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Direct detection of methicillin-resistant *Staphylococcus aureus* in clinical specimens by a nucleic acid-based hybridisation assay

M. Holfelder[1], U. Eigner[1], A.-M. Turnwald[1], W. Witte[2], M. Weizenegger[1] and A. Fahr[1]

[1]Limbach Laboratory, Department of Microbiology and Hygiene, Heidelberg and [2]Robert Koch Institute, Branch Wernigerode, National Reference Center for Staphylococci, Wernigerode, Germany

ABSTRACT

The occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) is still increasing worldwide and is associated with significant morbidity, mortality and hospital costs. Screening for MRSA plays a key role in limiting further nosocomial spread of this organism. Control measures require a rapid and sensitive test for direct detection of MRSA carriage. This study evaluated an easy-to-use PCR-hybridisation assay for the direct detection of MRSA in clinical swab specimens. In total, 508 pairs of swabs from 242 patients at risk for MRSA carriage were analysed by the standard culture method and the PCR assay. One swab was used for PCR and culture, while the second was used for culture only. Of the 508 pairs tested, 37 were positive by culture and 35 were positive by PCR. Among the 471 culture-negative specimens, 465 were negative by PCR and six were PCR-positive. The PCR assay had a sensitivity of 94.59%, a specificity of 98.73%, a positive predictive value of 85.37%, and a negative predictive value of 99.57%. The PCR-hybridisation assay enabled reliable detection of MRSA carriage in *c.* 4 h, thereby allowing its effective use in an MRSA control strategy.

Keywords Detection, hybridisation assay, MRSA, PCR, screening swabs, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus is one of the most important human pathogens in terms of nosocomial and community-acquired infections. Methicillin-resistant *S. aureus* (MRSA) has spread worldwide, with a mean prevalence among *S. aureus* blood culture isolates during 2002 of 1% in The Netherlands, 19.2% in Germany, 44.5% in the UK [1] and 57.1% among intensive care unit patients in the USA [2,3]. The associated morbidity and mortality for patients with MRSA infections is twice as high as for patients with methicillin-susceptible *S. aureus* (MSSA) infections [4]. In addition, the costs of management of patients with MRSA infection are 1.5–3-fold higher than for patients with MSSA infection [4,5]. In The Netherlands, studies have shown that the spread of MRSA can be controlled by implementing effective preventive measures, and that these measures are cost-effective [2]. Screening of patients with risk-factors for MRSA carriage is an important component of a successful MRSA control policy [2,6]. In order to limit the spread of MRSA, a rapid and sensitive test for detection of MRSA colonisation is required. However, standard culture methods for identification of MRSA require at least 48 h to complete. A screening test for MRSA that provides accurate results in less than a single day would enable earlier MRSA control measures. A negative result, embedded in a policy of screening upon admission and isolation of patients suspected of MRSA carriage, would shorten the time of patient isolation to <12 h and would therefore make the test cost-effective.

Several DNA-based tests have been developed for the rapid detection of MRSA. Most of these tests are based on simultaneous detection of a *S. aureus*-specific target and the *mecA* gene [7–10]. However, results may be non-specific when these tests are used for direct detection of MRSA in clinical specimens containing both coagulase-negative staphylococci (CoNS) and *S. aureus*, both of

Corresponding author and reprint requests: A. Fahr, Limbach Laboratory, Department of Microbiology and Hygiene, Heildeberg, Germany
E-mail: A.Fahr@docnet.de

which can carry the *mecA* gene. By using MRSA-specific chromosomal sequences, as described in the present study, it is possible to detect MRSA directly from clinical specimens, and to discriminate MRSA from CoNS carrying the *mecA* gene.

MATERIALS AND METHODS

Analytical sensitivity and specificity of the nucleic acid-based assay

The present study evaluated a PCR-based hybridisation assay (GenoType MRSA Direct; Hain Lifescience, Nehren, Germany) for the direct detection of MRSA from clinical swab specimens obtained upon hospital admission from patients who had risk-factors for MRSA carriage. GenoType MRSA Direct contains a set of primers specific to staphylococcal cassette chromosome *mec* (SCC*mec*) types I, II, III and IV, including the newly recognised community-acquired MRSA strain. The results of the test are available in *c.* 4 h.

