Rapid differentiation of *Staphylococcus aureus*, *Staphylococcus epidermidis* and other coagulase-negative staphylococci and meticillin susceptibility testing directly from growth-positive blood cultures by multiplex real-time PCR

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This study evaluated a multiplex real-time PCR method specific for the *mecA*, *femA*-SA and *femA*-SE genes for rapid identification of *Staphylococcus aureus*, *Staphylococcus epidermidis* and non-*S. epidermidis* coagulase-negative staphylococci (CoNS), and meticillin susceptibility testing directly in positive blood cultures that grew Gram-positive cocci in clusters. A total of 100 positive blood cultures produced: 39 *S. aureus* [12 meticillin-resistant *S. aureus* (MRSA), 31% of all the *S. aureus*]; 30 *S. epidermidis* (56.6% of the CoNS), 8 *Staphylococcus capitis* (15.1%), 3 *Staphylococcus saprophyticus* (5.7%), 4 *Staphylococcus hominis* (7.5%), 3 *Staphylococcus haemolyticus* (5.7%), 2 *Staphylococcus warneri* (3.8%), 1 *Staphylococcus cohnii* (1.9%) and 2 unidentified *Staphylococcus* spp. (3.8%); and 1 *Micrococcus luteus* in pure culture. Two blood cultures had no growth on subculture and five blood cultures grew mixed CoNS. For the 95 blood cultures with pure growth or no growth on subculture, there was very good agreement between real-time PCR and the BD Phoenix identification system for staphylococcal species categorization in *S. aureus*, *S. epidermidis* and non-*S. epidermidis* CoNS and meticillin-resistance determination (Cohen’s unweighted kappa coefficient $k = 0.882$). All MRSA and meticillin-susceptible *S. aureus* were correctly identified by *mecA* amplification. PCR amplification of *mecA* was more sensitive for direct detection of meticillin-resistant CoNS in positive blood cultures than testing with the BD Phoenix system. There were no major errors when identifying staphylococcal isolates and their meticillin susceptibility within 2.5 h. Further studies are needed to evaluate the clinical benefit of using such a rapid test on the consumption of glycopeptide antibiotics and the alteration of empiric therapy in the situation of positive blood cultures growing staphylococci, and the respective clinical outcomes.

INTRODUCTION

Blood culture is a cornerstone investigation in a clinical microbiology laboratory. The most common isolates are staphylococci, namely *Staphylococcus aureus*, either meticillin-resistant *S. aureus* (MRSA) or meticillin-susceptible *S. aureus* (MSSA), and coagulase-negative staphylococci (CoNS) including *Staphylococcus epidermidis*. Turnaround times for positive results are important so that appropriate antimicrobial agents can be selected immediately, unnecessary treatment of likely contaminants and antibiotic exposure can be avoided, and expenditure on antimicrobials decreased. Timely detection, distinction of *S. aureus* from CoNS and meticillin-susceptibility results have great therapeutic, prognostic and economic significance (Beekmann et al., 2003; Cosgrove et al., 2003; Annane et al., 2005). Conventional identification and susceptibility...
testing of positive blood cultures can take up to a further 48 h from the preliminary Gram stain. Of blood cultures growing staphylococci, approximately 60–80% contain CoNS (Weinstein et al., 1997), and about 80% of these are S. epidermidis (Mack et al., 2006). CoNS may be contaminants arising from the skin, but are also now being recognized as important nosocomial pathogens, particularly with the ever-increasing use of prosthetic devices such as intravenous catheters (Weinstein et al., 1997).

