in the case of orthopedic prosthesis-associated infections, the proportion of *S. aureus* isolates positive for both *icaA* and *icaD* increased to 92%. We are convinced that the proportion of *ica*-positive and slime-producing strains among *S. aureus* clinical isolates varies with the clinical origin of the infection, being higher in orthopedic prosthesis-associated than in catheter-associated infections, as if the site and the indwelling material act as selective factors for strains with different and alternate adhesion mechanisms, either slime or microbial surface components recognizing adhesive matrix molecules.

In our previous published work on catheter-associated infections (2) and in a recent survey (our unpublished data) on orthopedic prosthesis-associated infections, a strict consistency was observed between the detection of *ica* genes and the in vitro slime production revealed by the Congo red agar plate method, in the case both of *S. epidermidis* and of *S. aureus*.

We agree with the Rohde's warning that the standard, i.e., frequently used, biofilm assay with Trypticase soy broth gives false-negative results. This is true for *S. aureus*, and we were able to demonstrate (1) that, unlike that of *S. epidermidis*, the ability of *S. aureus* to produce slime is dramatically affected by the presence of an additional carbohydrate source in the medium. The addition of 1% glucose increased the percentage of slime-producing *S. aureus* from 34.4% to 83.3%, and the carbohydrate effect was never detected for other staphylococcal species. Recently we have shown that, like glucose addition to Trypticase soy broth, iron limitation in the same medium stimulates slime production (3).

In our experience, the Congo red agar plate method ensures a strict correspondence between the phenotypic characterization of slime production and the genotypic detection of *ica* locus. The presence of 0.1 M saccharose (3.6% [wt/vol]) as a carbohydrate source and the observation of the plates at between 48 and 72 h for the full development of the black color are important in the case of *S. aureus*, as highlighted in our cited paper (2).

We are grateful to Rohde et al. for their challenging letter, and we intend to present soon in this journal the detailed results of our investigation on staphylococcal clinical isolates from orthopedic prosthesis infections.

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