The analytical sensitivity of the assay was evaluated using 22 MRSA isolates provided by the National Reference Centre for Staphylococci, Wernigerode, Germany. The following strains were tested: RKI ID 134/93 (ST 247); ID 3441/02 (ST 5); ID 1442/98 (ST 254); ID 1417/97 (ST 45); ID 809/96 (ST 134); ID 1155/98-2 (ST 228); ID 131/98 (ST 228); ID 2594/97 (ST 228); ID 872/98 (ST 254); ID 1450/94 (ST 134); ID 234/95 (ST 247); ID 406/98 (ST 247); ID 1678/96 (ST 22); ID 1293/00 (ST 22); ID 773/96 (ST 239); ID 1000/93 (ST 254); ID 3391/02 (ST 8); ID 2773/03 (ST 1); ID 1150/93 (ST 45); ID 1816/03 (ST 30); ID 1085/04 (ST 8); and ID 949/04 (ST 254). These reference strains represent SCC*mec* types I, II, III and IVa, c and d, and include the clonal lineages that are prevalent among MRSA strains from infections in hospitals and in the community in Europe and in North America [11].

The analytical specificity of the assay was determined using 53 methicillin-resistant CoNS and methicillin-susceptible CoNS provided by the National Reference Centre for Staphylococci, comprising 16 methicillin-resistant *Staphylococcus epidermidis*, 14 methicillin-susceptible *S. epidermidis*, eight *Staphylococcus haemolyticus*, and one each of the following *Staphylococcus* spp.: *S. hominis*, *S. lugdunesis*, *S. capitis*, *S. auricularis*, *S. caprae*, *S. carnosus*, *S. cohnii*, *S. delphinii*, *S. equorum*, *S. gallinarum*, *S. intermedius*, *S. sciurii*, *S. xylosus*, *S. warneri* and *S. schleiferi*. In addition, two MSSA strains were analysed, including ATCC 25923, which is a *S. aureus* strain that carries an SCC*mec*-type element lacking the *mecA* gene. This strain carries a DNA fragment inserted at the same nucleotide position in *orfX* as three different SCC*mec* elements, with structural characteristics similar to SCC*mec* elements at both ends that show high similarity to those of type III SCC*mec* [12].

The lower detection limit of the assay was determined by serial dilution of four MRSA strains representing SCC*mec* types I, II, III and IV, namely RKI ID 134/93 SCC*mec* type I, RKI ID 3441/02 SCC*mec* type II, RKI ID 1442/98 SCC*mec* type III, and RKI ID 1417/97 SCC*mec* type IV. After incubation for 24 h on Columbia blood agar, a suspension equivalent to a 0.5× MacFarland standard was prepared in sterile NaCl 0.9% w/v, and then serially diluted in parallel in sterile NaCl 0.9% v/v and in lysis buffer (see below) to a final concentration of 10 CFU/mL. Aliquots (500 μL) of each dilution were incubated at 95°C for 10 min, after which 5-μL portions were used for PCRs (see below); 100 μL of each dilution were also plated on Columbia blood agar and incubated at 37°C for 24 h to determine the number of CFUs.

Patient enrolment and collection of clinical specimens

Patients were selected at 12 hospitals (11 secondary- and tertiary-care hospitals with 80–667 beds, and one hospital for neurological long-term treatment with 102 beds). Patients were considered for inclusion in the study if they had known risk-factors for colonisation with MRSA, e.g., transfer from another hospital or region with a high incidence of MRSA, antibiotic treatment within the previous 14 days, or chronic wounds. Patients were excluded from the study if they had received treatment for MRSA colonisation or infection within the previous 4 weeks. Two swabs (Venturi Transsystem, Copan, Italy) were collected from each body site sampled of the patients included in the study. In total, 508 pairs of swab specimens were obtained from 242 patients between November 2004 and February 2005. The swabs were taken from the following body sites: nose (n = 209), throat (n = 101), skin (n = 80), groin (n = 46), axilla (n = 12), wound (n = 34) and other sites (n = 26).