Real-time PCR is significantly faster than conventional PCR and other detection methods. The combination of excellent sensitivity and specificity, low contamination risk, ease of performance and speed has made real-time PCR technology appealing to the clinical microbiology laboratory (Espy et al., 2006). Studies targeting the nuc, mecA and tuf genes and the sa442 DNA fragment, in simplex, duplex and multiplex situations, have reported promising sensitivities and specificities directly from positive blood cultures (Tan et al., 2001; Palomares et al., 2003; Sakai et al., 2004; Adams, 2005; Paule et al., 2005; Größer & Kempf, 2007; Thomas et al., 2007; Hogg et al., 2008; Kilic et al., 2010). However, most of these studies only identified S. aureus and its meticillin susceptibility.

The aim of this study was to develop a protocol that could differentiate the majority of blood culture staphylococcal isolates into S. aureus, S. epidermidis and other CoNS, and determine their meticillin susceptibility, within hours, by targeting the mecA gene, femA-SA gene (specific for S. aureus) and the femA-SE gene (specific for S. epidermidis), using a multiplex real-time PCR assay directly from growth-positive blood culture bottles using specific fluorescently labelled probes (Francois et al., 2003). One hundred growth-positive blood cultures containing Gram-positive cocci in clusters were investigated by real-time PCR and compared with the results of testing with the BD Phoenix (Becton Dickinson) automated identification system and susceptibility test results.

**METHODS**

**Study setting and processing of positive blood cultures.** Public Health Wales Microbiology ABM Swansea laboratory serves a population of approximately 350,000 including three major acute hospitals of the Abertawe-Bro Morgannwg University Health Board (~1570 beds) with multiple secondary and tertiary services including haematology, with a regional bone-marrow transplant centre, neonatal intensive care unit subregional centre, cardiac surgery and the Welsh Centre for Burns and Plastic Surgery, making evaluation of positive blood cultures that grow CoNS a challenge. One hundred blood cultures positive for Gram-positive cocci in clusters were selected at random for study during the 4 months from February to May 2008. Blood culture vials were incubated in an automated fluorescent series BACTEC 9240 instrument for up to 5 days. Blood from positive vials was Gram stained and those cultures containing Gram-positive cocci in clusters were inoculated onto Columbia blood agar with 5% horse blood and processed further according to standard microbiological procedures. A 0.5 ml aliquot of the samples was taken from the blood culture vials for PCR testing.

**RESULTS**

**Identification and susceptibility testing of staphylococcal isolates by real-time PCR compared with culture**

Of the 100 positive blood cultures with Gram-positive cocci in clusters, 39 contained S. aureus. A total of 53 contained pure growth of CoNS identified by BD Phoenix as the following species: 30 S. epidermidis (56.6% of the CoNS), 6 Staphylococcus capitis (11.3%), 2 S. capitis subsp. urealyticus (3.8%), 3 Staphylococcus saprophyticus (5.7%), 4 Staphylococcus hominis (7.5%), 3 Staphylococcus haemolyticus (5.7%), 2 Staphylococcus warneri (3.8%), 1

**Identification and susceptibility test results.** Staphylococcal colonies were identified using a Prolex Staph latex kit agglutination reaction (Pro-Lab), the presence or absence of DNase enzyme was determined on DNase agar, and the susceptibility of the isolate to oxacillin was interpreted based on British Society for Antimicrobial Chemotherapy guidelines. Isolates were finally tested using the BD Phoenix automated identification and susceptibility testing system panels for Gram-positive isolates (BD Phoenix PMIC/ID-63; Becton Dickinson), following standard operating procedures, and real-time PCR results were compared with the BD Phoenix identification and susceptibility results as the reference.

**Bacterial lysis and DNA extraction directly from positive blood cultures.** DNA was extracted from positive blood culture supernatants as described by Louie et al. (2002). Briefly, 1 ml sterile distilled water was added to a 100 μl aliquot of each sample, mixed by inversion and incubated at room temperature for 5 min. After centrifugation at 16 000 g for 1 min, the supernatant was discarded and the pellet resuspended in 100 μl lysis buffer [20 mM Tris/HCl (pH 8.0), 2 mM EDTA, 1.2% (w/v) Triton X-100, Lysostaphin (5 μg, 1 mg ml⁻¹) was added, and the sample was mixed and incubated at 37 °C for 10 min. This suspension was then boiled for 10 min. After cooling to room temperature for 5 min, the sample was centrifuged at 16 000 g for 1 min.

