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Rapid Identification of Methicillin-Resistant *Staphylococcus aureus* from Positive Blood Cultures by Real-Time Fluorescence PCR

THEAN YEN TAN,[1]* SALLY CORDEN,[2] ROSEMARY BARNES,[1] AND BARRY COOKSON[3]

Department of Microbiology, Public Health Laboratory Service,[1] and Department of Virology, Public Health Laboratory Service,[2] Cardiff, and Laboratory of Hospital Infections, Central Public Health Laboratory, London,[3] United Kingdom

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Methicillin-resistant *Staphylococcus aureus* septicemia is associated with significant morbidity and mortality and requires treatment with intravenous glycopeptides. For blood cultures positive for gram-positive cocci, 24 to 48 h is required for the detection of *S. aureus* bacteremia and the provision of antibiotic susceptibility testing results. We describe a molecular biology-based assay that requires 2 h from the time of initial positivity of blood cultures. The assay correctly detected 96% of the *S. aureus* isolates including all methicillin-resistant *S. aureus* isolates. Clinical data collected during the study suggest that 28% of patients with *S. aureus* bacteremia do not receive early and appropriate treatment and that 10% of patients may initially be receiving inappropriate glycopeptide treatment.

Staphylococcus aureus septicemia is associated with a mortality rate of 15 to 30% (17). The increasing proportion of infections caused by methicillin-resistant *S. aureus* (MRSA) (8) has resulted in the widespread empirical use of glycopeptides, which increases the pressure for selection of vancomycin resistance (19, 24). The use of vancomycin also requires therapeutic monitoring (15) to reduce the potential for use of a suboptimal dosage (5, 10) and side effects. From the time that a blood culture is positive, conventional methods of culture and antibiotic susceptibility testing require 48 h for the detection of *S. aureus* bacteremia and the provision of antibiotic susceptibility testing results. Rapid identification of MRSA from blood cultures would accelerate the diagnosis of *S. aureus* bacteremia and reduce the level of empirical use of vancomycin.

This paper reports on a method for the detection of *S. aureus* and MRSA directly from positive blood cultures by rapid real-time fluorescence PCR and also attempts to measure the potential clinical impact of the more rapid provision of test results.

BACTEC 9240 (Becton Dickinson, Le Pont de Claix, France) blood culture bottles were inoculated with 5 to 10 ml of blood from patients with suspected bacteremia and were incubated in the BACTEC 9240 automated continuous monitoring system. When a positive growth index was achieved, aliquots of blood were taken from 141 positive blood cultures showing gram-positive cocci in clusters for culture and PCR.

Clinical data were requested from the clinicians directly involved in the care of the patient. All isolates of *S. aureus* were considered clinically significant. Coagulase-negative staphylococci (CoNS) were considered clinically significant if all of the following factors were present: (i) the patient had had multiple episodes of bacteremia (26); (ii) intravascular catheters, prosthetic heart valves, or other risk factors were present in situ (13, 25); or (iii) the patient had pyrexia, peripheral leukocytosis, or hypotension. Therapy was considered effective if the patient was receiving an antibiotic that was recognized to have activity against the organism isolated from blood cultures.

Lysis and DNA extraction were achieved for each positive blood culture with the Generation DNA Purification Capture Column kit (Gentra Corporation, Minneapolis, Minn.). Oligonucleotide primers and fluorescence-labeled probes were designed for amplification and sequence-specific detection of a 179-bp fragment within the *S. aureus* sequence (16) and a 98-bp fragment within the *mecA* gene (Table 1). Two separate PCRs were performed with each sample; the first one detected the *Sa442* fragment, and the second one detected the *mecA* gene. PCR was performed with the LightCycler device (Biogene, Kimbleton, United Kingdom), which combines rapid thermal cycling and probe-specific detection of the amplified product.

The amplification mixture for the *Sa442* fragment consisted of 5 μl of a 2× *Taq*-based master mixture containing 2 mM $MgCl_2$, 0.5 μl each of primers Sa442-F and Sa442-R (final concentrations, 0.4 μM), 1 μl of cyanine 5 (Cy5)-labeled probe *Sa442*-P (final concentration, 0.3 μM), 1 μl of 1× SYBR Green I, and 2 μl of template DNA. The amplification mixture for the *mecA* fragment consisted of 5 μl of a 2× *Taq*-based master mixture containing 3 mM $MgCl_2$, 0.5 μl each of primers mecA-F and mecA-R (final concentrations, 0.4 μM), 1 μl of Cy5-labeled probe MecA-P (final concentration, 0.2 μM), 0.5 μl of 1× SYBR Green I, and 2 μl of template DNA.

Details of the 50-cycle amplification profiles are listed in Table 2.

Overnight culture of the 141 blood cultures on Columbia agar (Oxoid, Basingstoke, England) isolated 20 strains of methicillin-susceptible *S. aureus* (MSSA), 30 strains of MRSA, and 93 strains of CoNS. Two blood cultures were positive for a mixture of methicillin-resistant CoNS and MRSA. Methicillin susceptibility was determined by oxacillin antibiotic disk testing, in line with the guidelines issued by the British Society for Antimicrobial Chemotherapy (3, 4). Identification of *S. aureus* was achieved by colonial morphology, with a latex ag-

* Corresponding author. Mailing address: Department of Microbiology, Cardiff Public Health Laboratory, University Hospital Wales, Cardiff CF14 4XW, United Kingdom. Phone: 44-029-20742718. Fax: 44-029-20742161. E-mail: theanyen.tan@phls.wales.nhs.uk.

