Real-Time PCR Can Rapidly Detect Methicillin-Susceptible and Methicillin-Resistant Staphylococcus aureus Directly From Positive Blood Culture Bottles

Suzanne M. Paule,1 Anna C. Pasquariello,1 Richard B. Thomson, Jr,1,2 Karen L. Kaul,1,2 and Lance R. Peterson1,2

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Abstract

We developed, validated, and implemented real-time polymerase chain reaction (PCR) detection of the femA gene for Staphylococcus aureus and the mecA gene for methicillin resistance directly from BACTEC (Becton Dickinson, Sparks, MD) blood culture bottles showing gram-positive cocci in clusters. For the 332 positive blood cultures tested, the assay had 100% sensitivity and specificity for identifying methicillin-susceptible (n = 28) and methicillin-resistant (n = 28) S aureus, and overall was 98% sensitive and 94% specific, with 3 uninterpretable test results when identification of coagulase-negative staphylococci was included. PCR detection yields rapid (2-3 hours) results and accurate identification of S aureus directly from signal-positive blood culture bottle samples.

Staphylococcus aureus is an important cause of bloodstream infections1 and a leading cause of severe health care–associated infections.2 Infection results in significant morbidity and mortality and longer hospital stays if not treated early with effective antibiotics.3 The prevalence of methicillin resistance in S aureus causing infection now exceeds 49% in US hospitals and continues to increase.4 Thus, improved methods are needed for rapid detection and differentiation of methicillin-susceptible S aureus (MSSA) and methicillin-resistant S aureus (MRSA) bacteremia to ensure optimal treatment early in the infection.

Initial antimicrobial treatment for bacteremia often is based on the results of the Gram stain from a positive blood culture bottle. In 48 to 72 hours, results of conventional culture and susceptibility testing permit a change to pathogen-specific antimicrobial therapy. Although vancomycin remains the treatment of choice for MRSA bacteremia, unnecessary use should be avoided to prevent further emergence of resistance as well as colonization or infection by organisms such as vancomycin-resistant enterococci. In addition, treatment of MSSA infections with vancomycin might be inferior to treatment with antistaphylococcal penicillins.5 The earlier one knows whether the positive blood culture contains S aureus and whether it is methicillin-resistant or susceptible, the more quickly appropriate therapy can be initiated.

Materials and Methods

From July 2003 through March 2005, in cases at Evanston Northwestern Healthcare, Evanston, IL, with a signal-positive blood culture bottle showing gram-positive...
coci in clusters, an aliquot was taken from the bottle for real-time polymerase chain reaction (PCR). All positive blood culture bottles (BACTEC 9240, Becton Dickinson, Sparks, MD) continued to have conventional culture performed consisting of plating to blood and chocolate agar (Remel, Lenexa, KS) and then incubation for 18 to 48 hours at 35°C in 5% carbon dioxide. For the culture-based identification, *S. aureus* was identified by colony morphologic features, catalase, and the Staphaurex latex agglutination test (Murex Biotech, Dartford, England). Oxacillin susceptibility testing using oxacillin disk diffusion and oxacillin agar screen (July 2003-September 2004) or cefoxitin disk diffusion (September 2004-March 2005) was performed following the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) guidelines.6,7

The blood aliquot for real-time PCR was stored at 4°C for up to 24 hours when 200 µL was removed and applied to a Generation Capture Column (Genta, Minneapolis, MN) and washed following the manufacturer’s protocol. The DNA was eluted off the column in 50 to 100 µL of elution solution and used directly in real-time PCR. Extracted DNA (2 µL) was added to a hot-start reaction mixture for each test. The final 20 µL real-time PCR reaction contained 1x LightCycler FastSart DNA Master SYBR Green I (Roche Applied Science, Indianapolis, IN), 2% DMSO (Sigma, St Louis, MO), 5 µmol/L of magnesium chloride, and for the *fema* test, 0.25 µmol/L of each primer *fema*-2F and *fema*-2R,8 which were designed to amplify a uniquely conserved region of the *fema* gene found only in *S. aureus*, or for the *meca* test, 0.5 µmol/L of each primer *meca*-4up: 5’-AAGCGACTTCACATCTATTAGGTTAT and *meca*-4dn: 5’-TATATTCTTCGTTACTCATGCCCATAc, yielding a 402-base-pair product. Included in each run for both tests were blank (water), negative (methicillin-resistant *S. aureus*, MSSA, and a mixture of MSSA and MRCNS). By using this strategy, a specimen can be called positive for MRSA only if the *fema/meca* C_T ratio was between 0.9 and 1.1.