**PCR amplification and analysis of results.** Real-time PCR was performed with primers exactly as described by Francois et al. (2003) and TaqMan probes specific for mecA (5′-TGGAGATTAGATTGGATCATGCGTCA-BHQ1-3′), femA-SA (5′-ROX-TCAT-TTACGCGAAGCTTGGCAGCTAT-BHQ2-3′) and femA-SE (5′-FAM-TACTACGCTGGTGGAACTTCAAATCGTTATCG-BHQ1-3′) (Eurogentec) with fluorophores and quenchers as required for the detection of S. aureus, femA-SA and femA-SE, respectively. DNA was extracted from positive blood culture supernatants as described by Louie et al. (2002). Briefly, 1 ml sterile distilled water was added to a 100 μl aliquot of each sample, mixed by inversion and incubated at room temperature for 5 min. After centrifugation at 16 000 g for 1 min, the supernatant was discarded and the pellet resuspended in 100 μl lysis buffer [20 mM Tris/HCl (pH 8.0), 2 mM EDTA, 1.2% (w/v) Triton X-100, Lysostaphin (5 μg, 1 mg ml⁻¹) was added, and the sample was mixed and incubated at 37 °C for 10 min. This suspension was then boiled for 10 min. After cooling to room temperature for 5 min, the sample was centrifuged at 16 000 g for 1 min.

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Staphylococcus cohnii (1.9%) and 2 Staphylococcus spp. (unidentified) (3.8%). The latter two strains were identified as S. haemolyticus and S. capitis using Bruker Daltonik MALDI Biotyper matrix-assisted laser desorption ionization—time of flight (MALDI-TOF) MS (Harris et al., 2010). Five cultures contained mixed CoNS (three with S. haemolyticus/S. epidermidis, one with Staphylococcus schleiferi subsp. coagulans/Staphylococcus equorum and one with S. hominis/S. epidermidis). One culture grew Micrococcus luteus and two cultures yielded no growth on subculture.

Real-time PCR identification (femA-SA probe positive) correlated with the BD Phoenix method for all MSSA and MRSA isolates. Thirty out of thirty-two blood cultures containing S. epidermidis by real-time PCR (femA-SE probe positive) were confirmed to grow S. epidermidis by BD Phoenix, whilst the remaining two were identified as non-S. epidermidis CoNS. Retesting of DNA extracted from the latter two cultured isolates confirmed the BD Phoenix identification, as both isolates were femA-SE negative on retesting. The most likely explanation is the presence of S. epidermidis DNA in the original extracts (mixed blood culture, but undetected as such by culture). A total of 21 out of 24 blood cultures containing non-S. epidermidis CoNS as determined by real-time PCR (both femA-SE and femA-SA negative) were confirmed to grow non-S. epidermidis CoNS with the BD Phoenix system, whilst one isolate was identified as M. luteus and two cultures had no growth on subculture, indicating reliable categorization of CoNS into S. epidermidis and non-S. epidermidis CoNS by multiplex real-time PCR (Table 1). For the 95 blood cultures with pure growth or no growth on subculture, there was very good agreement between real-time PCR and BD Phoenix for staphylococcal species categorization into S. aureus, S. epidermidis and non-S. epidermidis CoNS (Cohen’s unweighted kappa coefficient $\kappa=0.9207$).

