Rapid Detection of Methicillin Resistance in Coagulase-Negative Staphylococci by a Penicillin-Binding Protein 2a-Specific Latex Agglutination Test

MATTHIAS A. HORSTKOTTE, JOHANNES K.-M. KNOBLOCH, HOLGER ROHDE, AND DIETRICH MACK*

Institut für Medizinische Mikrobiologie und Immunologie, Universitätsklinikum Hamburg-Eppendorf, D-20246 Hamburg, Germany

Received 29 May 2001/Returned for modification 4 July 2001/Accepted 15 July 2001

Coagulase-negative staphylococci (CoNS) are major nosocomial pathogens (10, 12, 18, 22, 24), and treatment of infections caused by CoNS is increasingly problematic due to the frequent occurrence of isolates resistant to multiple antibiotics (2).

The majority of clinical CoNS harbor the mecA gene, which encodes an additional penicillin-binding protein (PBP), PBP 2a, essential for expression of methicillin resistance (3). Phenotypic detection of methicillin resistance in CoNS is difficult due to the heterogeneous expression of mecA (6, 16, 21, 27). mecA detection by PCR is very sensitive but is not feasible for the busy clinical microbiology laboratory.

Thus, rapid, sensitive, and specific procedures for the detection of methicillin resistance in CoNS are urgently needed. A latex agglutination (LA) test (MRSA-Screen) detects PBP 2a by using latex particles sensitized with monoclonal antibodies specific for PBP 2a of Staphylococcus aureus (17). This test was evaluated for S. aureus with overall favorable results for sensitive and specific detection of methicillin-resistant S. aureus (MRSA) (5, 11, 15, 17, 25, 26).

(Part of this work will appear in the doctoral thesis of M. A. Horstkotte, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.)

CoNS strains (n = 197) were consecutively collected at the University Hospital Hamburg-Eppendorf from November 1997 to January 1998, including blood culture (n = 77), infected catheter (n = 91), wound (n = 15), and urine (n = 14) isolates. A single isolate per patient was included. S. epidermidis 1457, 1057, 9225, and RP62A were included as reference isolates. A single isolate per patient was included.

S. epidermidis were subcultured twice onto Columbia blood-agar (CBA) and 80°C and kept at


0099-1132/01/$04.00

Oxacillin MICs were determined by the broth microdilution method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (9).

PCR detection of mecA was performed by using the conditions described previously (13, 20) with the primers mecA sense (181–5′-GAA ATG ACT GTA CGT CCG AT-3′) and mecA antisense (330–5′-GGC ATC AAC ATG TA ACC GTA GT-3′) (19). S. epidermidis 1457 and RP62A were included as negative and positive controls, respectively.

For the MRSA-Screen (Denka Seiken Co., Niigata, Japan), isolates were grown overnight on CBA. The manufacturer’s instructions were essentially followed; however, a larger inoculum was used by suspending a loopful of bacteria to at least a McFarland no. 6 standard in extraction buffer. Agglutination results were read after 3 min.

A total of 201 isolates of CoNS (142 S. epidermidis, 15 S. haemolyticus, 10 S. hominis, 9 S. saprophyticus, 6 S. capitis, 4 S. lugdunensis, 4 S. warneri, 4 S. xylosus, 2 S. schleiferi, 2 S. cohnii, 1 S. chromogenes, 1 S. simulans, and 1 S. kloosii isolates) were tested. mecA was detected by PCR in 126 (62.7%) of all strains, of which 102 were S. epidermidis strains and 24 were non-S. epidermidis strains (Table 1). By the MRSA-Screen, 119 of 126 mecA-positive strains were LA positive. On initial testing 2 mecA-positive S. epidermidis strains were LA negative and 5 strains displayed weakly positive agglutination (Tables 1 and 2).

When the weakly positive reactions were also counted as positive, 2 mecA-positive S. epidermidis strains were LA negative and 5 strains displayed weakly positive agglutination (Tables 1 and 2). Initially, 67 of 75 mecA-negative strains were LA negative, but 8 non-S. epidermidis strains displayed a positive (n = 1) or weakly positive (n = 7) agglutination result (Tables 1 and 2).

When the weakly positive reactions were also counted as positive, 2 mecA-negative strains were counted as positive, 2 mecA-negative strains were counted as positive, 2 mecA-negative strains were counted as positive, 2 mecA-negative strains were counted as positive, 2 mecA-negative strains were counted as positive, 2 mecA-negative strains were counted as positive, 2 mecA-negative strains were counted as positive, 2 mecA-negative strains were counted as positive. PBP 2a was detected with sensitivities of 98.4, 98.0, and 77.1% in all strains of CoNS, S. epidermidis strains only, and non-S. epidermidis strains, respectively.

