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BRIEF REPORT

Rapid detection of methicillin-resistant staphylococci by real-time PCR directly from positive blood culture bottles

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Abstract For the rapid detection of methicillin-resistant staphylococci directly from blood cultures containing gram-positive cocci in clusters, we implemented a real-time (LightCycler) polymerase chain reaction (PCR) specific for the *Staphylococcus aureus nuc* gene encoding nuclease and the *mecA* gene encoding methicillin resistance. For the 475 positive blood cultures tested, the assay turned out to have 100% sensitivity and 100% specificity for the identification of methicillin-susceptible (n=108) and methicillin-resistant (n=34) *S. aureus*. When coagulase-negative staphylococci (CoNS) were included, the overall sensitivity for the detection of methicillin resistance was 93% and the specificity was 99%. Real-time PCR for *nuc* and *mecA* from blood culture bottles with staphylococci yields therefore a rapid (2–3 h) identification of *S. aureus* and CoNS including methicillin resistance.

Introduction

Staphylococci are the most frequently isolated bacteria from blood [1]. Since *Staphylococcus aureus* septicaemia is associated with a high mortality [2] and an increased length of hospital stay [3], timely detection and identification of *S. aureus* or coagulase-negative staphylococci (CoNS) including methicillin resistance from the patient's blood has great therapeutic, prognostic and economic significance.

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Vancomycin is the empiric drug of choice for the treatment of bacteraemia caused by *S. aureus* or CoNS [4]. The use of real-time polymerase chain reaction (PCR) for the detection of methicillin resistance directly from signal-positive blood cultures showing gram-positive cocci in clusters could help to reduce the level of empiric use of vancomycin by quick adaption of an appropriate antibiotic treatment. This is even more relevant since it has been reported that 6% of methicillin-resistant *S. aureus* (MRSA) blood culture isolates exhibited a heterogeneous intermediate resistance to vancomycin (hVISA) [5]. Moreover, vancomycin is considered to be inferior to penicillins for the treatment of endocarditis caused by methicillin-susceptible *S. aureus* (MSSA) [6].

In this study, we performed real-time PCRs for the *S. aureus*-specific *nuc* gene encoding nuclease and the *mecA* gene encoding methicillin resistance [7] directly from positive blood culture bottles. This method, which was originally implemented for the identification of MRSA from agar-grown cultures, allowed us to rapidly identify MRSA, MSSA and CoNS and represents therefore a powerful diagnostic approach for a fast identification of staphylococci from blood cultures.

Material and methods

To perform real-time PCRs for the *nuc* and the *mecA* genes directly from signal-positive blood culture bottles (BACTEC 9240, Becton Dickinson, Heidelberg, Germany), 8 ml blood were transferred into a BD Vacutainer SST II Advance tube (Becton Dickinson, Heidelberg, Germany) and centrifuged for 10 min at 2,000 rpm. The supernatant was removed and DNA was extracted by addition of 200 µl extraction buffer containing 1% (v/v) Triton X-100, 0.5% (v/v) Tween 20, 10 mM

Table 1 Results obtained by real-time PCR compared to standard microbiological methods (n=475). *PCR* polymerase chain reaction, *MRSA* methicillin-resistant *S. aureus*, *MSSA* methicillin-susceptible *S. aureus*, *MRCoNS* methicillin-resistant coagulase-negative staphylococci, *MSCoNS* methicillin-susceptible coagulase-negative staphylococci

	Genotype (real-time PCR)			
Phenotype	*nuc*+/ *mecA*+	*nuc*+/ *mecA*−	*nuc*−/ *mecA*+	*nuc*−/ *mecA*−
MRSA	34	–	–	–
MSSA	–	108	–	–
MRCoNS	–	–	189	18
MSCoNS	–	–	2	109
Other organisms[a]	–	–	–	15

[a] Nonstaphylococcal organisms: *Corynebacterium* species (n=2), *Enterococcus faecium* (n=2), *Lactobacillus* species (n=1), *Micrococcus luteus* (n=3), *Streptococcus mitis* (n=2), *Streptococcus mutans* (n=1) *Streptococcus oralis* (n=1), *Streptococcus salivarius* (n=1), *Peptoniphilus asaccharolyticus* (n=1) and *Propionibacterium* species (n=1)

Tris-HCl (pH 8) and 1 mM ethylenediaminetetraacetate (EDTA) (all from Sigma, Deisenhofen, Germany) as described [7]. After shaking the tube slightly, the extracted DNA (200 µl) was transferred into a 1.5-ml tube, heated for 10 min at 95°C and centrifuged at 1,600 *g* for 5 min. For the detection of *mecA* or *nuc*, 2 µl each of the supernatants were added directly into a LightCycler (Roche, Mannheim, Germany) to 18 µl amplification mixture containing the primers Mec-R and Mec-F (each 0.5 µM) and the hybridization probes Mec-HP-1 and Mec-HP-2 (each 0.2 µM) or the primers Sa442-R and Sa442-F (each 0.25 µM) and the hybridization probes Sa442-HP-1 and Sa442-HP-2 (each 0.2 µM; all from TibMolBiol, Berlin, Germany), 5 mM $MgCl_2$ and 2 µl of 10× LightCycler FastStart DNA Master Hybridization Probes mixture (Roche) in millipore-purified water as described with minor modifications [7]. *S. aureus* (ATCC 25923), MRSA (ATCC 33592) and *S. epidermidis* (ATCC 12228) were used as controls. A potential inhibition of real-time PCRs by blood culture ingredients was excluded for each single specimen by adding blood culture samples to control DNA (data not shown).

