PCR for the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains using a single primer pair specific for SCC*mec* elements and the neighbouring chromosome-borne *orfX*

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ABSTRACT

The chromosomal location of the SCC*mec* elements containing *mecA* allows the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains by PCR amplification of a sequence covering the right junction of the SCC*mec* elements and the adjacent chromosomal region encoding the species-specific ORFX. MRSA strains can be identified specifically using one forward primer, with only one or two mismatches, targeting the SCC*mec* elements of different types, and one reverse primer targeting the *orfX* region.

Keywords Methicillin-resistant *Staphylococcus aureus*, MRSA, *orfX*, PCR, typing, SCC*mec*

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The emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA), both as a nosocomial pathogen and, more recently, in the community, are matters of concern worldwide. *S. aureus* acquires methicillin resistance by insertion into the chromosome of a mobile genetic element, designated staphylococcal cassette chromosome (SCC*mec*), carrying the *mecA* gene. This gene encodes an additional penicillin-binding protein, PBP2a, which is not inhibited by existing β-lactam antibiotics [1]. At least five different types of SCC*mec* elements have been described, characterised by different classes of site-specific recombinase genes (*ccr* complex), the *mec* gene complex, insertion sequences and, in two cases, transposon Tn*554* [2,3]. SCC*mec* elements are flanked by incomplete inverted repeats, and are integrated at a specific site (*attB*SCC) in the *S. aureus* chromosome, which is located at the 3′-end of *orfX* [4].

PCR-based detection of MRSA has mostly been based on individual amplification of an *S. aureus*-specific gene and *mecA*, as described recently for a duplex real-time PCR with *mecA*- and *nuc*-specific primers [5]. This approach is not immediately applicable to the detection of MRSA from non-sterile clinical specimens, which often contain both *S. aureus* and methicillin-resistant coagulase-negative staphylococci (CNS), thereby potentially yielding false-positive results. An alternative approach involves the selective immunomagnetic enrichment of *S. aureus* and a subsequent multiplex PCR [6]. A further possibility is the amplification of a sequence covering both SCC*mec* and the *S. aureus*-specific *orfX* region. Because of the sequence differences at the right extremity of the SCC*mec* elements, the assay described by Huletsky *et al.* [7] uses five different primers to target SCC*mec* types I–V and one primer for *orfX*. However, the emergence of new SCC*mec* types, especially among community-acquired MRSA [8], may require the use of additional primers.

Following comparison of sequences at the right junction of SCC*mec* and *orfX*, a 16-bp region of relatively high homology was found, with only 1 or 2 bp differences between the SCC*mec* elements of types I–V (Table 1). The present study describes the use of a PCR assay, with this sequence as the forward primer and a highly conserved sequence in *orfX* as the reverse primer, to identify MRSA containing different types of SCC*mec* elements. The primer sequences used in this assay were: rjmec (forward), 5′-TATGATATGCTTCTCC (positions 57641–57656, accession number D86934); and ORFX1r (reverse), 5′-AACGTTTAGGCCCATACACCA (58042–58022, D86934).

The *S. aureus* reference strain used was NCTC 8325. Reference strains of MRSA containing SCC*mec* elements of types I–V were NCTC 10442 (I), N315 (II), 85/2082 (III), 1155/93 (IV) and WIS (V). The reference strain for *S. aureus* containing SCC*mec cap1* was strain M. Reference strains of CNS were: *S. auricularis*, ATCC33753; *S. capitis*, CCM2734; *S. capitis* subsp. *ureolyticus*, ATCC49326; *S. caprae*, CCM3573; *S. carnosus*,

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Table 1. DNA sequences at the right junction of different types of SCC*mec* elements

SCC*mec* type	Reference strain	Sequence	Position; accession number[a]
I	NCTC 10442	5′-TATGATAAGCTTCTCC	38855–38870; AB033763
II	N315	5′-TATGATATGCTTCTCC	57651–57656; D86934
III	85/2082	5′-TATGATACGCTTCTCC	29582–29597; AB047089
IVa	CA05	5′-TATGATATGCTTCTCC	25210–25225; AB063172
Ivb	JCSC1978	5′-TATGATATGCTTCTCC	21207–21222; AB063173
Ivc	MR108	5′-TATGATATGCTTCTCC	30555–30570; AB096217
V	WIS	5′-TATGATACGCCTCTCC	892–877; AB121219

[a]Accession numbers: AB033763, *S. aureus* DNA, type I SCC*mec*; D86934, *S. aureus* DNA, resistance island type II SCC*mec*; AB047089, *S. aureus* DNA, right extremity of type III SCC*mec*; AB063172, *S. aureus* DNA, type IV SCC*mec*, strain CA05; AB063173, *S. aureus* DNA, type IV SCC*mec*, strain JCSC1978; AB09621, *S. aureus* DNA, type IVc SCC*mec*, strain MR108; AB121219, *S. aureus* DNA, type V SCC*mec*, strain JCSC3624.

