

Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Blood with the EVIGENE MRSA Detection Kit

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A total of 200 blood cultures containing putative staphylococci were analyzed by a commercial gene probe hybridization assay (EVIGENE; Statens Serum Institut, Copenhagen, Denmark), and 18 were identified as methicillin-resistant *Staphylococcus aureus* (MRSA) positive. Of these, 17 were positive by PCR and 16 were positive by culture. Detailed analysis of the discrepant results showed that the EVIGENE kit allowed specific identification of MRSA in blood cultures without any of the drawbacks associated with PCR.

Staphylococcus aureus is an important cause of bacteremia worldwide, with an increasing incidence of multiresistant strains (2, 5). In most clinical microbiology laboratories, positive blood cultures identified with automated systems are examined microscopically for gram-positive cocci in clusters (GPCC), followed by conventional tests to differentiate between *S. aureus* and coagulase-negative staphylococci and to confirm the identity of methicillin-resistant *S. aureus* (MRSA). As definitive results require at least 48 h from the recognition of a positive blood culture, empirical treatment with glycopeptides is common (9, 13, 15), resulting in undesirable selection pressure for the development and spread of vancomycin resistance.

Several groups have identified *S. aureus* and MRSA from blood cultures by using DNA probes (4, 7), peptide nucleic acid probes (12), gel-based PCR (1, 8–11), and real-time PCR (13, 15). However, such molecular assays are associated with specialized equipment and expertise, increased cost, and specific laboratory organization into pre- and postamplification areas. Nevertheless, there is a need for rapid diagnosis of MRSA bacteremia so that appropriate therapeutic decisions can be made. This study evaluated a commercial gene probe hybridization assay (EVIGENE MRSA Detection Kit; Statens Serum Institut, Copenhagen, Denmark) that targets staphylococcus-specific 16S rRNA, *mecA*, and *nuc* gene sequences, thereby differentiating coagulase-negative staphylococci and *S. aureus* and determining methicillin resistance (14).

The contents of aerobic (Plus Aerobic/F), anaerobic (LYTIC/10), and pediatric (PEDS PLUS/F) BACTEC blood culture bottles (Becton Dickinson, Oxford, United Kingdom) were inoculated with blood from patients at University Hospital, Nottingham, United Kingdom, and were incubated on the BACTEC system (Becton Dickinson). Following recognition of a positive growth curve, 200 random blood cultures containing GPCC were analyzed by EVIGENE, PCR, and conventional culture (as described below) over a period of 4 months from August to November 2002.

Samples (1 ml) were mixed with 5 ml of sterile water to lyse red blood cells and were then transferred to 25 ml of Mueller-Hinton broth. Following incubation at 37°C with constant shaking (200 rpm) for 3 h, 10 ml of broth was centrifuged at 3,000 × *g* for 15 min and the pellet was tested with the EVIGENE MRSA Detection Kit, according to the manufacturer's instructions. Briefly, samples underwent enzymatic and heat lysis before the addition of *mecA*, *nuc*, and 16S rRNA probes. Hybridization, capture, and detection of probe target complexes were carried out in a microwell strip, with colorimetric results obtained in <3.5 h (14). The manufacturer's recommended cutoff value of optical density at 405 nm (OD₄₀₅) (≥0.8) was used.

Cultures were also processed (50 μl) with the MD_x InstaGene Whole Blood Kit (Bio-Rad, Hemel Hempstead, United Kingdom) to extract DNA, according to the manufacturer's instructions, followed by amplification in a *mecA/femB* multiplex PCR (16). Specific PCR products were visualized with ethidium bromide staining following electrophoresis on agarose 2% (wt/vol) gels. Cultures were also spread (100 μl) onto Oxacillin Resistance Screening Agar (ORSA; Oxoid, Basingstoke, United Kingdom), which is a nutritious and selective indicator medium containing 5.5% (wt/vol) NaCl and oxacillin (2 mg/liter). Following incubation at 37°C, plates were inspected for deep blue colonies (indicative of MRSA) after 24 and 48 h. Putative MRSA colonies were confirmed by latex agglutination tests for *S. aureus* (Slidex Staph Kit; bioMérieux, Marcy l'Etoile, France) and oxacillin resistance (PBP2' Latex Agglutination Test; Oxoid). Any further identification of colonies was accomplished with the ID32 Staph Kit (bioMérieux). Conventional microbiological tests were used to identify non-staphylococci.