Real-time PCR correctly identified all 12 positive blood cultures that grew MRSA (31% of all S. aureus) and all 27 MSSA as mecA positive and mecA negative, respectively. A total of 34 and 15 cultures with CoNS had positive and negative mecA amplification results, respectively, which were in agreement with the meticillin susceptibility results as detected by BD Phoenix. Four isolates of CoNS were judged to be meticillin resistant by PCR but susceptible by BD Phoenix (Table 1). As the M. luteus isolate was negative for all three target genes, the real-time PCR result was interpreted as a meticillin-susceptible CoNS. The BD Phoenix system does not give antimicrobial susceptibility results on Micrococcus species. The two positive blood cultures showing Gram-positive cocci in clusters that were subsequently negative on subculture were also negative for all three target genes, which was interpreted as meticillin-sensitive CoNS other than S. epidermidis.

Taken together for the 95 blood cultures with pure growth or no growth on subculture, there was very good agreement between the real-time PCR method and BD Phoenix with respect to staphylococcal species categorization and meticillin-resistance determination (Table 1; Cohen’s unweighted kappa coefficient $\kappa=0.882$).

When more than one target gene was amplified, usually mecA and femA-SA or femA-SE, threshold cycle ($C_T$) values were within a range of three cycles in most cases of MRSA.

### Table 1. Comparison of results by real-time PCR with the BD Phoenix reference

<table>
<thead>
<tr>
<th>BD Phoenix identification and susceptibility result</th>
<th>MSSA</th>
<th>MRSA</th>
<th>MSSE</th>
<th>MRSE</th>
<th>MS-CoNS</th>
<th>MR-CoNS</th>
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<tbody>
<tr>
<td>MSSA</td>
<td>27</td>
<td>–</td>
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<td>22</td>
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<tr>
<td>MS-CoNS</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>1</td>
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<tr>
<td>MR-CoNS</td>
<td>–</td>
<td>–</td>
<td>2*</td>
<td>–</td>
<td>10</td>
<td>–</td>
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<tr>
<td>Other</td>
<td>–</td>
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MS-CoNS, Meticillin-susceptible non-S. epidermidis CoNS; MR-CoNS, meticillin-resistant non-S. epidermidis CoNS.

*Two blood cultures contained MRSE according to the real-time PCR result, whilst BD Phoenix identification revealed meticillin-resistant S. haemolyticus and S. warneri, respectively. PCR testing using DNA from the cultured bacteria showed positive mecA but negative femA-SE results, indicating the presence of very low amounts of S. epidermidis DNA in the original blood culture extract, rather than non-specific amplification of the femA gene of another staphylococcal species or inefficient hybridization with the femA-SE probe.

†A blood culture growing M. luteus had negative amplification results for all three targets and was interpreted as ‘MS-CoNS’ by real-time PCR.

‡Two blood cultures were negative on subculture. Amplification results were negative for all three targets and were interpreted as ‘MS-CoNS’ by real-time PCR.
or meticillin-resistant CoNS. In six cases with $\Delta C_t = 3$ cycles (range 3.63–13.08 cycles), the results for five of these correlated with the BD Phoenix results (two MRSA, three MRSE) despite the discrepancy in $C_t$ values, whilst one case identified as MRSE ($\Delta C_t = 6.28$ cycles) by the multiplex real-time PCR test was identified as *S. haemolyticus* by BD Phoenix. Retesting of DNA from the cultured isolate gave a positive result for *mecA* and negative amplification of *femA*-SA as stated above, indicating the presence of very low levels of *S. epidermidis* DNA in the original blood culture extract, rather than non-specific amplification of the *femA* gene of another staphylococcal species or inefficient hybridization with the *femA*-SA probe. Nevertheless, discrepancies in $C_t$ of $>3$ cycles between *mecA* and *femA*-SA or *femA*-SE should lead to further testing and more cautious interpretation and reporting pending the full results.

