Letters to the Editor

Identification of 2,600 Clinical Methicillin-Resistant *Staphylococcus aureus* Strains in The Netherlands Yielded Sporadic Cases of Strains Negative for the Species-Specific Sa442 Gene Fragment

Staphylococcus aureus is well known as a major pathogen, causing a variety of nosocomial and hospital-acquired infections. Rapid and reliable detection of methicillin-resistant *S. aureus* (MRSA) is important for initiation of appropriate antibiotic therapy and prevention of the spread of the organism.

In the clinical laboratory, identification of *S. aureus* is based primarily on growth characteristics, Gram staining, and the subsequent detection of catalase and coagulase activities (Staphaurex; Remel Europe Ltd., Dartford, Kent, United Kingdom); in the case of undetermined strains, additional biochemical tests may be required, e.g., VITEK or API Staph (bioMérieux S.A., Marcy l'Etoile, France). *S. aureus* strains may yield a false-negative or indeterminate result when commercially available kits for coagulase testing (1, 5) are used. Conventional susceptibility testing of *S. aureus* detects resistance to methicillin or oxacillin by methods used according to the standards of the NCCLS (3, 4).

In clinical laboratories today, genotypic methods for species determination and detection of the methicillin-resistant gene *mecA* are being used more often to increase the accuracy of identification and to obtain reliable results more rapidly. Since the *mecA* gene is not exclusive to *S. aureus*, these methods include a species-specific detection test as well. The Sa442 fragment has proven to be a useful test for detection of *S. aureus* (2; K. Sütterlin, R. Englert, T. Schmidt-Wieland, J. Schmitt, U. Reischl, and N. Lehn, Letter, J. Clin. Microbiol. 41:3449, 2003). To our knowledge only three exceptions have been described (C. H. W. Klaassen, H. A. de Valk, and A. M. Horrevorts, Letter, J. Clin. Microbiol. 41:4493, 2003; Sütterlin et al., letter), resulting in a sensitivity of 99.9% (Sütterlin et al., letter).

Misidentification based on a single gene, as suggested by Klaassen et al. (Klaassen et al., letter), is not likely to happen. In The Netherlands the medical microbiological first-line routine laboratories together with the second-line reference methods of the National Institute of Public Health and the Environment (RIVM) led to identification of the MRSA strains on the basis of both multiple phenotypic and multiple genotypic determination characteristics.

Nearly all isolated MRSA strains are sent to the RIVM, the national reference laboratory for epidemiology of *S. aureus*, which applies genotypic methods for *S. aureus* identification as confirmation of the phenotypic results and DNA fingerprinting for epidemiological purposes. From November 2001 to July 2003 we screened approximately 2,600 isolates belonging to nearly 400 pulsed-field gel electrophoresis clusters of putative *S. aureus* organisms cultured from a variety of clinical specimens for the presence of a species-specific 442-bp chromosomal fragment (2) and the *mecA* gene. In the case of a discrepancy between the genotypic and phenotypic results, the

strains were identified by an extended number of biochemical tests, fatty acid analysis (Microbial Identification System; MIDI, Newark, Del.), and alignment of the first part of the sequence of the 16S rRNA gene (fragment nucleotides 8 to 574 [Escherichia coli numbering]) with GenBank sequences of *S. aureus* type strains.

In the period of the study, we encountered seven *S. aureus* isolates which tested negative for the Sa442 fragment, resulting in a sensitivity of 99.7%, which is in accordance with the conclusions of Sütterlin et al. (Sütterlin et al., letter). All seven isolates which were negative for both *mecA* and Martineau PCR were pulsed-field gel electrophoresis cluster 44 or its derivatives: two were cluster 44, one was cluster 44a, and four were cluster 44b. In The Netherlands, *S. aureus* isolates assigned to the whole cluster 44 are not epidemic.

REFERENCES

1. Frebourg, N. B., D. Nouet, L. Lemée, E. Martin, and J. F. Lemeland. 1998. Comparison of ATB Staph, Rapid ATB Staph, Vitek, and E-test methods for detection of oxacillin heteroresistance in staphylococci possessing *mecA*. J. Clin. Microbiol. 36:52–57.
2. Martineau, F., F. J. Picard, P. H. Roy, M. Ouellette, and M. G. Bergeron. 1998. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. J. Clin. Microbiol. 36:618–623.
3. National Committee for Clinical Laboratory Standards. 1993. Approved standard M2-A5. Performance standards for antimicrobial disk susceptibility tests. National Committee for Clinical Laboratory Standards, Villanova, Pa.
4. NCCLS. 1999. Performance standards for antimicrobial susceptibility testing. 9th informational supplement M100-S9. NCCLS, Wayne, Pa.
5. Wilkerson, M., S. McAllister, J. M. Miller, B. J. Heiter, and P. P. Bourbeau. 1997. Comparison of five agglutination tests for identification of *Staphylococcus aureus*. J. Clin. Microbiol. 35:148–151.

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