

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/51168452>

Detection of novel chromosome-SCCmec variants in Methicillin Resistant Staphylococcus aureus and their inclusion in PCR based screening

Article in BMC Research Notes · May 2011

DOI: 10.1186/1756-0500-4-150 · Source: PubMed

CITATIONS

7

READS

29

5 authors, including:



[Anneke van der Zee](#)

Maasstad Ziekenhuis

41 PUBLICATIONS 807 CITATIONS

[SEE PROFILE](#)



[Lieuwe Roorda](#)

Maasstad Ziekenhuis

9 PUBLICATIONS 81 CITATIONS

[SEE PROFILE](#)

SHORT REPORT

Open Access

Detection of novel chromosome-SCC*mec* variants in Methicillin Resistant *Staphylococcus aureus* and their inclusion in PCR based screening

Anneke van der Zee^{1*}, Lieuwe Roorda¹, Willem DH Hendriks¹, Jacobus M Ossewaarde^{1,2} and Johannes Buitenwerf¹

Abstract

Findings: To facilitate automation, a novel DNA extraction method for MRSA was adopted. The MRSA specific chromosome-SCC*mec* PCR was adapted, additional primers were added, and the performance was validated. From various laboratories in The Netherlands we received a total of 86 MRSA clinical isolates, that were negative in commercially available tests. We identified 14 MRSA strains with new variant chromosome-SCC*mec* junctions by sequence analysis. These MRSA strains appeared to carry SCC*mec* sequences with a high degree of homology to SCC regions of *S. epidermidis* and *S. haemolyticus*. All were included for detection in chromosome-SCC*mec* based PCR.

Background: Efficient management of Methicillin Resistant *Staphylococcus aureus* (MRSA) in the hospital is needed to prevent dissemination. It is important that MRSA can be rapidly identified, and effective infection control measures can be initiated. Equally important is a rapid MRSA negative report, especially for patients in isolation. For negative screening we implemented fully automated high through-put molecular screening for MRSA.

Conclusions: Fourteen variant chromosome-SCC*mec* junctions in MRSA, that are not detected in commercially available MRSA detection kits were added to our PCR to detect all currently known variant SCC-*mec* types of MRSA.

Background

The dissemination of Methicillin Resistant *Staphylococcus aureus* (MRSA) in hospitals is a growing problem worldwide. In The Netherlands, a search and destroy policy is implemented [1]. Patients colonized with MRSA are kept in isolation until they are culture negative. A MRSA negative report can faster be obtained by PCR. Therefore, a molecular approach for negative screening of MRSA was exploited. Molecular detection of the *mecA* gene, which confers resistance to all β -lactams, has often been used in combination with other *S. aureus* specific genes in a multiplex PCR. Genes that are specific for *S. aureus* comprise for example of the sequence published by Martineau et al. [2], the nuclease gene (*nuc*) [3,4], or the coagulase gene (*coa*) [5]. When clinical samples contain a mixture of coagulase negative staphylococci (CNS), methicillin sensitive

S. aureus (MSSA), and MRSA, a positive *mecA* PCR can be generated by CNS while both MSSA and MRSA generate a positive PCR for the *coa* or the *nuc* gene. Only culturing could confirm MRSA. Another approach for detection of MRSA was presented by a multiplex PCR described by Huletsky et al. [6]. This PCR specifically targets the junction between a conserved open reading frame *orfX* in *S. aureus*, and the staphylococcal cassette chromosome containing the *mecA* gene (SCC*mec*). For MRSA, 8 different types of SCC*mec* elements have been classified [7]. The SCC is known to be a mobile heterogeneous genetic element that integrates site specifically into *orfX*. SCC*mec* is an SCC containing the *mecA* gene. SCC can also be present in CNS, not containing *mecA* but integrated into the analogous chromosomal location. MSSA can contain non-*mecA*-SCC or SCC*mec* elements which have lost the region containing *mecA*. Several commercially available molecular screening tests are based on PCR amplification of the chromosome-SCC*mec* junction.