**Mixed cultures**

Among the four mixed cultures containing *S. epidermidis* strains, three had a *femA*-SE-positive amplification result, whilst the *S. epidermidis* of the fourth mixed culture (as identified by BD Phoenix) was identified as *S. hominis* by the MALDI Biotyper and was *femA*-SE negative on retesting. The *S. schleiferi/S. equorum* mixed culture was *femA*-SE negative. Four of the five mixed blood cultures had positive *mecA* amplification results. All six CoNS isolates from three mixed blood cultures with meticillin-resistance multiplex PCR results were *mecA* negative when testing the cultured isolates, indicating that DNA from additional uncultured strains was present initially in these, most probably contaminated, blood cultures. Unlike culture-based methods, real-time PCR would be unable to indicate a mixed culture. For all cultures including those with mixed CoNS, there was very good agreement between the real-time PCR method and BD Phoenix with respect to staphylococcal species categorization and meticillin resistance determination (Cohen’s unweighted kappa coefficient $\kappa = 0.823$).

**DISCUSSION**

More rapid methods for species identification and susceptibility testing directly from blood cultures are urgently sought. PCR amplification directly from positive blood cultures targeting the *mecA*, *nuc* and *tuf* genes, and the sa442 DNA fragment, in simplex, duplex and multiplex situations, have reported promising sensitivities and specificities. However, they predominantly address only identification of *S. aureus* and its meticillin susceptibility (Tan et al., 2001; Palomares et al., 2003; Sakai et al., 2004; Adams, 2005; Paule et al., 2005; Gröbner & Kempf, 2007; Thomas et al., 2007; Hogg et al., 2008; Kilic et al., 2010). Other approaches include fluorescence in situ hybridization using peptide nucleic acid probes (Ly et al., 2008; Poppert et al., 2010) and MALDI-TOF MS (La Scola & Raoul, 2009; Christner et al., 2010; Stevenson et al., 2010). However, these are limited in that, although rapid and able to distinguish *S. aureus* from CoNS, they cannot identify the presence of meticillin resistance.

Using a multiplex real-time PCR assay detecting the *mecA*, *femA*-SA and *femA*-SE genes, the major staphylococcal species *S. aureus*, *S. epidermidis* and non-*S. epidermidis* CoNS, and meticillin susceptibility, could be ascertained within 2.5 h with very good agreement with BD Phoenix identification and susceptibility results. A recently described alternative method only discriminates *S. aureus* from CoNS, together with testing for the presence of *mecA* (Kilic et al., 2010). The hands-on time for the extraction process was 30 min, with a 95 min PCR run time. Thus, even within the constraints of the routine working hours of a clinical diagnostic laboratory, it would be possible to report all results of blood culture samples that were positive in the morning by noon, and all results from blood cultures that were positive by approximately 2 p.m. before 5 p.m. This has potential significance for patient management and clinical decision-making, as it was found in previous studies that the Gram-stain result of positive blood cultures has higher impact for patient management than the full identification and susceptibility result (Munson et al., 2003), which is available only 24–48 h later. With the real-time PCR assay as described here, the Gram-stain result of almost 50% of positive blood cultures could be rapidly complemented with the clinically significant differentiation between *S. aureus*, *S. epidermidis* and other non-*S. epidermidis* CoNS, and their respective meticillin susceptibilities (Weinstein et al., 1997; Munson et al., 2003).

A variety of methods has been described for the extraction of DNA from positive blood cultures containing staphylococci (Tan et al., 2001; Palomares et al., 2003; Sakai et al., 2004; Adams, 2005; Paule et al., 2005; Gröbner & Kempf, 2007; Thomas et al., 2007; Hogg et al., 2008). Our study used centrifugation and lysis buffer with lysostaphin (Louie et al., 2002), which was found to be an easy and reliable method for extracting DNA from BACTEC Standard/10 Aerobic/F, BACTEC Plus Aerobic/F*, BACTEC Peds Plus/ F and BACTEC Lytic/10 Anaerobic/F culture vials (data not shown). The method described by Adams (2005) was not able, in our hands, to overcome the PCR inhibition by the blood culture medium of the BACTEC system. Notably, Adams (2005) used the BacT/ALERT blood culture system. Lysostaphin was used to aid lysis in our study, thus releasing more DNA from the bacteria than simply boiling, allowing dilution to overcome the inhibition problems (Louie et al., 2002; Adams, 2005), underlining the fact that methodology is not necessarily transferable between systems. However, use of a triplex real-time PCR assay targeting *nuc*, *tuf* and *mecA* was very reliable with BACTEC blood culture bottles, where 10 μl blood culture fluid was diluted in 990 μl water and 1 μl of this was used directly as a template in 25 μl reactions (Kilic et al., 2010). Bearing these problems in mind, further
development of our method could include the *tuf* gene as described by Kilic *et al.* (2010) using the fourth channel of the Rotor-Gene 6000, which would add definite identification of a staphylococcal gene in each assay.

