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RESEARCH NOTE

A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCC*mec* types I–V

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ABSTRACT

A multiplex PCR with four primer-pairs was designed to identify the five main known SCC*mec* types. A clear and easily discriminated band pattern was obtained for all five types. The SCC*mec* type was identified for 98% of 312 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). SCC*mec* type IV was by far the most common SCC*mec* type among both hospital- and community-acquired MRSA isolates in Denmark.

Keywords Identification, molecular, MRSA, multiplex PCR, SCC*mec* type, *Staphylococcus aureus*

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The worldwide increase in the number of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has emphasised the need for fast and reliable identification and typing methods. In addition to genotyping, characterisation of the staphylococcal cassette chromosome (SCC) *mec* type has led to better discrimination of hospital-acquired MRSA (HA-MRSA) and community-onset MRSA (CO-MRSA) [1–5]. The multiplex PCR for discrimination of SCC*mec* types I–IV developed by Oliveira and de Lencastre [6] has been used widely, but was designed primarily to characterise HA-MRSA carrying SCC*mec* types I–III. Currently, an increase in CO-MRSA is occurring worldwide, caused mainly by CO-MRSA belonging to the same multilocus sequence type lineage as HA-MRSA and carrying the smaller SCC*mec* types IV or V [2–4,7]. In order to discriminate the classic (HA-)MRSA with SCC*mec* types I–III from (CO-)MRSA with SCC*mec* types IV and V, the present study developed a new multiplex PCR for use in routine SCC*mec* type determinations.

In total, 312 clinical isolates of MRSA from hospitalised patients or patients attending general practitioners during 2003–2005 (one isolate per patient) were used to test the multiplex PCR. The isolates were all *mecA*-positive by PCR [6] (results not shown). Four primer sets were designed to ensure amplification of two DNA targets from SCC*mec* type IV and two targets from SCC*mec* type V. The targets were chosen so that one target would be amplified from each of SCC*mec* types I–III (Table 1). To validate the multiplex PCR method, 50 isolates were also amplified using the multiplex PCR of Oliveira and de Lencastre [6]. SCC*mec* type I isolates were also retested by *ccrA1* PCR [8], and SCC*mec* type II and III isolates were retested by *mecI* PCR [6]. PCR was performed in a total volume of 50 μL containing 1 × Ampli*Taq* PCR buffer, 1.5 mM $MgCl_2$, 200 μM each dNTP and 1 U of Ampli*Taq* DNA polymerase. Based on optimisation experiments, primer concentrations were as follows: primers β and α3, 0.2 μM each; ccrCF and ccrCR, 0.25 μM each; 1272F1 and 1272R1, 0.08 μM each; and 5RmecA and 5R431, 0.1 μM each. Amplification comprised 4 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C and 60 s at 72°C, with a final extension for 4 min at 72°C. PCR products (5 μL) were analysed by electrophoresis on agarose 1.5% w/v gels, followed by staining with ethidium bromide. The SCC*mec* type was determined on the basis of the band pattern obtained (Table 1; Fig. 1). Isolates with no visible bands, or with a band pattern that was not in agreement with one of the five predicted band patterns, were classified as non-typeable (NT).

A clear and easily discriminated band pattern was obtained for all five of the main SCC*mec* types using the multiplex PCR (Fig. 1). The presence of SCC*mec* types I–III, which yielded only one band in the multiplex PCR, was confirmed by the additional PCRs described above. The 50 isolates tested by the multiplex PCR of Oliveira and de Lancastre [6] yielded results that

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Table 1. Primers used in the multiplex SCC*mec* PCR and the resulting gel band patterns of SCC*mec* types I–V

Name	Primer sequence (5′ → 3′)	Length	Target	SCC*mec* type				
				I	II	III	IV	V
β	ATTGCCTTGATAATAGCCYTCT[a]	937 bp	*ccrA2-B*		X		X	
α3	TAAAGGCATCAATGCACAAACACT[a]							
ccrCF	CGTCTATTACAAGATGTTAAGGATAAT[b]	518 bp	*ccrC*			X		X
ccrCR	CCTTTATAGACTGGATTATTCAAAATAT[b]							
1272F1	GCCACTCATAACATATGGAA[c]	415 bp	IS*1272*	X			X	
1272R1	CATCCGAGTGAAACCCAAA[c]							
5RmecA	TATACCAAACCCGACAACTAC[c]	359 bp	*mecA*–IS*431*					X
5R431	CGGCTACAGTGATAACATCC[c]							

[a]Ito *et al*. [11].
[b]Ito *et al*. [12].
[c]This study.