The 12 weakly positive strains and the 3 strains with LA results discordant with the mecA PCR result were retested. All seven mecA-positive strains were LA positive on retesting.
TABLE 1. Detection of methicillin resistance by latex agglutination of PBP 2a compared to mecA PCR

<table>
<thead>
<tr>
<th>Isolate and mecA status</th>
<th>No. (%) of isolates tested</th>
<th>No. of isolates with the following MRSA-Screen result:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Weakly positive</td>
</tr>
<tr>
<td>All CoNS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mecA positive</td>
<td>201 (100)</td>
<td></td>
</tr>
<tr>
<td>mecA negative</td>
<td>75 (37.5)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mecA positive</td>
<td>142 (70.6)</td>
<td>95 (100)</td>
</tr>
<tr>
<td>mecA negative</td>
<td>40 (28.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Non-S. epidermidis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mecA positive</td>
<td>59 (29.4)</td>
<td>24 (24)</td>
</tr>
<tr>
<td>mecA negative</td>
<td>35 (59.3)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

a Each value in parentheses is the number of strains with the given MRSA-Screen result upon retesting of weakly positive strains.

The present study detection of PBP 2a by MRSA-Screen was highly sensitive and specific and at least equivalent to other phenotypic techniques for the detection of methicillin resistance in CoNS (6, 16, 21, 27). Compared to the results of PCR, MRSA-Screen was superior to the standard broth microdilution assay when the present oxacillin breakpoint (0.5 μg/ml) suggested by NCCLS (9) was used. The latter method misidentified 66 of 75 mecA-negative strains of CoNS as oxacillin resistant. In contrast, an excellent specificity of the new breakpoint was reported for S. epidermidis, S. hominis, and S. haemolyticus, but the breakpoint was less accurate when it was applied to other species of CoNS (8). A lack of specificity of the new breakpoint could jeopardize the efforts directed at curtailing the overuse of glycopeptide antibiotics. When we evaluated our MIC data with the old oxacillin breakpoint (4.0 μg/ml), one mecA-positive S. epidermidis isolate would have been misclassified as susceptible and one mecA-negative S. kloosii isolate would have been misclassified as resistant.

The principal difficulties of performance of the MRSA-Screen with CoNS were reported to be regarding sensitivity (7, 15) or specificity (1, 28), or both (4, 23). A low initial sensitivity was reported, with satisfactory results obtained only after induction of PBP 2a synthesis with an oxacillin disk during overnight subcultivation (7). In contrast, all 60 mecA-positive strains of CoNS were reported to be LA positive after 3 min without induction (28). Apparently, the sensitivity of the MRSA-Screen depends on the amount of bacteria used in the inoculum or additional enhancement of PBP 2a expression. We prefer using a rather heavy inoculum, as oxacillin induction of PBP 2a requires subcultivation and could delay the results by 24 h. Additionally, extended agglutination times for increased sensitivity (7) can lead to false-positive results (28).

When CoNS were evaluated, weakly positive LA results occurred (7, 28). In our study, retesting of weakly positive strains led to positive test results for all of the mecA-positive strains and to negative results for all but two mecA-negative strains. For use in the clinical laboratory, it seems reasonable...
to retest a weakly positive isolate and to interpret a concordant LA result (positive or weakly positive on retesting) as positive. If the second LA test gives a discordant (negative) result, either the species diagnosis could help in the decision, as all false-negative results occurred with non-\emph{S. epidermidis} strains, or an independent reference method could be used in parallel.

In our study two \emph{S. warneri} strains displayed false-positive LA test results. False-positive LA test results were reported with \emph{S. lugdunensis}, \emph{S. warneri}, \emph{S. simulans}, and \emph{S. hominis} (1, 7, 23, 28).

Apparently, the MRSA-Screen performs favorably with clinical isolates of CoNS, especially the most frequently encountered species, \emph{S. epidermidis}. False-positive results occur primarily with non-\emph{S. epidermidis} strains. With a turnaround time of about 30 min, this assay could replace other phenotypic methods for determination of methicillin resistance in CoNS in the clinical microbiology laboratory.

We thank Rainer Laufs for continuous support.

This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft (to D.M.).

\textbf{REFERENCES}