DSM20501; *S. chromogenes*, CCM3387; *S. cohnii cohnii*, CCM2736; *S. cohnii ureolyticus*, ATCC49330; *S. delphini*, DSM20771; *S. epidermidis*, CCM2124; *S. equorum*, DSM20674; *S. felis*, ATCC49168; *S. gallinarum*, CCM3572; *S. haemolyticus*, CCM2737; *S. hominis*, DSM20328; *S. hyicus*, CCM2368; *S. intermedius*, CCM5739; *S. kloosii*, DSM20676; *S. lugdunensis*, ATCC43809; *S. muscae*, CCM4175; *S. saprophyticus*, CCM883; *S. schleiferi coagulans*, GA211; *S. schleiferi schleiferi*, ATCC43808; *S. sciurii*, ATCC29062; *S. simulans*, CCM2705; *S. warneri*, CCM2370; *S. xylosus*, CCM2738; *S. caseolyticus*, ATCC13548; *S. lentus*, CCM2598; and *S. arlettae*, DSM20672.

The clinical isolates of methicillin-susceptible *S. aureus* (MSSA) and MRSA investigated were sent from representative regions in Germany to the authors' laboratory, which is the national reference centre for typing of staphylococci. The MRSA isolates were characterised by multilocus sequence typing according to Enright *et al.* [9] and by SCC*mec* typing using PCR according to Okuma *et al.* [10]. Subtypes of type IV (a–d) were determined as described by Huletzky *et al.* [7]. The clinical isolates of different species of CNS (n = 80) originated from blood cultures of patients showing symptoms of intravenous catheter-related septicaemia.

Total cellular DNA was prepared from staphylococci grown overnight on blood agar plates and suspended in NaCl 0.9% w/v to a density corresponding to a 0.5 × McFarland standard. After treatment with lysostaphin (200 μg/mL), DNA was prepared using the DNeasy tissue kit (Quiagen, Hilden, Germany) according to the manufacturer's instructions. Approximately 10 ng of DNA in a volume of 0.5 μL was added to a 25-μL PCR mixture containing 2.5 pmol of each primer and 200 μM each dNTP in 10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 500 mM KCl. The reaction was started by the addition of 1.3 U of r*Taq* DNA polymerase (Amersham Pharmacia Biotech, Freiburg, Germany). The thermal cycling protocol (MJ Research PTC-200 thermocycler; Biozym, Markrodendorf, Germany) comprised 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 4 min. The PCR products were visualised following agarose gel electrophoresis.

With the use of primers rjmec and ORFX1r, PCR products of *c.* 400 bp were obtained from the reference strains containing SCC*mec* elements of types I, II, III, IV and V, and from all clinical MRSA isolates investigated. A representative set of PCR products is shown in Fig. 1. The results

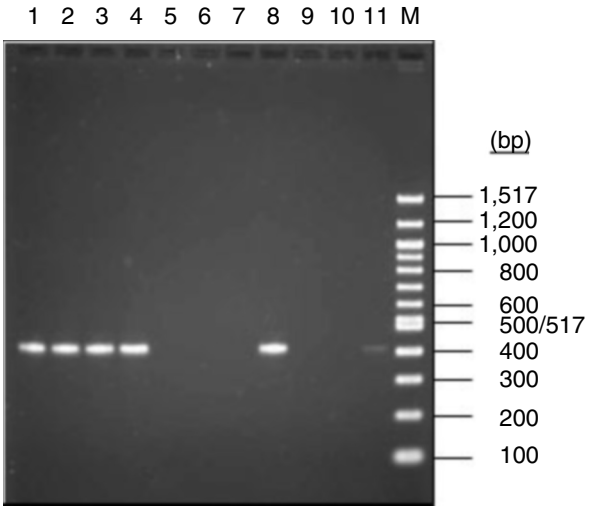

Fig. 1. PCR products obtained with the rjmec–ORFX1r primer set. Lanes: 1, NCTC 10442 (I); 2, N315 (II); 3, 85/2082 (III); 4, 1155/93 (IV); 5, methicillin-susceptible *Staphylococcus aureus* (MSSA), NCTC 8325; 6, MSSA, 2639/04; 7, MSSA, 1567/02; 8, MSSA, *S. aureus* M (SCC*cap1*); 9, methicillin-resistant *Staphylococcus epidermidis* (MRSE), *S. epidermidis* 208; 10, methicillin-susceptible *Staphylococcus epidermidis* (MSSE), *S. epidermidis* CCM2124; 11, *S. delphinii* DSM20771; M, molecular size standard. SCC*mec* types are given in parentheses.