Of 200 cultures positive for GPCC, 18 (10 aerobic and 8 anaerobic samples from 12 patients) were positive for the *mecA* and *nuc* targets according to the EVIGENE kit. OD readings for these samples are shown in Table 1. Multiplex *mecA/femB* PCR indicated that 17 of these were MRSA. The EVIGENE-positive but PCR-negative sample was also positive for MRSA by PCR and latex agglutination tests when isolated bacterial colonies were tested. Culture on ORSA, followed by latex agglutination tests on deep blue colonies, identified 16 cultures as MRSA positive. Isolated colonies from two

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TABLE 1. OD₄₀₅ of positive, negative, *mecA*, and *nuc* target wells of MRSA-positive samples in the EVIGENE MRSA Detection Kit

Sample no.	OD ₄₀₅ for:			
	Positive control	Negative control	<i>mecA</i> target	<i>nuc</i> target
9 ^b	1.938	0.208	3.227	1.823
10 ^a	2.159	0.200	2.859	1.258
36 ^b	2.620	0.223	3.576	2.228
37 ^a	1.433	0.214	3.924	1.943
38 ^a	1.825	0.236	2.138	1.463
39 ^b	3.170	0.215	3.500	2.265
56 ^a	1.836	0.224	2.854	1.880
58 ^b	1.927	0.215	2.201	1.767
65 ^a	3.439	0.218	2.766	2.662
80 ^a	1.950	0.220	1.746	1.750
81 ^b	1.990	0.223	1.662	1.590
82 ^a	2.634	0.220	2.451	2.020
83 ^b	2.587	0.214	1.996	1.869
84 ^a	2.364	0.220	2.333	1.986
85 ^b	2.566	0.206	2.297	1.808
88 ^a	2.623	0.212	3.098	2.536
125 ^a	1.529	0.328	1.872	1.373
190 ^b	0.916	0.231	1.683	0.798

^a Aerobic bottle.^b Anaerobic bottle.

cultures that were identified as MRSA positive by EVIGENE and PCR but did not give deep blue colonies on ORSA were later confirmed as MRSA by latex agglutination tests. Table 2 compares the efficacy of the EVIGENE kit and conventional culture with that of *mecA/femB* PCR for the specific identification of MRSA in blood cultures.

A few minor discrepancies were observed for non-MRSA strains. Two cultures were identified as containing methicillin-sensitive *S. aureus* on the basis of borderline *nuc* OD₄₀₅ signal readings of 0.864 and 0.758 in the EVIGENE kit, but this was not confirmed by PCR amplification of *femB* or by latex agglutination tests. One contained both *S. capitis* and *S. epidermidis*, while *S. capitis* was isolated from the other. One other culture was repeatedly *femB* positive by PCR but was negative by EVIGENE for *nuc*; no subsequent isolate of *S. aureus* was identified.

Twenty-five of the 200 samples containing GPCC were negative with the staphylococcus-specific 16S rRNA control in the EVIGENE kit. Of these, 14 yielded nonstaphylococci following conventional culture. Isolates from the remaining 11 were identified as staphylococci following conventional culture and

TABLE 2. Comparison of the efficacy of EVIGENE MRSA Detection Kit and conventional culture with that of *mecA/femB* PCR for detection of MRSA in blood cultures (*n* = 200)^c

Test being evaluated	Comparison (%) with <i>mecA/femB</i> PCR			
	PPV	NPV	Sensitivity	Specificity
EVIGENE	94.4 ^a	100	100	99.5 ^a
Culture	93.8 ^a	98.9 ^b	88.2 ^b	99.5 ^a

^a One EVIGENE and culture-positive sample were later also shown to be PCR positive for MRSA when the PCR was repeated on isolated colonies (i.e., an initial PCR false negative).^b Two samples failed to yield blue colonies following initial culture on ORSA but were positive for MRSA by PCR and subsequent latex agglutination tests (i.e., culture false negatives).^c PPV, positive predictive value; NPV, negative predictive value.

were from nine anaerobic bottles, one pediatric bottle, and one aerobic bottle. Nine of these negative samples were available for subsequent assays after a further 3 h of incubation, at which time specific 16S rRNA was detected in five of the nine samples and the *mecA/nuc* EVIGENE results were in agreement with the *mecA/femB* PCR. This suggested that growth had been slowed by the change from anaerobic to aerobic conditions. MRSA was not detected in these 11 samples by any method.

All EVIGENE MRSA-positive results were subsequently confirmed by latex agglutination tests on purified colonies. However, with any multiplex molecular method there is a possibility of false identification of MRSA in mixed cultures because of the simultaneous detection of *mecA* and an *S. aureus*-specific sequence from two separate organisms. Although not significant in the present study, this theoretical drawback could be overcome by the addition of oxacillin to the blood culture broth to kill susceptible *S. aureus*, a strategy that has been successfully employed to prevent false identification of MRSA from patient screening swabs by multiplex PCR (6, 16).

Overall, this study found that the EVIGENE kit was user friendly for the routine microbiology laboratory, with results available within 7 h of recognition of a blood culture positive for GPCC. Rapid and accurate testing of GPCC-positive blood culture samples should facilitate infection control measures, reduce empirical use of vancomycin, and improve the management of MRSA bacteremia; indeed, there is evidence that mortality rates of MRSA bacteremia drop during outbreaks, possibly because MRSA is suspected and identified more readily, thereby resulting in an earlier initiation of appropriate treatment (3).

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