* Correspondence: zeea@maasstadziekenhuis.nl

¹Maasstad Laboratory, Molecular Diagnostics Unit, Maasstad Hospital, Olympiaweg 350, 3870HT Rotterdam, The Netherlands

Full list of author information is available at the end of the article

In this study, a novel DNA extraction method for MRSA was adopted that virtually prevents PCR inhibition. The detection process was fully automated for high through-put of clinical materials. An extra 17 forward primers were added to PCR to detect several newly identified MRSA strains in this study carrying *SCCmec* variants and found to be present in The Netherlands, and possibly elsewhere. An adaptable PCR format is needed for reliable detection of all MRSA.

Findings

Implementation and evaluation of *orfX/SCCmec* PCR

The PCR as described by Huletsky et al. [6], was slightly adapted (Table 1).

To allow PCR detection of more MRSA types, a literature search was conducted. Forward primer F7 was derived from the sequence of *S. aureus* strain JCSC 3624 (WIS), accession number AB121219 [8], and was included in the PCR. Primer F10 was designed based on the sequence of *S. aureus* U10927 [9] (Table 1).

With the expanded *orfX/SCCmec* PCR a total of 1906 samples was investigated with high through-put screening; 303 were PCR positive, no inhibition of PCR was found. To verify whether a positive signal was due to viable or dead MRSA, all were cultured; 141 were culture positive, and 22% of 141 were found to be MSSA. The latter may have lost *mecA* regions or contain non-*mecA* SCC elements. All culture positive MRSA were confirmed by *mecA/coa* PCR. Thus, PCR is highly non specific. However, since all suspect samples are subsequently cultured, this is acceptable. With negative screening it is important that the negative predictive value is 100%.

One MRSA isolate found by routine culture was negative in PCR. We analyzed the sequence of this strain (303480, Table 2) by genomic DNA sequencing with primer R1. A sequence of 345 bp was obtained which was aligned to Genbank/EMBL DNA sequences using BLAST. No significant sequence homology was found except that the 35 bp flanking *orfX* showed 97%

Table 1 Primer and probe sequences used in *OrfX-SCC* PCR

primer/probe	Sequence 5'-3'	Reference
F1	GTCAAAAATCATGAACCTCATTACTTATG	according to Huletsky et al.
F2	AATATTTTCATATATGTAATTCCTCCACATCTC	adapted from Huletsky et al.
F3	CTTCAAATATTATCTCGTAATTTACCTTGTTT	adapted from Huletsky et al.
F4	CTCTGCTTTATATTATAAAATTACGGCTG	according to Huletsky et al.
F5	TCACTTTTTATTCTTCAAAGATTTGAGC	adapted from Huletsky et al.
F7	CCATTTCTCCAAAAATATATTTACTTTAGTC	This study
F8	TTTCATAATATGTGCTACGCAACCTA	This study
F9	CGAGTTAATTTTTATTTAGAGCGCTTAC	This study
F10	CCGCTCCTTTATATTATACACAACCTATT	This study
F11	GCCATATTAATGCCTCACGAAAC	This study
F12	CATTCATTAACATCGTACTCTGCATTT	This study
F13	TCCCTTTATGAAGCGGCTGAA	This study
F14	AAGCTATAGTTCAGCATTATCGTAAGTAACT	This study
F15	TGCCAATCACAGTTCAATCAATTATT	This study
F16	TCCTTTCTAATTATATTATGCGCAACCT	This study
F17	ACTCTGATAAGCCATTCATTCATCCA	This study
F18	ACAATCCTAACATAAGATTGTGGCTTT	This study
F20	GCATATTCACITTTGATAAGCCATTCAT	This study
F21	CGGTTCTGATATCTTTTCAACCATT	This study
F23	CCCCTCCATTAACCTCCGTATAT	This study
F24	CCCAAATCTTAACITTTCTTCAATACATT	This study
F25	TTCTAAGGTAGTCTCCCTTTCAATAATTT	This study
R1	CGTCATTGGCGGATCAAAC	adapted from Huletsky et al.
R2	CGTCATTGGTGGATCAAACG	adapted from Huletsky et al.
probe2	FAM-CACAAGGATGTCTTACAACG-MGB	adapted from Huletsky et al.
probe3	FAM-CACAAGGACGTCTTACAACG-MGB	adapted from Huletsky et al.
probe4	FAM-CACAAAGACGTCTTACAACG-MGB	adapted from Huletsky et al.

