Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit

Ane B. Poulsen*, Robert Skov and Lars V. Pallesen

Department of Microbiological R&D, Statens Serum Institut, Copenhagen, Denmark

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The EVIGENE MRSA Detection Kit was evaluated on coagulase-negative staphylococci (CoNS) from agar plates and on staphylococci directly from positive spiked blood cultures. For the CoNS study, a total of 242 isolates were tested, and of these 237 gave valid test results. For the 237 valid tests, all gave correct mecA classification. For the blood culture procedure, a collection of 51 mecA-positive Staphylococcus aureus, 21 mecA-negative S. aureus, 31 mecA-positive CoNS and 28 mecA-negative CoNS were used for the simulated blood cultures. For the S. aureus strains, all gave valid test results and correct mecA classification. One of the MRSA isolates gave a very faint nuc signal, and another four isolates gave results close to the cut-off of the kit; however, these were still clearly positive when read by the naked eye. For the CoNS isolates, 51 of the 59 strains gave valid results. All of these 51 strains gave correct mecA status. Thus the EVIGENE MRSA Detection Kit can provide fast and accurate determination of methicillin resistance in CoNS. This preliminary study of the blood culture procedure indicates that it is possible to achieve determination of methicillin resistance in staphylococci 8 h after positivity of the blood culture, making same-day detection of methicillin resistance possible.

Keywords: MRSA, blood culture, coagulase-negative staphylococci, mecA, methicillin resistance determination

Introduction

With the spread of methicillin-resistant staphylococci in hospitals and in the community, fast and reliable methods for detection of resistance are warranted. Such detection in coagulase-negative staphylococci (CoNS) has been very difficult, and therefore detection of the mecA gene has been recommended as the gold standard.1 Fast methods for use on samples drawn from blood cultures positive for staphylococci are needed in order to speed up response time, which for conventional methods is 24–48 h. The EVIGENE MRSA Detection Kit is a commercially available fast kit for detection of the mecA gene in Staphylococcus aureus, for which a sensitivity and specificity of 100% has been found.2 The aim of this study was two-fold: first to validate the EVIGENE MRSA Detection Kit on CoNS; and secondly to evaluate a procedure making same-day determination of methicillin resistance in staphylococci directly from positive blood cultures possible.

Materials and methods

The EVIGENE MRSA Detection Kit is a DNA probe-based hybridization assay performed in a microwell format, as described previously in detail.2 The kit is ready to use in routine laboratories requiring a shaker–incubator and heating block. An ELISA reader is optional, as a positive result is visible by eye. Briefly, the kit procedure is as follows. A loopful of bacterial growth (10 µL) is collected from a pure agar plate culture and incubated in a lysis reagent at 37°C. The bacteria are completely lysed and the chromosomal DNA is denatured. A hybridization reagent containing biotinylated detection probes is added to the sample, which is then transferred to four wells in a microtitre plate, each well containing capture probes specific for the target gene of that well. After hybridization at 50°C and 400 rpm in a shaker–incubator, the wells are washed and incubated with an alkaline

*Corresponding author. Tel: +45-32-68-82-10; Fax: +45-32-68-32-31; E-mail: abp@ssi.dk

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phosphatase-conjugated anti-biotin antibody. After a second wash, substrate is added to all wells and a colour develops, corresponding to a positive result for each well. The kit result is clearly readable with the unaided eye; however, the result can also be read in an ELISA reader at 405 nm. The cut-off value for positive signals in the kit is set at OD_{405} = 0.8. The four wells of each test comprise (1) a positive control (16S rDNA detection), (2) a negative control, (3) meca gene detection and (4) nuc gene detection for differentiation between S. aureus and CoNS. The kit procedure takes 3.5 h to complete with ~45 min of hands-on time.

For the study of CoNS, 242 epidemiologically unrelated strains previously collected from patient samples were used. The collection consisted of 128 meca-positive and 114 meca-negative strains, and comprised 163 Staphylococcus epidermidis, 27 Staphylococcus hominis, 26 Staphylococcus haemolyticus, 10 Staphylococcus warneri, five Staphylococcus lugdunensis, four Staphylococcus capitis, three Staphylococcus sciuri, two Staphylococcus colonii, one Staphylococcus simulans and one Staphylococcus schleiferi, identified to the species level by API ID32 Staph (bioMérieux, Marcy l’Étoile, France). The meca status was established using multiplex PCR, as described previously, complemented with a primer set for detection of the nuc gene, encoding a thermostable nuclease specific for S. aureus. This multiplex PCR resulted in a 16S rDNA band of 886 bp, a meca band of 527 bp and a nuc band of 255 bp, as analysed on normal agarose gels (data not shown). In brief, each PCR contained 5 µL of 10× PCR Buffer II (Applied Biosystems, Foster City, CA, USA), 3.75 mM MgCl₂, 0.4 mM of each dNTP, 1 µM of each primer, 1.25 U AmpliTaq (Applied Biosystems) and 5 µL of template in a total reaction volume of 50 µL. Template DNA was purified from overnight cultures using the QIAamp DNA mini kit (QIAGen GmbH, Hilden, Germany). The primers used were: for 16S rDNA, 16Sup1: 5'-GTGCGCAGCAGCCGGGTAATA-3' and 16Sup2: 5'-AGACCCCAGGAACGTATTCC-3'; for the meca gene, mecup1: 5'-GGGATCATAGCCTATTTC-3' and mecup2: 5'-AAGGATGTGACACGATAGCC-3'; for the nuc gene, nucPCR1: 5'-TCAGAAATGCAATCAAAACAG-3' and nucPCR2: 5'-CTGAAATGCACTTGGTTCAAG-3'. The PCR profile was: denaturation for 5 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, and finally elongation for 2 min at 72°C.