Processing of clinical specimens

One swab was initially agitated for 30 s in the lysis buffer solution provided with the assay and was then used to inoculate thioglycolate broth (Becton Dickinson, Heidelberg, Germany) and Columbia-CNA agar (Becton Dickinson), containing sheep blood 5% v/v, colistin and nalidixic acid. The second swab was used to inoculate thioglycolate broth and CNA agar only. CNA agar plates were incubated for 48 h at 35°C and then examined for growth. The thioglycolate broths were incubated for 20–24 h at 35°C, subcultured to CNA agar, and examined for growth after incubation for 20–24 h at 35°C. The identification of suspected *S. aureus* colonies was confirmed by tests with clumping factor, protein A and a group-specific antigen bound to *S. aureus*-specific peripheral structures (Slidex Staph Plus; bioMérieux, Marcy-l'Etoile, France).

Confirmed *S. aureus* isolates were tested for growth on oxacillin screen agar (Becton Dickinson) according to CLSI recommendations [13]. Each isolate was also analysed using the VITEK 2 instrument (bioMérieux), and MRSA was confirmed by detection of the *mecA* gene and a *S. aureus*-specific target with the GenoType MRSA assay (Hain Lifescience).

GenoType MRSA Direct assay

DNA extraction and amplification. Specimens were processed for PCR analysis according to the manufacturer's instructions. The swabs were washed in 300 μL of lysis buffer before cultivation. Bacterial DNA was released by incubation of the lysis buffer for 10 min at 95°C, followed by centrifugation for 5 min at 6000 *g*. Portions (5 μL) of the supernatant were used for amplification. In brief, 45 μL of primer nucleotide mix (provided with the kit), $MgCl_2$ to a final concentration of 2.5 mM and 1 U of HotStar*Taq* polymerase (Qiagen, Hilden, Germany) were added, followed by amplification on a PE 9700 thermocycler (Applied Biosystems, Weiterstadt, Germany) for 15 min at 95°C, 35 cycles of 95°C for 30 s, 55°C for 40 s and 72°C for 40 s, and a final extension at 70°C for 8 min. Each run included a negative control sample to demonstrate the absence of

contaminating DNA. The sensitivity of amplification and hybridisation was monitored using an internal control.

Hybridisation protocol. In brief, the assay uses a specific oligonucleotide probe, targeting the SCC*mec* chromosomal cassette of MRSA, that is immobilised on membrane strips. PCR amplicons hybridise with this probe during the detection process. Hybridisation and detection were performed in an automated washing and shaking device (Profiblot; Tecan, Maennedorf, Switzerland). PCR products (20 μL) were mixed for 5 min with 20 μL of denaturing reagent (provided with the kit) at room temperature in separate troughs of a plastic tray. After addition of 1 mL of pre-warmed hybridisation buffer, the membrane strips in the kit were added to every trough. Hybridisation was at 45°C for 30 min, followed by two washing steps at 45°C for 30 min with 1 mL of pre-warmed stringent wash solution. For colourimetric detection of hybridised amplicons, streptavidin-conjugated alkaline phosphatase and the appropriate substrate were added. After final washing, the strips were air-dried and fixed on a data sheet. The individual steps in the process, i.e., DNA isolation, amplification and hybridisation, were monitored using an internal control to improve the reliability of the test.

RESULTS

Analytical sensitivity and specificity

All 22 MRSA reference strains, representing SCC*mec* types I, II, III and IVa, c and d, including the prevalent MRSA strains in Europe and North America, tested positive by the GenoType MRSA Direct assay. All 53 CoNS tested, including *mecA*-positive CoNS, as well as two MSSA strains, were negative. The lower detection limit of the assay for serially diluted MRSA strains revealed a detection limit of 20–30 CFU/5 μL.

