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Rapid Detection of Methicillin Resistance in *Staphylococcus aureus* Isolates by the MRSA-Screen Latex Agglutination Test

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The slide agglutination test MRSA-Screen (Denka Seiken Co., Niigata, Japan) was compared with the *mecA* PCR ("gold standard") for the detection of methicillin resistance in *Staphylococcus aureus*. The MRSA-Screen test detected the penicillin-binding protein 2a (PBP2a) antigen in 87 of 90 genetically diverse methicillin-resistant *S. aureus* (MRSA) stock culture strains, leading to a sensitivity of 97%. The three discrepant MRSA strains displayed positive results only after induction of the *mecA* gene by exposure to methicillin. Both *mecA* PCR and MRSA-Screen displayed negative results among the methicillin-susceptible *S. aureus* strains ($n = 106$), as well as for *Micrococcus* spp. ($n = 10$), members of the family *Enterobacteriaceae* ($n = 10$), *Streptococcus pneumoniae* ($n = 10$), and *Enterococcus* spp. ($n = 10$) (specificity = 100%). Producing the same PBP2a antigen, all 10 methicillin-resistant *Staphylococcus epidermidis* strains score positived in both the latex test and the *mecA* PCR. Consequently, the MRSA-Screen test should be applied only after identification of the MRSA strain to the species level to rule out coagulase-negative staphylococci. In conclusion, due to excellent specificity and sensitivity the MRSA-Screen latex test has the potential to be successfully used for routine applications in the microbiology laboratory.

In most routine microbiological settings, the detection of methicillin resistance among *Staphylococcus aureus* isolates is based on phenotypic assays. Genetic confirmation of positive findings based on the detection of the *mecA* gene (4) is performed in reference laboratories only, as these techniques are not yet generally available. Methicillin resistance in staphylococci is mediated by the *mecA* gene, encoding the penicillin-binding protein 2a (PBP2a), which has a reduced affinity for beta-lactam antibiotics. Denka Seiken has developed a simple latex agglutination assay for the detection of methicillin resistance, which makes use of a specific monoclonal antibody directed toward the PBP2a antigen (5). In order to get insight into the sensitivity of the assay, 90 genetically unrelated methicillin-resistant *S. aureus* (MRSA) strains were included as well as 106 epidemiologically unrelated methicillin-susceptible *S. aureus* (MSSA) strains to control the specificity of the assay. In order to test the extent of cross-reactivity of the MRSA-Screen assay, other species were also included: 10 methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains and 10 isolates each of species of the family *Enterobacteriaceae*, *Micrococcus* spp., *Enterococcus* spp., and *Streptococcus pneumoniae* (Table 1).

S. aureus strains were identified by the AccuProbe *S. aureus* culture identification test (Gen-Probe, Inc., San Diego, Calif.), performed in accordance with the manufacturer's protocol. Methicillin resistance of all *S. aureus* (MRSA and MSSA) and *S. epidermidis* (MRSE) strains was analyzed by means of the disk diffusion test in accordance with National Committee for Clinical Laboratory Standards guidelines (6), and the *mecA* gene was detected by PCR as the "gold standard" (4). Coagulase-negative staphylococci were identified as *S. epidermidis* by Staphaurex (Murex Biotech Ltd., Dartford, United Kingdom) and API-Staph (bioMérieux, Marcy l'Etoile, France). The complete panel of strains (Table 1) was tested with MRSA-Screen, and the test was performed in accordance with the manufacturer's protocol. In short, a loopful of bacterial cells was suspended in 200 μl of extraction reagent 1 and subsequently lysed by boiling for 3 min. After cooling to room temperature, 50 μl of extraction reagent 2 was added to 200 μl of the lysate and samples were mixed well. After 5 min of centrifugation (1,500 × *g*), 50 μl of the supernatant was used for testing agglutination with sensitized latex particles (1 drop) and another 50 μl of the supernatant was used for testing with the control latex particles (1 drop). The test slides were mixed by rotating them for 3 min, after which agglutination was assessed visually. *S. aureus* ATCC 29213 was used as a negative control in both the *mecA* PCR and the MRSA-Screen test. A *mecA*-positive clinical isolate was used as a positive control strain.