The overall results of our conventional culture and real-time PCR comparison for detection of staphylococci in blood culture bottles in a clinical laboratory setting are given in Table 1. Incorporating the real-time PCR test into the blood culture workup involved incorporation of the results into the microbiology culture report Table 2. From July 2003 through March 2005, a total of 332 positive blood culture bottles with gram-positive cocci in clusters were tested. Real-time PCR identified 28 as MRSA, 28 as MSSA, 179 as MRCNS, 94 as not *S. aureus* (*fema*– and *meca*–), and 3 as equivocal (Table 1). Comparison of real-time PCR showed complete correlation with culture for all MRSA, MSSA, and MRCNS cases. All MRCNS detected by real-time PCR were confirmed to be coagulate-negative staphylococci (CNS) by culture, but susceptibility testing was performed for only 69 of these. For the 3 samples, 2 for *fema* and 1 for *meca*, that were identified as equivocal, the C_T was repeatedly in the indeterminate zone, and these grew CNS in culture. Two samples contained both MRSA and CNS and were identified correctly using our C_T ratio rule (ratios of 1.00 and 0.94).

Based on the lack of results for 3 of 332 samples, the assay has an indeterminate rate of 0.90%. Analysis of the 329 samples with *fema* results found the *fema* test had a sensitivity and specificity of 100%. For the 90 samples that had CNS with susceptibility testing performed, the *meca* test had a sensitivity of 97% and a specificity of 86%, although the actual results might be better because the current phenotypic assay for methicillin resistance in CNS can
provide false-positive and false-negative test results. When \textit{femA} and \textit{mecA} test results were combined, the overall assay for the 329 tests showed a sensitivity of 98% and a specificity of 94% Table 3I.

**Discussion**

The use of PCR has increased the speed and accuracy for identification of \textit{S. aureus} and confirmation of MRSA by detection of \textit{mecA}.\textsuperscript{10} It is important to note that our assay correctly detected all MSSA and MRSA directly from signal-positive blood culture bottles. For the 3 blood samples with discrepant results (\textit{femA}– and \textit{mecA}–; identified by culture as MRCNS), based on our reporting format (Table 2), the discrepant results would not have led to a therapeutic error. In addition, for the 3 indeterminate samples, which were considered contaminants on clinical grounds, the only results reported were from traditional culture that detected CNS.

For the routine diagnostic laboratory, a multiplex PCR assay using detection of the \textit{mec} and \textit{fem} genes has been suggested as one approach to rapidly identify MRSA from cultures. This was found to be more accurate and sensitive than routine, culture-based analysis, with results available from the initial use of this technology in 24 hours.\textsuperscript{11} Similar suggestions have been made for the routine diagnostic laboratory to speed the accurate detection of MRSA in blood cultures,\textsuperscript{12} including the use of real-time PCR for this application.\textsuperscript{13,14}

**Table 3I**

**Sensitivity, Specificity, and Predictive Values of Positive and Negative Real-Time PCR Assay Results**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive Value of a Positive Test Result</th>
<th>Predictive Value of a Negative Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{femA} (n = 329)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>\textit{mecA} in \textit{femA}+ samples (n = 56)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>\textit{mecA} in \textit{femA}– samples (n = 90)</td>
<td>97</td>
<td>86</td>
<td>96</td>
<td>90</td>
</tr>
<tr>
<td>All tests (n = 329)</td>
<td>98</td>
<td>94</td>
<td>98</td>
<td>94</td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction; +, positive; –, negative.

\textsuperscript{*} Results are given as percentages. Three uninterpretable results were excluded from analysis.
Since implementing this described protocol in routine practice, we have tested 332 blood culture bottles by a real-time PCR assay that detected all MSSA and MRSA cases within a possible timeframe of 2 to 3 hours after the bottle was identified as positive. This testing was performed daily initially by 2 technologists from the molecular epidemiology section of clinical microbiology, and now it is run by the technologists in the molecular diagnostics laboratory. The addition of this real-time PCR test to blood cultures has increased the workload for the molecular diagnostics laboratory by 1 run a day that costs $8.00 (reagents and supplies only) per blood sample and has minimally increased the workload for microbiology but greatly increased the speed of reporting results.

Other testing strategies such as a multiplex assay and the addition of an internal control can be used during PCR assay development. However, each additional set of primers adds to the cost of the test and also might reduce detection sensitivity. Our assay was able to accurately address the particular question we were interested in: whether \textit{S. aureus} or MRSA was present in the blood culture of the patient; and the assay answered the question at a very reasonable expense. Therefore, the detection of additional targets was not needed. With the benefit of obtaining reliable results in a few hours as opposed to days, real-time PCR can be used for rapid, accurate detection of MSSA and MRSA bacteremia that can lead to optimal therapeutic treatment at the earliest time.

From the 1Department of Pathology and Laboratory Medicine, Evanston Northwestern Healthcare, Evanston, IL; and 2Northwestern University Feinberg School of Medicine, Chicago, IL. Supported by the Department of Pathology and Laboratory Medicine, Evanston Northwestern Healthcare.


Address reprint requests to Ms Paule: Dept of Pathology and Laboratory Medicine, Evanston Northwestern Healthcare, 2650 Ridge Ave, Evanston, IL 60201.

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References


