Rapid Detection of Epidemic Strains of Methicillin-Resistant
Staphylococcus aureus

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Fifty methicillin-resistant Staphylococcus aureus (MRSA) initial isolates obtained from patients hospitalized in the orthopedic clinic of the Frankfurt University Hospital and 150 methicillin-sensitive Staphylococcus aureus (MSSA) isolates were investigated in this study to determine whether the Slidex Staph-Kit is capable of differentiating between MRSA and MSSA owing to its unique performance characteristics. The Slidex Staph-Kit is a combined latex hemagglutination test designed to detect clumping factor, protein A, and a specific surface immunogen for S. aureus. Clumping factor-positive strains cause erythrocytes sensitized with fibrinogen to hemagglutinate, thereby resulting in visible red clumps. S. aureus strains deficient in clumping factor agglutinate latex particles sensitized with specific antibodies against surface proteins of S. aureus, thereby resulting in visible white clumps. Our results demonstrate that while clumping has a 99% specificity as well as a 98% positive predictive value for MRSA. Clumping factor-negative MRSA, which have been reported to occur in several countries, are epidemic in the Frankfurt area and account for 80% of all MRSA initial isolates in the orthopedic clinic of the Frankfurt University Hospital. Genotyping of all MRSA isolates by macrorestriction analysis of chromosomal DNA revealed that 83% of clumping factor-negative MRSA are closely related to the "southern-German" epidemic strain. This is the first study demonstrating the Slidex Staph-Kit's capability for identifying epidemic clumping factor-negative S. aureus strains as methicillin resistant even prior to antimicrobial susceptibility testing.

MATERIALS AND METHODS

Bacterial strains. A total of 50 MRSA isolates and 150 MSSA isolates were investigated in this study. The MRSA initial isolates were cultured from clinical specimens obtained from the orthopedic clinic of the Frankfurt University Hospital between 1993 and 1997. All isolates had been classified as MRSA during routine investigations and were stored in stock cultures prior to the study, as described previously (29). The MSSA isolates were cultured consecutively from clinical specimens obtained from the Frankfurt University Hospital. S. aureus ATCC 25923 was employed as the reference strain.

RESULTS

Strain characterization. Coagulase production and hyaluronidase activity was demonstrated for all S. aureus isolates in
this study. Methicillin resistance was proved genotypically for all isolates by amplification of the \textit{mecA} gene (Fig. 1) as well as phenotypically by growth on Mueller-Hinton agar supplemented with 6 \(\mu\)g of oxacillin/ml and 4\% NaCl.

**Genotyping.** Macrorestriction analysis of 50 MRSA initial isolates obtained from the orthopedic clinic of the Frankfurt University Hospital in a 5-year period revealed 18 different genotypes (Fig. 2). Fifty percent of all isolates belonged to type 1, and strongly corresponding restriction fragment patterns indicating close clonal relatedness between MRSA types 1, 7, 71, and 72 were obvious (Fig. 3).

**Agglutination performance.** Table 1 compares the performance of the Slidex Staph-Kit with that of the Staphylase agglutination test. It could be demonstrated that all MRSA initial isolates closely related to the epidemic strain, as well as isolates belonging to MRSA types 6, 76, 82, and 83, exhibited white-clumping behavior (Fig. 4). In every instance where the Slidex Staph-Kit exhibited white clumping the Staphylase agglutination test was negative, which indicates that these strains were at least phenotypically clumping factor negative. In contrast, 149 of 150 MSSA isolates classified during routine investigation were determined to be clumping factor positive, i.e., they exhibited red-clumping behavior (Fig. 5).

