

Clinical *Staphylococcus aureus* Isolate Negative for the Sa442 Fragment

The Sa442 DNA fragment, originally described by Martin-eau et al. (3), is a popular DNA target for identification of *Staphylococcus aureus* by PCR (2–6). Several PCR-based assays targeting this DNA fragment alone or in combination with a *mecA* PCR for identification of a methicillin-resistant *S. aureus* have been described (3, 4). Apart from having been established as being unique for *S. aureus*, the Sa442 fragment has not been further characterized.

Recently, we cultured a coagulase-positive staphylococcal isolate (strain 550226) identified as *S. aureus* by standard procedures. However, this particular isolate had tested negative in two previously published Sa442 assays (3, 4), suggesting that it was not *S. aureus*. Because of this ambiguous result, DNA sequence analysis of part of the 16S rRNA gene was performed. The obtained DNA sequence was identical and unique to *S. aureus*. Hence, we concluded that this strain was indeed *S. aureus*.

The fact that this strain tested negative in the Sa442 PCR could be the result of a sequence variation in the primer binding site(s) or a deletion of (part of) the corresponding gene from the genome. To our knowledge, this has not been reported before. In order to design another primer set to amplify the Sa442 fragment, we looked for flanking sequences in the *S. aureus* genome. Therefore, the nucleotide sequence of the Sa442 fragment was compared to genomic sequences of *S. aureus* by using BLAST analysis (1). Interestingly, we found that the first 256 nucleotides of the originally described fragment (containing the target for the previously described Sa442 assays) could be mapped to a hypothetical *S. aureus* gene coding for a protein with similarity to glutamate synthase whereas the last 190 nucleotides correspond to part of the *S. aureus* phosphomevalonate kinase gene (*mvaK2*) with a 4-bp overlap between the two parts. These two genes are located in different regions of the *S. aureus* chromosome, which implies that the original Sa442 fragment must have been a cloning artifact. A novel primer set was used to amplify a 920-bp fragment containing the first 256 nucleotides of the Sa442 fragment. The primer binding sites were chosen so as not to include sequence variations in the corresponding gene among three *S. aureus* genomes available in the public domain (N315, Mu50, and MW2). The sequences of the forward and reverse primer were 5'-TCGTTGTAGGCTTCCTGCTT-3' and 5'-CAACTGATCGACGAAACGAA-3', respectively. We ran-

domly tested 20 *S. aureus* isolates, including strain 550226. All isolates, with the exception of strain 550226, tested positive, indicating deletion of the whole or a major part of the corresponding gene in strain 550226.

Our results show that the use of a single gene- or DNA fragment-specific PCR for identification of microbial isolates may result in misidentification if one is not aware of caveats as described here. Moreover, a number of *S. aureus* isolates may have been misidentified in the past or the presence of *S. aureus* in a clinical sample may have been overlooked when identification was solely based on the Sa442 PCR assay.

REFERENCES

1. Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
2. Grisold, A. J., E. Leitner, G. Muhlauer, E. Marth, and H. H. Kessler. 2002. Detection of methicillin-resistant *Staphylococcus aureus* and simultaneous confirmation by automated nucleic acid extraction and real-time PCR. *J. Clin. Microbiol.* **40**:2392–2397.
3. Martineau, F., F. J. Picard, P. H. Roy, M. Couellette, and M. G. Bergeron. 1998. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. *J. Clin. Microbiol.* **36**:618–623.
4. Reischl, U., H.-J. Linde, M. Metz, B. Leppmeier, and N. Lehn. 2000. Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous species confirmation using real-time fluorescence PCR. *J. Clin. Microbiol.* **38**:2429–2433.
5. Shrestha, N. K., M. J. Tuohy, G. S. Hall, C. M. Isada, and G. W. Procop. 2002. Rapid identification of *Staphylococcus aureus* and the *mecA* gene from BacT/ALERT blood culture bottles by using the LightCycler system. *J. Clin. Microbiol.* **40**:2659–2661.
6. Tan, T. Y., S. Corden, R. Barnes, and B. Cookson. 2001. Rapid identification of methicillin-resistant *Staphylococcus aureus* from positive blood cultures by real-time fluorescence PCR. *J. Clin. Microbiol.* **39**:4529–4531.

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