For the evaluation of the blood culture procedure, a total of 131 epidemiologically unrelated staphylococcal strains (S. aureus and CoNS), with meca and nuc status previously characterized by PCR, were tested. The collection consisted of 51 meca-positive S. aureus (MRSA), 21 meca-negative S. aureus (MSSA), 31 meca-positive coagulase-negative staphylococci (MRSE) and 28 meca-negative coagulase-negative staphylococci (MSSE). Pilot studies showed that the number of bacteria per mL, when a blood culture becomes positive, was too low to give a readable signal in the kit if tested directly. Therefore, an enrichment step was introduced in the protocol described here. Simulated blood cultures were used in this study because of the very low number of MRSA in Denmark. For each bacterial isolate tested, a BacTec Plus Aerobic/F blood culture bottle (BacTec, Sparks, MD, USA), containing ~7 mL of fresh whole blood from healthy volunteers, was inoculated with 2–4 cfu, and incubated in a BacTec 9240 instrument. When detected positive by the BacTec instrument (usually after 14–18 h), the blood cultures were processed within a maximum of 5 h. After decontamination of the blood culture bottle, 1 mL of blood culture was withdrawn with a syringe and mixed with 5 mL of sterile water in order to lyse the red blood cells. The mixture was transferred to 25 mL of Mueller–Hinton broth, and the culture incubated at 37°C for 3 h while gently shaking. Ten mL of the culture was centrifuged at 4500 g for 10 min and the total bacterial pellet obtained was used as starting material in the EVIGENE MRSA Detection Kit, substituting the 10 µL of bacterial growth recommended by the manufacturer in the kit protocol. The entire procedure, from positive blood culture to final result, took ~8 h.

**Results**

Of the 242 CoNS tested following the normal kit procedure, 237 gave valid results. There was a 100% correlation between the results obtained with the kit and those obtained with the multiplex PCR. The remaining five strains (two meca-positive S. epidermidis, two meca-positive S. haemolyticus and one meca-negative S. simulans) all gave signals in the positive control well below the cut-off of the kit, thus rendering the tests invalid.

For the evaluation of the blood culture procedure, all of the 72 S. aureus isolates tested gave correct meca results. For the CoNS, 51 of 59 strains gave valid test results and all of these gave correct meca results. One MRSA strain produced a very faint nuc signal. This strain also produced a low (but clearly positive) signal when run in the normal kit procedure and is thought to be mutated in the nuclease gene sequence, supported by the fact that several attempts to sequence the gene have been unsuccessful (data not shown). In four other MRSA strains, the nuc signals were just below the cut-off of the kit; however, the signals were clearly positive when read by the naked eye. These four strains gave normal positive nuc signals when run in the normal kit procedure. The results of the studies are summarized in Tables 1 and 2.

**Discussion**

The EVIGENE MRSA Detection Kit gave 100% sensitivity and specificity for detection of methicillin resistance in CoNS using the normal kit procedure. Valid test results were
Detection of methicillin resistance

Table 1. Results for CoNS in the normal kit procedure

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
<th>No. of strains</th>
<th>No. of valid tests</th>
<th>meca (no. correct/no. total)</th>
<th>nuc (no. correct/no. total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoNS</td>
<td></td>
<td>242</td>
<td>237 (98%)</td>
<td>237/237</td>
<td>237/237</td>
</tr>
</tbody>
</table>

Table 2. Results from the evaluation of the blood culture procedure

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
<th>No. of strains</th>
<th>No. of valid tests</th>
<th>meca (no. correct/no. total)</th>
<th>nuc (no. correct/no. total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td></td>
<td>72</td>
<td>72 (100%)</td>
<td>72/72</td>
<td>67/72</td>
</tr>
<tr>
<td>CoNS</td>
<td></td>
<td>59</td>
<td>51 (86%)</td>
<td>51/51</td>
<td>51/51</td>
</tr>
</tbody>
</table>

obtained for 98% of the 242 isolates. The EVIGENE MRSA Detection Kit can thus provide accurate genotypic meca classification of staphylococci in routine laboratories; furthermore, the result is ready 1–2 days earlier than phenotypic methods. The kit simultaneously provides a differentiation between S. aureus and CoNS by detection of the nuc gene specific for S. aureus.

This preliminary evaluation of the blood culture procedure on seeded blood cultures demonstrates that after a short enrichment step the EVIGENE MRSA Detection Kit can be used on samples taken directly from positive blood cultures, making same-day determination of methicillin resistance in staphylococci possible. Five S. aureus isolates produced nuc signals that were visually clearly positive but had OD values below the cut-off, making the results ambiguous. In addition, when using the EVIGENE MRSA Detection Kit directly on blood cultures, false results can emerge in cases of mixed cultures. If, for example, a culture contains both a meca-negative S. aureus and a meca-positive CoNS in approximately equal amounts, the kit result will read MRSA, thus giving rise to a falsely positive result. On the other hand, if the S. aureus has outgrown the CoNS, the kit will not detect the methicillin-resistant CoNS. The result of a specimen taken directly from a positive blood culture bottle is therefore only preliminary and cannot replace concomitant conventional identification. A notice regarding this issue should be given to the clinician when reporting the result.

As the blood culture procedure was evaluated on seeded blood cultures only, further evaluations of the blood culture procedure, using true clinical samples, are necessary.

References