Table 2. PCR results obtained with the rjmec–ORFX1r primer set for clinical isolates of MRSA, MSSA and coagulase-negative methicilllin-susceptible or -resistant staphylococci

Species	MLST type	SCC*mec* type	Number of isolates	PCR result
MRSA	ST254	IVc	4	+
	ST247	I	10	+
	ST228	I	10	+
	ST05	II	10	+
	ST22	IVc	10	+
	ST22	IVa	5	+
	ST239	III	5	+
	ST45	IVa	10	+
	ST30	IVa	10	+
	ST80	IV	10	+
	ST01	IVa	1	+
	ST08	IVa	6	+
	ST07, ST30, ST154	Not identified	4	+
	ST254	IVd	5	+
MSSA	ND[a]	–	100	–
S. epidermidis				
MRSE	ND	ND	50	–
MSSE	ND	ND	30	–
S. haemolyticus mecA$^+$	ND	ND	30	–
S. hominis mecA$^+$	ND	ND	10	–
S. hominis mecA$^-$	ND	ND	10	–

[a] Different *Sma*I macrorestriction patterns.
MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; MRSE, methicillin-resistant *S. epidermidis*; MSSE, methicillin-susceptible *S. epidermidis*; ND, not determined.

obtained with DNA from clinical MRSA and MSSA isolates, as well as CNS, are shown in Table 2. MRSA strains of MLST types ST05, ST22, ST45, ST228, ST254 and ST247 are the major epidemic clones in Central Europe. MRSA strains of types ST01, 30 and 80 are community-acquired MRSA strains that have emerged recently [8]. The PCR approach also allowed the detection of MRSA strains with as yet unidentified SCC*mec* elements (Table 2) that yielded negative results with PCRs targeting the known *ccr* complexes [3,7] and sequences of types IVa, IVb, IVc, IVd and V. The PCR produced no amplification product for 100 clinical MSSA isolates of different origins with different *Sma*I macrorestriction patterns. The PCR was also negative with the reference strains of CNS, with the exception of the *S. delphinii* reference strain, which gave a weak positive reaction (Fig. 1); however, this PCR product did not hybridise with a *mecA*-specific probe (data not shown). Furthermore, the PCR was negative with 130 clinical isolates of CNS, 90 of which contained *mecA*.

Detection systems for MRSA that are based on reverse blotting and use an MRSA-specific capture probe require smaller amplimers. This can be achieved by using primer ORFX2r (5′-ATGACATTCCCACATCAAATC; positions 38942–38922; accession number AB033767), which yields an 89-bp amplimer.

The advantage of the PCR test described in this study is the use of only one primer set for the detection of MRSA belonging to a variety of different SCC*mec* types. However, the specificity of this method may be an issue for strains of *S. aureus* containing a staphylococcal cassette chromosome lacking *mecA*, as described for SCC*cap1*. This element harbours the *cap1* genes responsible for production of a type 1 capsular polysaccharide [11]. As expected, the PCR was positive for *S. aureus* strain M harbouring *SCCcap1* (Fig. 1). However, *S. aureus* strains of capsular type 1 are rare, with the most frequent being types 5 and 8 [12]. None of the 100 MSSA isolates of different origins and with different *Sma*I macrorestriction patterns were PCR-positive, yielding the *c.* 400 bp product. Other SCC elements have been described for CNS [13]. However, this will not interfere with the PCR approach described here, since these species differ from *S. aureus* in the chromosomal sequences next to the 3′ junction of the cassette.

Rapid detection of MRSA directly from clinical specimens and nasal swabs is important for timely and appropriate treatment and infection control. In particular, screening of patients for MRSA upon hospital admission must produce quick results in order to be effective [14]. The possible use of the PCR assay described here for the direct detection of MRSA in clinical samples is currently being investigated.

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RESEARCH NOTE

Change in bacterial aetiology of peritoneal dialysis-related peritonitis over 10 years: experience from a centre in south-east Asia

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ABSTRACT

This study reviewed 1787 episodes of peritoneal dialysis (PD)-related peritonitis in 544 patients between 1994 and 2003. The overall rate of peritonitis was 0.68 episodes/year of PD, but decreased from 1.10 to 0.46 episodes/year between 1994 and 2003. The incidence of peritonitis caused by coagulase-negative staphylococci declined between 1994 and 1998 from 0.21 to 0.06 episodes/year of PD, coinciding with a reduction in the use of spike PD sets. There was a 60.1% response rate to antibiotics throughout the period, but the percentage of cases that required modification of the initial empirical antibiotic regimen rose from 13.6% to 58.7%, indicating that treatment should be individualised.

Keywords Coagulase-negative staphylococci, dialysis, infection, peritoneal dialysis, peritonitis, renal failure

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Peritonitis is the most important cause of treatment failure in peritoneal dialysis (PD) patients [1]. Peritonitis was common following the initial development of PD, but the incidence has decreased markedly during the past 15 years, probably as a result of improvements in connection technology [2,3]. Peritonitis rates nowadays are less than one episode/20 patient-months in most series [4,5]. The present study describes the change in distribution of causative organisms in PD-related peritonitis during a 10-year period in a single centre in Hong Kong.

All episodes of PD-related peritonitis between 1994 and 2003 in the renal unit of the Prince of Wales Hospital, Hong Kong, were reviewed. In total, 1787 episodes of peritonitis in 544 patients were identified. Data completeness was assured by the computerised Renal Registry. A diagnosis of peritonitis was based on at least two of the following criteria [6]: (1) abdominal pain or cloudy peritoneal dialysis effluent (PDE); (2) leukocytosis in PDE (white blood cells >100/mL); and (3) positive Gram-stain or culture from PDE. Episodes with peritoneal eosinophilia but with a negative bacterial culture were excluded. Bacterial culture of PDE was performed