Table 2 Observed sequence homologies of analyzed MRSA strains from various locations, length of DNA sequence analysis reads, and PCR primers based on these sequences

Strain	Location	bp	Homology (%) with accession number (species) corresponding to bp	primer
02M023064	Breda	222	94% U10927 (<i>S. aureus</i>);bp 648-426	F9
JBZ12	Den Bosch	235	93% AB539727 (<i>S. aureus</i>);bp 84645-84507	F13
261207168	Enschede	834	91% AB063172 (<i>S. aureus</i>); bp 169-879	F15
JBZ33	Den Bosch	396	99% EU272080 (<i>S. aureus</i>);bp 794-399	F23
S0121	Utrecht	280	100% EU263618 (<i>S. aureus</i>);bp 655-376	F17
CC8	Denmark ()	422	99% BK001539 (<i>S. epidermidis</i>);bp 19437-19016	F20
434-1819	Eindhoven	416	99% BK001539 (<i>S. epidermidis</i>);bp 34917-34205	F24
S0089	Utrecht	409	100% AP006716 (<i>S. haemolyticus</i>);bp 52718-52358	F16
582	Utrecht	344	98% AP006716 (<i>S. haemolyticus</i>);bp 91374-91031	F18
060120	Leiden	912	98% AP006716 (<i>S. haemolyticus</i>);bp 30663-29755	F25
303480	Rotterdam	345	CGCAACCTATTTTTAGTTTTATTGTGATAtGCT	F8
251110219	Enschede	900	CAACtATTTTTAGTTTTATTGTGATACGCTTCT	F14
JBZ54	Den Bosch	688	No significant homology found	F21
40295861	Breda	417	No significant homology found	F11
40461611	Breda	762	No significant homology found	F12

homology with a repeated sequence found in *S. haemolyticus* (AP006716;bp 52313, and 91034), also present in *S. saprophyticus* (AP08934;bp 50105). A new primer in PCR (primer F8, Table 1) was derived from the obtained sequence. To minimize the chance for more false-negative PCR results for MRSA, other laboratories in The Netherlands were asked for MRSA strains that were negative in commercial molecular tests used by these laboratories. We received a total of 86 clinical isolates or DNA. Whenever a strain was also negative in our *orfX*/SCC*mec* PCR, DNA was sequenced and a new primer was added to PCR. Of 86 strains, another 14 additional forward primers were designed, apart from 2 literature based primers, and 1 primer based on a MRSA strain from our own hospital. All 17 primers were included in PCR to a total of 22 forward primers (Table 1).

Sequence analysis of PCR negative MRSA strains

All 14 strains mentioned above were verified to be MRSA using *mecA/coa* PCR. All were positive in both PCRs. The results of DNA sequence analysis are presented in Table 2. BLAST comparisons were made with the sequences cut off at the *orfX* according to Ito et al. [10]. Within the *orfX* gene, the obtained sequences were highly homologous and consistently aligned to *S. aureus orfX*.

The SCC*mec* of 5 strains showed various degrees of homology (91-100%) with different parts of SCC*mec* sequences of *S. aureus* strains. Two MRSA strains showed 99% homology with *S. epidermidis* SCC, but with different regions. Another 3 strains were homologous to *S. haemolyticus* in their *orfX* flanking sequences. BLAST alignments of strains 303480 and 251110219

showed no significant sequence homologies except for a 35 bp repeat (CAACtATTTTTTAGTTTTATTGTGATACGCTTCT) found present in *S. haemolyticus* and *S. saprophyticus*. The *orfX* flanking regions of 3 other strains showed no significant homology to any Genbank/EMBL sequences.

Comparison of right SCC*mec*-Orf*X* junctions

The alignment of right SCC*mec*-Orf*X* junctions is shown in Figure 1. The direct repeat consensus (-A-TT-TGATA-GC-TC, [10]) is largely intact, suggesting that SCC sequences from *S. epidermidis* and *S. haemolyticus* were acquired by recombination rather than by transposition.