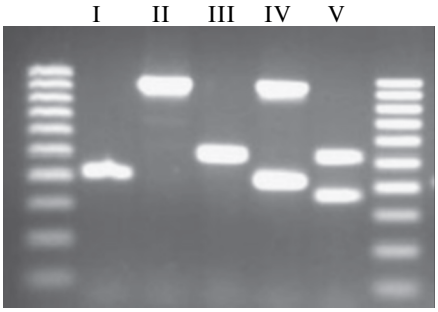

Fig. 1. Examples of PCR amplification products obtained with SCC*mec* types I–V. Lanes on the left and right of the gel contain 100-bp ladder molecular size markers.

agreed with those obtained by the new method described above, although, as expected, SCC*mec* type V was not identified by the method of Oliveira and de Lancastre [6].

Of the 312 MRSA isolates examined, 306 (98%) were typeable by the new multiplex PCR. SCC*mec* type IV was the most common type (84% of the isolates), followed by type V (6%), type I (4%) and type II (3%). SCC*mec* type III was found only in three imported isolates. The 312 isolates belonged to nine multilocus sequence type-based clonal complexes (CCs), with CCs 5, 8, 30 and 80 constituting 86% of the isolates (results not shown). SCC*mec* typing was suitable for subtyping multilocus sequence type CCs and *spa* types. Four and five SCC*mec* types were found in CC5 and CC8, respectively. The majority of isolates in CC8, the largest complex, carried SCC*mec* type IV.

Six isolates were classified as NT according to the multiplex PCR. Of these, two isolates were also NT by the method of Oliveira and de Lencastre [6]. These isolates may represent new or variant SCC*mec* types, and their SCC*mec* cassettes require further analysis. The remaining four isolates were typeable by the method of Oliveira and de Lancastre [6], and yielded a corresponding band in the novel multiplex PCR; however, either bands from other SCC*mec* types were also obtained, or the SCC*mec* type was not confirmed by the further confirmatory PCRs. These isolates might carry new recombinant cassettes and require further study.

Of 143 MRSA isolates recorded during 2003 and 2004, 70% were CO-MRSA and 30% were HA-MRSA. All SCC*mec* types were represented in both categories, except for SCC*mec* type V, which was detected only among the CO-MRSA isolates. SCC*mec* type IV was found in 86% of the CO-MRSA isolates and in 84% of the HA-MRSA isolates. Based on these findings, SCC*mec* typing is not useful for discriminating between CO-MRSA and HA-MRSA in Copenhagen, since SCC*mec* type IV predominated among both community and hospital isolates.

Isolates representing known, pandemic, international MRSA clones [9] were identified in the MRSA collection. These included ten isolates of ST5-MRSA-II (the New York/Japan clone) and nine isolates of ST5-MRSA-IV (the Paediatric clone). There were three isolates of ST239-MRSA-III (the Brazilian or Hungarian clone; the SCC*mec* PCR does not differentiate between SCC*mec* type III and IIIA). Two isolates might correspond to the Iberian clone (ST247-MRSA-I; SCC*mec* types I and IA are not distinguished by the multiplex PCR), and the Oceanian clone was represented by 38 isolates of ST30-MRSA-IV, *spa* type t019. The ST30 isolates were typically imported and caused small outbreaks in private households.

The SCC*mec* multiplex PCR developed by Oliveira and de Lencastre [6] can be used to differentiate among SCC*mec* types I–IV [6]. The PCR described in the present study has the advantage of detecting type V, thereby reducing the number of NT isolates and mistyping of type V as type III. Zhang *et al*. [10] have described a

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nine-gene multiplex PCR that contains one primer set for each of the main types I, II, III and V, and one primer set for each of subtypes IVa, b, c and d. Thus, most of the common type IV subtypes were discriminated, but new subtypes might be missed. The new multiplex PCR described in the present study identified SCC*mec* types for 98% of the predominantly CO-MRSA isolates investigated, and adds further information to the results obtained by determination of MLST and *spa* types.

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