Clinical specimens

Of the 508 pairs of swabs tested, 37 were positive by culture and 41 were positive by the GenoType MRSA Direct assay. Thirty-five specimens positive by both culture and PCR were from 20 patients, of whom 12 had one specimen tested, three had two specimens tested, three had three specimens tested, and two had four specimens tested. Table 1 summarises the efficiency of the GenoType MRSA Direct assay in comparison with culture for the detection of MRSA. In order to resolve discrepant results, the assay was also performed using the enrichment broths. Of the six specimens from six patients that were PCR-positive but culture-negative, none was culture-positive after subculture from the enrichment broth, but three remained PCR-positive. One of these six patients had four other specimens taken that were positive by both culture and PCR; the other five patients had only one specimen taken. Of the two specimens from two patients that were PCR-negative but culture-positive, one specimen remained PCR-negative, but two other specimens from this patient were positive by both culture and PCR. The other specimen was PCR-positive when using the enrichment broth.

Table 1. Sensitivity and specificity of the GenoType MRSA Direct assay in comparison with culture for the detection of methicillin-resistant *Staphylococcus aureus*

	GenoType MRSA Direct		Compared with culture			
Culture	MRSA-positive	MRSA-negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Positive	35	2	94.59	98.73	85.37	99.57
Negative	6	465				

PPV, positive predictive value; NPV, negative predictive value.

DISCUSSION

One important approach for controlling MRSA transmission in hospitals involves the rapid identification of MRSA-carrying patients. Delays in detection of MRSA lead to increased transmission of MRSA among patients, higher numbers of MRSA infections, and increased hospital costs. The GenoType MRSA Direct assay provides a rapid, sensitive and specific method, in comparison with selective culture, for direct detection of MRSA in clinical swab specimens. In combination with a simple DNA extraction method and detection procedure, the results of the test are available in *c.* 4 h.

A previous study used a commercially available PCR kit to detect MRSA directly from the nasal swabs of 288 patients [14], and reported a sensitivity of 91.7% and a specificity of 93.5%, with a positive predictive value of 82.5% and a negative predictive value of 97.1%. Huletsky *et al.* [15,16] reported similar results with the same system during a hospital surveillance programme involving 331 nasal swab specimens from 162 patients at risk for MRSA colonisation, with a sensitivity of 100%, a specificity of 96.5%, a positive predictive value of 89.4% and a negative predictive value of 100%. A higher number of positive PCR results was found compared with

culture, perhaps because of the presence of non-viable or non-cultivable MRSA, or an MRSA load that was below the detection limit for the culture method. More recently, a real-time PCR assay for rapid identification of MRSA from clinical samples that targets the integration site (*orfX*) of the SCC*mec* region has been described [17]. The assay showed a sensitivity and specificity of 93% and 100%, respectively, when swabs were tested after overnight incubation in brain–heart infusion broth. The overnight incubation prolonged the time required for a result to two working days. The detection limit of the assay was <10 CFU/swab, compared with the 20–30 CFU/5 μL detected in the present study, but this did not affect the clinical sensitivity.

One sample in the present study remained PCR-negative after repetition of the assay, despite the fact that the MRSA isolate from culture carried the *mecA* gene, as shown in a separate PCR for the *mecA* gene only (results not shown), and the isolate itself was positive by the GenoType MRSA Direct assay. Possibly, the number of organisms present was below the detection limit of the assay. The second swab was positive by PCR after repeated testing.

The sensitivity of a direct DNA-based assay may also be influenced by the type of swab. The present study used a swab with a gel carrier and charcoal, which is also suitable for culture. Further studies are required to determine whether the use of swabs suitable for culture methods is optimal for direct DNA-based assays.

In conclusion, the GenoType MRSA Direct assay appears to be a rapid, sensitive and specific test for direct detection of MRSA in clinical swab specimens, but simultaneous culture of the same swab is recommended. The assay provides same-day results, thereby improving the efficiency and effectiveness of infection control measures to prevent the spread of MRSA in a hospital setting. Even a negative test result is cost-effective, as it reduces the isolation time required for patients at risk of MRSA carriage.

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