In parallel, standard microbiological identification and antibiotic susceptibility testing was performed. For this purpose, an aliquot of blood cultures from BACTEC Plus Aerobic/F or BACTEC Plus Anaerobic/F blood culture bottles (Becton Dickinson) was plated on brain heart agar (Becton Dickinson) and incubated for 18–48 h at 37°C in 5% CO_2, or, for anaerobic bottles, under anaerobic conditions. The staphylococcal isolates were identified by standard laboratory methods [e.g. colony morphology, catalase and DNase testing, mannitol fermentation, glucose fermentation, latex slide agglutination test (Staphytect Plus, Oxoid, Basingstoke, UK)] and by Vitek2 or by the API Staph System (bioMérieux, Nürtingen, Germany). Preliminary susceptibility testing was performed directly from positive blood cultures by agar disk diffusion and confirmed by Vitek2 or agar diffusion testing from brain heart agar-grown colonies following the Clinical and Laboratory Standards Institute (CLSI) guidelines [8–10].

Results and discussion

From August 2004 to March 2007, overall 475 signal-positive blood culture bottles with growth of gram-positive cocci in clusters detected by Gram staining were analysed by real-time PCRs for the presence of *mecA* gene encoding methicillin resistance and for the *S. aureus*-specific *nuc* gene. All 142 *S. aureus* isolates were correctly identified when compared with standard laboratory procedures. The identification "MRSA" was made for 34 isolates; the remaining 108 isolates were identified as MSSA (Table 1). Therefore, for all *S. aureus* isolates, the approach had 100% sensitivity and specificity and predictive values of 100% for positive or negative test results (Table 2). Our data are consistent with those of two other similar studies published recently [11, 12]. No *nuc* and *mecA* genes were amplified in the 15 nonstaphylococcal isolates (see Table 1). Our results confirm that "detection of gram-positive cocci in clusters" directly from blood cultures cannot necessarily be equated with "detection of staphylococci". Moreover, the results underline the high specificity of the *nuc* gene for the identification of staphylococci [7].

Table 2 Sensitivity, specificity and predictive values (in %) of positive and negative real-time PCRs for blood cultures with staphylococcal organisms detected by Gram stain (n=460)

	Sensitivity	Specificity	Predictive value of positive test result	Predictive value of negative test result
nuc in all samples (n=460)	100	100	100	100
mecA in *nuc*+ samples (n=142)	100	100	100	100
mecA in *nuc*- samples (n=318)	91	98	99	87
mecA in all samples (n=460)	93	99	99	91

However, the predictive value of a positive *mecA* real-time PCR indicating oxacillin resistance was a little lower (99%) when CoNS were included. It is important to notice that in 2 of the 318 CoNS isolates oxacillin resistance was only detectable by *mecA* real-time PCR although these strains were phenotypically susceptible to oxacillin. Such discrepant results might be due to heterogeneous resistance gene expression of *mecA* in CoNS as described earlier [13] underlining that the molecular detection of *mecA* represents the gold standard for oxacillin susceptibility testing.

Remarkably, 18 CoNS (all *S. epidermidis*) turned out to be phenotypically oxacillin resistant although these isolates were negative in *mecA* PCRs directly from blood cultures (predictive value: 87%). It is unlikely that a problem in DNA extraction is causative of this phenomenon since this should also have emerged in *S. aureus*-positive blood cultures, and PCR inhibition could also be excluded (see "Material and methods"). Possibly, only a small minority of *mecA*-positive CoNS were present in these samples and the sensitivity of the *mecA* PCR was simply too low (sensitivities of the herein described real-time PCRs $\sim 1 \times 10^5$ bacteria). A further possible explanation might be that oxacillin resistance of CoNS was caused by alterations in penicillin binding proteins (PBP) other than PBP2a (which is encoded by *mecA*). This phenomenon leads to elevated minimum inhibitory concentration (MIC) values (0.5–2.0 μg/ml) and was described to be responsible for phenotypical resistance of CoNS independently from *mecA* [14]. Another possible explanation might be the overexpression of beta-lactamases by these isolates resulting in a so-called borderline oxacillin resistance *S. aureus* phenotype [15]; unfortunately, these isolates were not tested for the BORSA phenotype.

A limitation of the correct detection of MRSA from positive blood cultures by real-time PCRs might be a simultaneous detection of *mecA* and *nuc* resulting from a mixture of methicillin-resistant CoNS and MSSA in blood culture bottles. However, although mixed blood cultures cannot be totally excluded, we never observed such a misleading result in any of the 475 blood cultures. For the future, methods detecting *mecA* coupled with a *S. aureus*-specific gene (*orfX*) in a single PCR reaction [16] might be implemented in our laboratory algorithm.

A variety of genotypic methods has been described for the rapid detection of MSSA, MRSA and CoNS. These methods include the use of nucleic acid amplification-based methods, conventional and peptide nucleic acid fluorescence in situ hybridization [12, 17–19]. The benefit of all these methods is the rapid result compared with conventional identification or susceptibility testing from positive blood cultures (requiring at least ~18 h). With the use of real-time PCR directly from blood cultures with gram-positive cocci in clusters, a rapid result is available after 2–3 h. Hence, at least for *mecA*-negative and *nuc*-positive blood cultures with *S. aureus*, empiric therapy could be timely adapted to isoxazolyl penicillins avoiding unnecessary expenditures for antibiotics such as linezolid or vancomycin.

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