Discussion

The staphylococcal cassette chromosome SCC*mec* is a genetic mobile element that contains the methicillin resistant *mecA* gene [8]. Its site-specific insertion or excision is mediated by cassette chromosome recombinase (*ccr*) complex and by the presence of direct and inverted repeats at the SCC*mec* extremities. The combination of classes of *mec* gene complex and *ccr* gene complex forms the basis of their classification [10]. At present 8 types of SCC*mec* elements have been classified [7,11]. In addition, SCC*mec* can harbour other drug resistant genes, and insertion sequence elements (*IS431*, *IS1272*), transposons (Tn4001, Tn554) or plasmids (pUB110, pT181). The chromosome flanking regions (junction or J-regions, formerly, junkyard regions) of SCC*mec* are most variable in their DNA sequences.

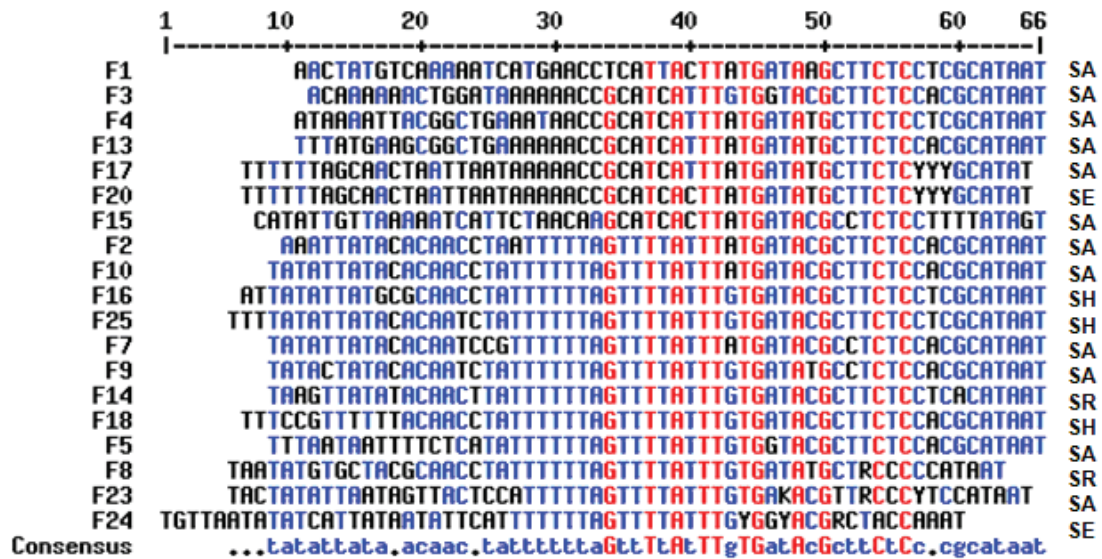


Figure 1 Alignment of right SCC-OrfX junctions. Strains are designated by the primers based on their sequences. OrfX starts at bp 55 (according to Ito et al. [10]). SA; *S. aureus*, SE; *S. epidermidis*, SH; *S. haemolyticus*, SR; Staphylococcal repeats. Strains without any homology to known sequences were omitted from the comparison.

We adapted the PCR described by Huletsky et al. [6], based on amplification of the chromosome-SCCmec junction. We initially included 2 more forward primers to expand detection to MRSA known from literature. After sequence analysis of 14 MRSA strains that were not recognized by the initial primer set, we included additional primers resulting in a megaplex PCR with 14 primers based on sequences of newly identified MRSA with variant SCCmec sequences to a total of 21 forward primers. No adverse effects on PCR were seen with this number of primers.

From 3 strains the sequenced SCCmec region did not match any known DNA sequence, presuming a non-human relation. In The Netherlands live-stock associated MRSA is increasingly isolated [12]. Reischl et al. [13] have shown that live-stock associated MRSA can be discriminated on the basis of a G→A mutation in *orfX*. Based on the finding that these strains were only positive in PCR when the probe harbouring this mutation (probe 2) was used, we confirmed that these 3 strains were most probably live-stock associated MRSA.

