

Be Aware of the Possibility of False-Positive Results in Single-Locus PCR Assays for Methicillin-Resistant *Staphylococcus aureus*

The emergence of methicillin-resistant *Staphylococcus aureus* as a nosocomial pathogen is an ever increasing problem. Prevention measures require the rapid detection of nasal carriage in patients and health care workers, and PCR is the fastest method fulfilling this task. The commonly used *mecA* gene detection method cannot be used in nonsterile specimens, because the possible presence of methicillin-resistant coagulase-negative staphylococci might result in false-positive reactions. In a new approach, a single locus, including the right extremity of the staphylococcal cassette chromosome genetic element (SCC*mec*) downstream of the *mecA* gene and a part of the adjacent *S. aureus*-specific *orfX* gene, was amplified (1, 2). These tests are based on the assumption that detection of this part of the SCC*mec* region is equivalent to the presence of the *mecA* gene; due to the detection of the *orfX* gene, positive reactions are restricted to *S. aureus* species. Commercial assays using this approach include the IDI-MRSA (Infecto Diagnostic, Inc., Sainte-Foy, Quebec, Canada) and GenoType MRSA Direct (Hain Lifescience, Nehren, Germany) tests. Here we report an *S. aureus* isolate which was methicillin susceptible despite a positive reaction in the GenoType MRSA Direct test (Hain Lifescience). The isolate was gained from a nasal swab of a health care worker at University Hospital Lübeck, Lübeck, Germany. It was identified as *S. aureus* by biochemical profiling (ApiStaph; bioMérieux, Nürtingen, Germany) and amplification of the Sa442 gene fragment (primers in reference 6). The isolate was resistant to penicillin only and susceptible to oxacillin, macrolides, aminoglycosides, fluoroquinolones, vancomycin, linezolid, and quinupristin-dalfopristin. A *mecA*-specific PCR was negative (primers in reference 6). Amplification of site-specific recombinase genes (*ccr* complex) was also negative (primers in reference 5). Type 1 capsule genes, known to be associated with a staphylococcal cassette chromosome genetic element (4), could not be amplified (cap1J-forward, 5'T ATTTTTGCCGCTGCTCTTT; cap1J-reverse, 5'TTTTTGGT CGTCCGTATTGTT). Pantone-Valentine leukocidin genes were not detected (primers in reference 3). We detected a 400-bp fragment spanning the right extremity of the SCC*mec* gene and parts of the *orfX* gene by a PCR described by Cuny and Witte (1) and confirmed the specificity by sequencing. Spa typing of the isolate revealed a rare pattern, described only once before in a methicillin-resistant *Staphylococcus aureus* (MRSA) isolate from Italy (Ridom StaphType t948; Ridom Bioinformatics GmbH, Würzburg, Germany).

In summary, we have described a methicillin-susceptible *S. aureus* isolate containing only small fragments of the right extremity of the SCC*mec* that resulted in false-positive reactions in "single-locus" PCRs for MRSA. The prevalence of these isolates in our population seems to be low, but false-positive reactions in other "single-locus" PCR assays for MRSA have been reported (2, 8). Nevertheless, these studies did not comment on the cause of the false-positive results. In our case, the phylogenetic origin of the SCC*mec* fragment detected remains unknown. It can be speculated that the

methicillin-resistant, Italian isolate was a parent strain from which wide parts of the SCC*mec* were deleted. Deletion of the SCC*mec* during prolonged storage has been reported (7), but our isolate was immediately analyzed. As the SCC*mec* harbors mobile genetic elements, it would be most interesting to know whether the remaining SCC fragments are still able to acquire resistance determinants and, thus, are of potential epidemiological concern. In conclusion, users of "single-locus" PCR assays for MRSA should be aware of the possibility of false-positive reactions. Nevertheless, the opportunity to rapidly screen potential MRSA carriers by nasal swabs and, thus, to prevent further nosocomial spread will probably outweigh this disadvantage.

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