**DISCUSSION**

Rapid and accurate identification of methicillin resistance in \textit{S. aureus} is of ongoing clinical importance for controlling the spread of this pathogen within hospital settings. The plasma coagulase test is generally acknowledged as the “gold standard” for the identification of \textit{S. aureus}. Nevertheless, the use of commercial agglutination kits for identifying \textit{S. aureus} is widespread in clinical microbiological laboratories, since these tests are easy to perform and the results are available within minutes. Whereas the first-generation agglutination kits are capable of detecting clumping factor and/or protein A for \textit{S. aureus} strains while failing to identify certain MRSA strains (3, 22), second-generation agglutination kits, such as the Slidex Staph-Kit, possess the additional feature of being able to detect specific surface antigens for \textit{S. aureus}. Numerous studies have been published confirming the high sensitivity and specificity of these second-generation kits for the identification of MSSA as well as MRSA (1, 4–6, 10, 17, 23, 30). Definitive detection of methicillin resistance, however, still requires time-consuming antimicrobial susceptibility testing.
This is the first study demonstrating that the Slidex Staph-Kit is a reliable and, above all, rapid method for identifying epidemic clumping factor-negative MRSA even prior to antimicrobial susceptibility testing. Owing to its unique design as a combined latex and hemagglutination test, it reacts, in contrast to all the other latex agglutination kits, in two different ways. Red clumps indicate the presence of clumping factor, with which \textit{S. aureus} causes erythrocytes sensitized with fibrinogen to agglutinate (Fig. 5). White clumps, on the other hand, signal both the absence of clumping factor and the presence of other immunogens, which trigger the agglutination of the latex particles (Fig. 4). This study shows that white clumping has a 99% specificity and a 98% positive predictive value for the detection of methicillin resistance in \textit{S. aureus}. Fifty percent of all initial isolates obtained from the orthopedic clinic of the Frankfurt University Hospital and more than 60% of all initial isolates in the Frankfurt metropolitan area, including six community hospitals and the University Hospital (29), belong to one genotype, which has been identified by the Robert Koch Institute, the German national institute of infectious diseases, as the so-called “southern-German” epidemic strain. This epidemic strain is clumping factor negative and exhibits white-clumping behavior. Furthermore, it is not only epidemic in the south but is also widespread in the northern parts of Germany (22, 31). Since bacteria do not respect international boundaries, it came as no surprise when epidemic MRSA strains kindly sent to our institution from Slovakia and Italy exhibited the same southern-German genotype and showed white-clumping behavior (data not shown). As demonstrated by PFGE in this study, it is not only the southern-German epidemic strain which is clumping factor negative; other MRSA genotypes also manifest this typical agglutination characteristic.

The Slidex Staph-Kit, of course, is neither designed nor licensed to detect resistance, and as such, white clumping can be regarded only as a clue to methicillin resistance. Nonetheless, to the extent that the white clumping serves as a strong indicator for MRSA, rapid tests can be conducted which confirm methicillin resistance and, upon request, species identity within 4 hours. This validation can be achieved genotypically, for example, by multiplex PCR, a method that enables the detection of the \textit{mecA} gene and a species-specific gene for \textit{S. aureus} within 5 to 6 h (2, 21). Quicker and less labor intensive is the phenotypical verification of MRSA with the BBL Crystal MRSA ID system from Becton Dickinson, which can be performed within 4 h (12, 18, 28). Finally, the phenotypical test for plasma coagulation can be performed to confirm spe-

\begin{table}
\centering
\caption{Performance of the Slidex Staph-Kit and Staphylase test for the identification of MRSA and MSSA}
\begin{tabular}{llll}
\hline
Organism & Type & \( n \) & Results \footnote{W, white clumping; R, red clumping; –, no clumping; ND, not determined.} \\
\hline
MRSA & 1 & 25 & W – \\
 & 7 & 2 & W – \\
 & 71 & 3 & W – \\
 & 42 & 1 & W – \\
 & 72 & 1 & W – \\
 & 73 & 1 & W – \\
 & 74 & 1 & W – \\
 & 75 & 1 & R R \\
 & 6 & 1 & W – \\
 & 76 & 1 & W – \\
 & 77 & 1 & R R \\
 & 78 & 2 & R R \\
 & 79 & 1 & R R \\
 & 80 & 2 & R R \\
 & 81 & 2 & R R \\
 & 41 & 1 & R R \\
 & 83 & 2 & W – \\
 & 82 & 2 & W – \\
MSSA & 150 & R/W & ND \\
\hline
\end{tabular}
\footnotetext{\( n \) = 149 & \( n \) = 1.}
\end{table}
cies specificity, analogous to the genotypic confirmation by multiplex PCR (7) (Fig. 6).

The results of this study, as well as the fact that a modern second-generation agglutination test was required owing to the rising incidence of clumping factor-negative MRSA, indicate that all clumping factor-negative MRSA strains are capable of being recognized by the Slidex Staph-Kit with its differentiated agglutination characteristics. In view of these findings, the Slidex Staph-Kit is a valuable tool in clinical laboratories with a frequent occurrence of clumping factor-negative MRSA.

In conclusion, this study confirms the Slidex Staph-Kit’s ability within the context of the clinical microbiological laboratory to pinpoint certain epidemic MRSA strains 24 h prior to the final results provided by antimicrobial susceptibility testing. In turn, the initiation of effective infection control measures may reduce the spread of this pathogen.

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