In the SCCmec of the other new strains, repeated sequences as IS431 and IS1272 were found. IS elements are mobile and can move by transposition. The similar but not identical repeats found in strain 303480 and 251110219, might be related to an IS1272 encoded truncated transposase as was indicated by BLAST homology. This suggested that the variant SCCmec sequences resulted from transposition. Therefore the right chromosome-SCCmec junctions carried by the new MRSA

strains were compared. The consensus represented the direct repeat (DR) involved in insertion and excision of SCCmec. Consequently, the variant SCCmec sequences were acquired by recombination rather than transposition. Mongkolrattanothai et al. [14], reported a non-mecA-SCC element present in *S. epidermidis* with 3 DR highly homologous to *S. aureus*, 2 at the extremities of the element and 1 in the middle. The 2 MRSA strains carrying different SCC homologs of *S. epidermidis* in this study, were each highly homologous to the SCC described by Mongkolrattanothai [14], but represented the sequence on either side of the middle DR. This finding may corroborate the recombination theory, regardless of the orientation of the inserted SCC in the 2 MRSA strains. Whether *mecA* is carried on the acquired SCC from *S. epidermidis* of the MRSA strains found in our study, as well as SCC typing, remains a topic for future studies.

In conclusion, it appears that MSSA can convert to MRSA by acceptance of SCC sequences from CNS. This process may be driven by selection pressure of antibiotics. Indeed, an isogenic pair of MSSA and MRSA was recently described that supports the occurrence of horizontal transfer of SCCmec between Staphylococcal species [15]. The 86 strains not detected as being MRSA in other laboratories by commercially available tests raises concern about their performance [16,17]. Exploiting the SCCmec junction as target in PCR used for detection of MRSA requires continuous awareness of possible variants. PCR based on *mecA/nuc* gene does

not seem to present an acceptable alternative because the *nuc* gene may be absent as has been found earlier [18]. The *mecA/coa* PCR, combined with chromosome-*SCCmec* junction PCR identifies other MRSA variants that may arise.

Conclusions

We identified 14 new variant chromosome-*SCCmec* junctions in MRSA, that are not detected in commercially available MRSA detection kits. We adapted our PCR to detect all known variant chromosome-*SCC-mec* types of MRSA.

Fully automated high through-put detection and robust DNA extraction support an adaptable PCR format for detection of these newly identified MRSA strains. Moreover, this system can easily be expanded with novel PCR primers when new variants of chromosome-*SCCmec* types in MRSA may arise.

Methods

Clinical samples

Samples were taken from nose, throat, and perineum, and if appropriate also from wounds, sputum, and catheters (Transwab, Medical Wire & Equipment Co. Ltd., Corsham, Wilts., England) in the context of patient and personnel screening as required by the Dutch policy for MRSA control. Since no extra action or sampling was requested than the medically indicated, informed consent was not asked and no ethical approval was required, in conformity to the guidelines of the Dutch Central Committee on Research involving Human Subjects. Swabs were inoculated into 5 ml phenyl mannitol broth (PHMB) containing ceftizoxime and aztreonam [19] for overnight incubation. After at least 18 hours of incubation, PHMB broth was subjected to PCR.

Automated DNA isolation, PCR detection, and data output by MRSA-screen with PCR amplification of OrfX-*SCCmec* junction

PHMB tubes were placed into the MultiPROBE II PLUS HT Expanded pipetting robot (PerkinElmer Life and Analytical Sciences). This system is equipped with a bar-code reader and automated plate sealer (RoboSeal). This system handles the DNA extraction, PCR assay setup, and sealing of the plate. Next, the plates are automatically transferred to a real-time ABI 7900HT thermocycler (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Thus, barcode scanning, DNA extraction, pipetting, sealing of the 384-well PCR Plate, creating a run file and starting the PCR, were done by the system without interference or need of presence of a technician. Results were automatically transferred to the Laboratory Information System.

Software was programmed by PerkinElmer to perform the following steps. After barcode reading, 5 ml of saline was added and mixed by pipetting. This 1:1 dilution served to elevate the fluid level and to prevent the 9.5 cm long probes touching the swabs in the 15 cm long tubes.

Template DNA was prepared by using the Extract-N-Amp™ Plant PCR Kit (SIGMA, Munich, Germany). In short, 50 µl sample was mixed with 100 µl SIGMA Extraction Solution (E7526). The mixture was incubated at 95 °C for 10 minutes, cooled to room temperature, 100 µl SIGMA Dilution Buffer (D5688) was added and mixed. This DNA sample is PCR ready when used in combination with the SIGMA Extract-N-Amp PCR ReadyMix™ (E3004).