The results are summarized in Table 2. For all 90 genetically diverse MRSA strains consisting of six different *mecA* types (2), the presence of the *mecA* gene was reconfirmed with PCR. As MRSA-Screen detects the PBP2a antigen in 87 of 90 MRSA strains, the sensitivity is 97%. Repeated testing after multiple subculturing did not reconcile the results for the three initial agglutination-negative, *mecA*-positive *S. aureus* isolates. These discrepancies are probably due to the suppression of *mecA* gene transcription (3). In order to bypass this problem, the three isolates were again incubated overnight in the presence of a disk containing 5 μg of methicillin. The MRSA-Screen assay was repeated with colonies taken within the zone of inhibition or at the border of the inhibition zone. All three isolates became positive, whereas the control, i.e., colonies taken from the same culture plate but not in the vicinity of the methicillin disk, remained negative. These findings actually indicate that the sensitivity of the assay can be improved by performing the agglutination assay with isolates previously exposed to methicillin. None of the 106 genetically diverse MSSA strains demonstrated positive results with MRSA-Screen or

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TABLE 1. Characterization of the strain collections used in this study

Species	No. of strains	Origin	Reference(s)
MRSA	90	Geographically and temporally diverse strains ($n = 40$)	2, 7
		Community-acquired strains from New York, N.Y., hospital ($n = 26$)	8
		Strains from outbreak clusters in EMCR[a] ($n = 24$)	
MSSA	106	Primary blood culture isolates from EMCR ($n = 91$)	
		Geographically and temporally diverse strains ($n = 15$)	2, 7
MRSE	10	Isolates from hematology department of EMCR	
Enterobacteriaceae member	10	Primary clinical isolates from blood cultures at EMCR	
S. pneumoniae	10	Primary clinical isolates from blood cultures at EMCR	
Enterococcus sp.	10	Primary clinical isolates from blood cultures at EMCR	
Micrococcus	10	Primary clinical isolates from blood cultures at EMCR	

[a] EMCR, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands.

the *mecA* PCR, directing the specificity to 100%. The *Enterobacteriaceae* strains, *S. pneumoniae*, *Enterococcus* spp., and *Micrococcus* spp. were identified with Microscan (Dade Behring, Leusden, The Netherlands), optochin disk diffusion (Oxoid, Basingstoke, United Kingdom), Vitek (bioMérieux), and API-Staph (bioMérieux), respectively. Among these genera, no false-positive results could be detected, ruling out the presence of cross-reacting antigens. Since the genetic mechanism of methicillin resistance in coagulase-negative staphylococci is identical to that in *S. aureus* (1), all MRSE strains ($n = 10$) displayed positive results in both *mecA* PCR and the MRSA-Screen test.

In our opinion, the assay should be applied in a routine setting by using the following consecutive steps. After isolation of a suspect MRSA strain, it first needs to be identified to the species level to exclude coagulase-negative staphylococci. Second, the potential MRSA strain is subjected to the MRSA-Screen test. In the event of a positive result, the identification is definitely MRSA. If a negative result is found, the assay has to be repeated after the strain has been exposed to methicillin in order to induce possible methicillin resistance. If the MRSA strain is then positive, MRSA has been identified. In the event of a negative result, the *mecA* PCR should be used as the final and conclusive confirmation assay.

TABLE 2. Results obtained with diagnostic tests on diverse strain collections[a]

Organism	No. of isolates	No. (%) of isolates positive by:		
		AccuProbe	*mecA* PCR	MRSA-Screen
MRSA	90	90 (100)	90 (100)	87 (97)
MSSA	106	106 (100)	0 (0)	0 (0)
MRSE	10	ND	10 (100)	10 (100)

[a] *Micrococcus* spp., *S. pneumoniae*, *Enterococcus* spp., and *Enterobacteriaceae* strains displayed no agglutination test results with the MRSA-Screen assay. The other diagnostic tests were not done for these strains. ND, not done.

In conclusion, the MRSA-Screen latex test has the potential for the detection of MRSA in a routine microbiology setting as it combines high speed and excellent specificity and sensitivity with limited requirements for special equipment or skilled personnel.

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