In this study, *mecA* was detected in four positive blood cultures where isolates were phenotypically sensitive to oxacillin by BD Phoenix [three *S. epidermidis* with MICs of ≤1 μg ml⁻¹ (two isolates) and 2 μg ml⁻¹ (one isolate); one *S. hominis* isolate with an MIC of 2 μg ml⁻¹] (BSAC, 2009). Meticillin susceptibility testing in CoNS is notoriously difficult, such that the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) makes special species-specific recommendations for *Staphylococcus lugdunensis* and re-emphasizes additional testing for non-*S. epidermidis* CoNS with an oxacillin MIC of 0.5–2 μg ml⁻¹ (Tenover *et al.*, 1999; NCCLS, 2003; Horstkotte *et al.*, 2004; CLSI, 2010). The existence of oxacillin-resistant strains that are not detected by PCR would be more troublesome, because these cases are more likely to result in treatment failure. Our study did not identify any staphylococcal isolates that were *mecA* negative by real-time PCR but meticillin resistant on subculture.

Mixed cultures of CoNS decreased the reliability of the rapid test, and an inability to detect mixed cultures is a limitation of the real-time PCR method in general. A unique feature of our method is that, by including primers to the *femA-SE* gene, it would be possible to detect a mixed culture with *S. aureus* and *S. epidermidis*. Whilst this approach helps alert the microbiologist to the possibility of a mixture of MSSA and MRSE, there remains the potential pitfall of reporting MRSA in a case of a mixed culture containing MSSA and meticillin-resistant non-*S. epidermidis* CoNS. No such case was observed in our or another study (Kilic *et al.*, 2010), and this possibility would not result in a therapeutic recommendation that would put a patient in danger, but would lead to the unnecessary use of glycopeptide antibiotic in rare cases. This drawback, however, is surely balanced by the prospect of being able to give definite same-day recommendations for therapy of *S. aureus* bloodstream infections, and to evaluate blood cultures positive for CoNS with confidence in susceptibility testing results and CoNS classification in *S. epidermidis* and non-*S. epidermidis* CoNS.

To conclude, our study used a simple and reliable method for DNA extraction with a multiplex real-time PCR assay for the rapid identification of *S. aureus*, *S. epidermidis* and non-*S. epidermidis* CoNS and meticillin susceptibility testing from growth-positive blood cultures containing Gram-positive cocci. There were no major errors when identifying staphylococcal isolates and their meticillin susceptibility within 2.5 h. The future aim is to incorporate this multiplex real-time PCR assay alongside conventional culture methods in the clinical microbiology laboratory for the reporting of growth-positive blood cultures containing Gram-positive cocci. Further studies should then evaluate the clinical benefit of using such a rapid test on consumption of glycopeptide antibiotics and alteration of empiric therapy in the situation of positive blood cultures growing staphylococci and the respective clinical outcome.

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REFERENCES


Hogg, G. M., McKenna, J. P. & Ong, G. (2008). Rapid detection of meticillin-susceptible and meticillin-resistant *Staphylococcus aureus* directly from positive Bact/Alert blood culture bottles using real-time