Primers and probes for detection of the OrfX-*SCCmec* junction were used in one primer-probe mixture with the following concentrations; 600 nM for each F and R primer, and 120 nM for each probe (Table 1). Each sample was tested with this primer-probe mixture. Amplification reactions were performed in a volume of 20 µl, with 10 µl SIGMA Extract-N-Amp PCR Reaction Mix™ (including 4% Reference Dye) in 6 µl primer-probe mix and 4 µl of the DNA sample. Amplification consisted of 3 min at 95 °C followed by 44 cycles of 15 s at 95 °C and 60 s at 60 °C. In each run a MRSA positive and a negative control was included. A sample was regarded suspect for MRSA in the MRSA-screen if the PCR was positive. Positive samples were subsequently cultured on blood agar plates.

MecA/coa gene PCR

The *mecA* PCR was carried out as described [4], with 800nM of each primer. PCR for *coa* was as described previously [5]. The concentration of primers Coag2: CGAGACCAAGATTCAACAAG, and Coag3: AAA-GAAAACCACTCACATCA was 800nM each. *MecA/coa* PCR was used to confirm MRSA isolates. Results of *mecA* and *coa* PCR were analyzed using agarose gel electrophoresis.

DNA sequence analysis

Sequence analysis was performed by Baseclear, Leiden, The Netherlands on total chromosomal DNA with primer R1 (Table 1). Partial sequences of variant *SCCmec* have not yet been submitted but will be classified in further studies according to the recommendations of the International working group on the classification of staphylococcal cassette chromosome elements [11].

Acknowledgements

We gratefully acknowledge our colleagues who kindly provided their strains; Anneke Bergmans PhD., and Jan Kluytmans PhD., Amphia Hospital, Breda, Adriaan van den Brule PhD., Lab for Medical Microbiology, PAMM Regional

Laboratory, Eindhoven/Veldhoven, The Netherlands, Eric Claas PhD., University Medical Center, Leiden, Ad Fluit PhD., and Adrienne Box BSc, University Medical Center Utrecht, Utrecht, Gerda Noordhoek PhD., Centre for Infectious Diseases Friesland, Leeuwarden, Ron Hendrix MD, PhD., Labmicta, Enschede, Mirjam Hermans PhD., Jeroen Bosch Hospital, Den Bosch, Ludo Oostendorp BSc, Laboratory for Infectious Diseases, Groningen, Dorte Ørnskov, PhD, Sygehus Lillebælt, Vejle (Denmark), Thera Trienekens PhD., VieCuri Medisch Centrum voor Noord-Limburg, Venlo.

Author details

¹Maasstad Laboratory, Molecular Diagnostics Unit, Maasstad Hospital, Olympiaweg 350, 3870HT Rotterdam, The Netherlands. ²Erasmus Medical Centre, Rotterdam, The Netherlands.

Authors' contributions

AZ drafted the manuscript and carried out the analysis of data. LR carried out the molecular genetic studies, and participated in the design of PCR. WDHH and JB conceived of the study and participated in its design. JMO helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 30 March 2011 Accepted: 26 May 2011