TABLE 1. Oligonucleotide primers and probes used in the study

Oligonucleotide	Sequence	Nucleotide position	Target gene	GenBank accession no.	Source or reference
Sa442-F	TCGGTACACGATATTCTTCAC	13–33	*Sa442*	X52593	This study
Sa442-R	ACTCTCGTATGACCAGCTTC	173–192	*Sa442*	X52593	This study
Sa442-P	TCTCATTACGTTGCATCGGAA-[Cy5]	103–123	*Sa442*	X52593	This study
MecA-F	CAAGATATGAAGTGGTAAATGGT	1471–1493	*mecA*	AF033191	21
MecA-R	ACTGCCTAATTCGAGTGCTAC	1555–1576	*mecA*	AF033191	This study
MecA-P	AAACAAGCAATAGAATCATCAGAT-[Cy5]	1513–1536	*mecA*	AF033191	This study

glutination kit (Staphaurex; Murex BioTech Ltd., Dartford, England), and by tube coagulase tests.

Of the 50 blood cultures positive for *S. aureus*, 48 were positive for the *Sa442* gene fragment. PCR failed to identify a recurrent isolate of *S. aureus* from a patient with persistent bacteremia. The *Sa442* fragment was not detected in any of the blood cultures positive for CoNS. Two blood cultures were positive for a mixture of methicillin-resistant CoNS and MRSA and were positive by PCR for both the *Sa442* and the *mecA* genes. Identification of *S. aureus* in blood cultures by PCR showed a sensitivity of 96% and a specificity of 100%.

Detection of methicillin resistance in *S. aureus* isolates by PCR showed a sensitivity and a specificity of 100% each compared with the results of conventional susceptibility testing. In comparison, identification of *mecA* in CoNS showed a sensitivity of 97% and a specificity of 95%.

Fifty-two blood cultures positive for clinically significant organisms were available for evaluation. MSSA accounted for 13% ($n = 16$) of all bacteremias, and MRSA accounted for another 19% ($n = 23$). Eleven patients with *S. aureus* bacteremia remained on ineffective antibiotic therapy in the first 24 h following notification that the blood cultures were positive (Table 3). Patients with MRSA bacteremia were at a higher risk of initially receiving ineffective antibiotics. A total of 28% of the patients with *S. aureus* bacteremia remained on ineffective treatment, despite active clinical microbiological involvement.

Prescribed antibiotic therapy was evaluated for all patients with clinically significant bacteremia. Four patients (25%) with MSSA bacteremia were initially treated with vancomycin. Following notification of culture results, only one patient was continued on vancomycin therapy. Two patients (12.5%) with MSSA bacteremia were initially receiving cephalosporins. Seven patients with nonclinically significant pseudobacteremia were initially receiving vancomycin, but therapy was discontinued for only three patients.

S. aureus is a frequent cause of bacteremia (26) and is associated with increased rates of mortality and secondary complications (14, 18). The detection of the *mecA* gene from positive blood cultures by the conventional PCR process can take 4 to 5 h (6; Y. C. Lee, J. Wu, and P. Della-Latta, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 896, 2000). PCR with the LightCyler device provides species identification and methicillin susceptibility results within 2 h of detection of a positive blood culture, which allows faster clinical decision making.

Although the numbers of patients investigated in the present study was small, the study reveals that a significant proportion of cases of MRSA bacteremia remain untreated in the first 24 h following notification that a blood culture is positive. A PCR assay would allow early detection and treatment of such cases. Early and appropriate treatment of septicemia has been associated with a reduction in the rate of mortality (1, 9, 11). However, one study reported that although patients with MRSA bacteremia were less likely to receive effective antibiotic therapy, this was not significantly linked to an increased rate of mortality (22).

Rapid susceptibility testing may also reduce the rate of empirical use of glycopeptides. Although 10% of patients infected with methicillin-susceptible isolates in the present study were initially receiving vancomycin therapy, only half of these patients subsequently discontinued vancomycin therapy. In principle, rapid provision of antibiotic susceptibility testing results should reduce the rates of empirical use of vancomycin and cephalosporins, which are associated with additional side effects and costs (2, 23).

The use of PCR technology is likely to be significantly more expensive than the use of conventional culture methods. An

TABLE 2. Amplification parameters for *Sa442* and *mecA* PCRs

Cycle parameters	Temp (°C)		Ramp rate (°C/s)	Time(s)		Fluorescence acquisition	No. of cycles
	Sa442	*mecA*		*Sa442*	*mecA*		
Denaturation	95	95	20	10	10	None	1
Amplification	95	95	20	0	0	None	50
	55	57	20	2	1	None	
	74	74	3	2	1	Single	
Melt	95	95	20	0	0	None	1
	50	50	20	0	0	None	
	95	95	0.2			Continuous	

TABLE 3. Effectiveness of initial antibiotic therapy for clinically significant bacteremias

Patient treatment history	No. (%) of patients with the following organism from positive blood cultures:			
	MSSA (n = 16)	MRSA (n = 23)	CoNS (n = 13)	Total (n = 52)
Initially on effective antibiotics	13 (82)	11 (48)	8 (62)	32 (62)
Commenced on effective antibiotics after initial notification of positive blood culture	0 (0)	2 (9)	2 (15)	4 (8)
Not on effective treatment until culture results known	2 (12)	9 (39)	3 (23)	14 (27)
Antibiotic therapy not known[a]	1 (6)	1 (4)	0 (0)	2 (4)

[a] Antibiotic treatment data were not available for two patients with *S. aureus* bacteremia.

assay such as the one described here may be useful in patients with known risk factors for *S. aureus* bacteremia (20). However, the clinical and economic benefits to the provision of this information remain to be elucidated. Direct detection of *S. aureus* from blood (7, 12) may provide an even earlier diagnosis, but such techniques require further evaluation.

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