Published: 26 May 2011

References

1. Vos MC, Ott A, Verbrugh HA: **Successful Search-and-Destroy Policy for Methicillin-Resistant *Staphylococcus aureus* in The Netherlands.** *J Clin Microbiol* 2005, **43**(4):2034-2035.
2. Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG: **Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*.** *J Clin Microbiol* 1998, **36**:618-623.
3. Francois P, Bento M, Renzi G, Harbarth S, Pittet D, Schrenzel J: **Evaluation of Three Molecular Assays for Rapid Identification of Methicillin-Resistant *Staphylococcus aureus*.** *J Clin Microbiol* 2007, **45**:2011-2013.
4. Geha D J, Uhl J R, Gustafiero C A, Persing D H: **Multiplex PCR for identification of methicillin-resistant staphylococci in the clinical laboratory.** *J Clin Microbiol* 1994, **32**:1768-1772.
5. Goh S H, Byrne SK, Chow AW: **Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphisms.** *J Clin Microbiol* 1992, **30**:1642-1645.
6. Huletsky A, Giroux R, Rossbach V, Gagnon M, Vaillancourt M, Bernier M, Gagnon F, Truchon K, Bastien M, Picard FJ, van Belkum A, Ouellette M, Roy PH, Bergeron MG: **New real-time PCR assay for rapid detection of methicillin resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci.** *J Clin Microbiol* 2004, **42**:1875-1884.
7. Chongtrakool P, Ito T, Ma X X, Kondo Y, Trakulsomboon S, Tiensasitorn C, Jamklang M, Chavalit T, Song JH, Hiramatsu K: **Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Typing of Methicillin-Resistant *Staphylococcus aureus* Strains Isolated in 11 Asian Countries: a Proposal for a New Nomenclature for SCC*mec* Elements.** *Antimicrob Agents Therap* 2006, **50**(3):1001-1012.
8. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, Hiramatsu K: **Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*.** *Antimicrob Agents Therap* 2001, **45**:1323-1336.
9. Lin WS, Cunneen T, Lee CY: **Sequence analysis and molecular characterization of genes required for the biosynthesis of type 1 capsular polysaccharide in *Staphylococcus aureus*.** *J Bacteriol* 1994, **176**(22):7005-7016.
10. Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K: **Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*.** *Antimicrob Agents Therap* 2004, **48**:2637-2651.
11. International working group on the classification of staphylococcal cassette chromosome elements (IWG-SCC): **Classification of Staphylococcal Cassette chromosome *mec* (SCC*mec*): Guidelines for reporting novel SCC*mec* elements.** *Antimicrob Agents Therap* 2009, **53**(12):4961-4967.
12. De Neeling AJ, van den Broek MJ, Spalburg EC, van Santen-Verheuev MG, Dam-Deisz WDC, Boshuizen HC, van de Giessen AW, van Duijkeren E, Huijsdens XW: **High prevalence of methicillin resistant *Staphylococcus aureus* in pigs.** *Vet Microbiol* 2007, **122**(3-4):366-72.
13. Reischl U, Frick J, Hoermansdorfer S, Melzl H, Bollwein M, Linde HJ, Becker K, Köck R, Tuschak C, Busch U, Sing A: **Single-nucleotide polymorphism in the SCC*mec*-orfX junction distinguishes between livestock-associated MRSA CC398 and human epidemic MRSA strains.** *Euro Surveill* 2009, **14**(49):19436.
14. Mongkolrattanothai K, Boyle S, Murphy TV, Daum RS: **Novel non-*mecA*-containing staphylococcal chromosomal cassette composite island containing *pbp4* and *tagF* genes in a commensal staphylococcal species: A possible reservoir for antibiotic resistance islands in *Staphylococcus aureus*.** *Antimicrob Agents Therap* 2004, **48**:1823-1836.
15. Bloemendaal ALA, Brouwer EC, Fluit AC: **Methicillin Resistance transfer from *Staphylococcus epidermidis* to Methicillin-susceptible *Staphylococcus aureus* in a patient during antibiotic therapy.** *PLoS ONE* 2010, **5**(7):e11841.
16. Bartels MD, Boye K, Rohde SM, Larsen AR, Torfs H, Bouchy P, Skov R, Westh H: **A common variant of staphylococcal cassette chromosome *mec* type IVa in isolates from Copenhagen, Denmark, is not detected by the BD GeneOhm methicillin-resistant *Staphylococcus aureus* assay.** *J Clin Microbiol* 2009, **47**(5):1524-7.
17. Ornskøvd D, Kolmos B, Bendix Horn P, Nederby Nielsen J, Brandslund I, Schouenborg P: **Screening for methicillin-resistant *Staphylococcus aureus* in clinical swabs using a high-throughput real-time PCR-based method.** *Clin Microbiol Infect* 2008, **14**:22-28.
18. van Leeuwen W, Roorda L, Hendriks W, Francois P, Schrenzel J: **A nuc-deficient methicillin-resistant *Staphylococcus aureus* strain.** *FEMS Immunol Med Microbiol* 2008, **54**:157.
19. Wertheim H, Verbrugh HA, van Pelt C, de Man P, van Belkum A, Vos MC: **Improved detection of methicillin-resistant *Staphylococcus aureus* using phenyl mannitol broth containing aztreonam and ceftizoxime.** *J Clin Microbiol* 2001, **39**:2660-2662.

doi:10.1186/1756-0500-4-150

Cite this article as: van der Zee et al.: Detection of novel chromosome-SCC*mec* variants in Methicillin Resistant *Staphylococcus aureus* and their inclusion in PCR based screening. *BMC Research Notes* 2011 **4**:150.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

