Eur J Clin Microbiol Infect Dis (2014) 33:1967–1971
DOI 10.1007/s10096-014-2169-9

ARTICLE

Characterization of the staphylococcal cassette chromosome *mec* insertion site in 108 isolates lacking the *mecA* gene and identified as methicillin-resistant *Staphylococcus aureus* by the Xpert MRSA assay

M. Stojanov · D. S. Blanc

Received: 14 April 2014 / Accepted: 19 May 2014 / Published online: 7 June 2014

Abstract During a 3-year period, 848 patients were detected as carriers of methicillin-resistant *Staphylococcus aureus* (MRSA) by the Xpert MRSA assay (Cepheid). Among them, 108 patients (12.7 %) were colonized with strains showing methicillin-susceptible phenotypes and absence of the *mecA* gene, despite being positive with the rapid polymerase chain reaction (PCR) assay. DNA sequences of the staphylococcal cassette chromosome *mec* (SCC*mec*) insertion site of these "false-positive" strains was determined by direct sequencing of the genomic DNA. More than half (53.7 %) of the strains had DNA sequences unrelated to either SCC or SCC*mec* and one-third had DNA sequences related to non-*mec* SCC. Only 10.2 % of the strains carried sequences related to SCC*mec*, suggesting that a sequence containing the *mecA* gene was lost from an SCC*mec*. These findings differ from the general idea that all methicillin-susceptible *S. aureus* having positive Xpert MRSA assay results are essentially MRSA that lost the *mecA* gene.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of hospital-acquired infections and is associated with a high mortality rate. Patients with MRSA infections have prolonged length of stay in hospitals, which results in higher costs of hospitalization [1–4]. With this perspective, surveillance programs play an important role in preventing onward transmission from carriers, resulting in a decreased risk of infection for other patients and reduced economic impact for the hospital. Over the last decade, standard culture techniques have been replaced by more rapid molecular methods to detect MRSA in clinical samples.

Broad-spectrum resistance to β-lactam antibiotics is encoded by the *mecA* gene, which is located on the staphylococcal cassette chromosome *mec* (SCC*mec*), a genomic island inserted at the 3′ end of the *rlmH* gene (previously named *orfX*) [5]. Several commercially available polymerase chain reaction (PCR)-based kits detect the junction formed upon the insertion of SCC*mec* in the chromosome, using primers located in the conserved *rlmH* gene and several primers and probes detecting different SCC*mec* types. This rapid detection of MRSA-positive samples within a few hours might have an important impact on MRSA surveillance [6, 7]. However, since their introduction as tools for surveillance and diagnosis, a number of studies have shown that misinterpretation can often occur [8–14]. In a pilot study aiming to detect the chromosome–SCC*mec* junction by PCR, a false-positive signal was found in 26 strains (4.6 %) in a collection of 569 methicillin-sensitive *S. aureus* (MSSA) [15]. A more recent study showed that, among 251 Xpert MRSA-positive samples obtained during a 1-year period, 23 (9.2 %) were MSSA isolates [16]. Similarly, a 9-month study conducted in our hospital showed that, among 217 patients with a positive Xpert MRSA assay, 28 (12.9 %) were colonized by an MSSA isolate [8].

In this study, we characterized the SCC*mec* insertion site in 108 strains misidentified as MRSA by the Xpert MRSA assay (GeneXpert system, Cepheid, Sunnyvale, CA) during a 3-year period using direct sequencing of genomic DNA.

Electronic supplementary material The online version of this article (doi:10.1007/s10096-014-2169-9) contains supplementary material, which is available to authorized users.

M. Stojanov (✉) · D. S. Blanc
Hospital Preventive Medicine Service, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
e-mail: milos.stojanov@chuv.ch

Materials and methods

Bacterial strains

The University Hospital of Lausanne is a 1,000-bed tertiary care hospital where active surveillance cultures are part of its MRSA control program. The rapid PCR-based Xpert MRSA assay was introduced in June 2009 for MRSA screening. The detection and sampling methods have been described previously [17]. When a discrepant result was obtained, i.e., positive Xpert MRSA assay and absence of typical pink colonies on MRSASelect agar plates (Bio-Rad, Marnes-la-Coquette, France), the strain was tested on a chromogenic *S. aureus* agar plate (SAID, bioMérieux, Marcy-l'Etoile, France). If *S. aureus* characteristic green colonies grew on this plate, an Xpert MRSA assay was performed on the colonies and, if positive, the resistance to methicillin was assessed by an antibiogram using the Kirby–Bauer method with cefoxitin (30 μg) and oxacillin (1 μg) disks. Absence of the *mecA* gene was confirmed by PCR with a previously described method [18]. The presence of *ccrAB* genes was detected by M-PCR1 (as described by Kondo et al. [18]). Genotyping of all strains was performed by multilocus sequence typing (MLST) and double-locus sequence typing (DLST), as previously described [19–21].

Genomic DNA extraction

Genomic DNA was extracted using a protocol described previously [22]. In brief, 3 mL of an overnight culture were centrifuged and resuspended in 50 μL of lysostaphin solution (10 mM Tris-Cl, pH 7.5, and 1 mM EDTA, supplemented with lysostaphin at a final concentration of 0.5 μg/mL). After 30 min of incubation at 37 °C, 300 μL of "Nuclei Lysis Solution" (Promega Corporation, Madison, WI) were added and the cell suspensions were heated at 80 °C for at least 10 min. The samples were then cooled at room temperature and treated with RNase. Subsequently, 100 μL of "Protein Precipitation Solution" (Promega Corporation) were added to the samples, which were then incubated for 5 min on ice. After centrifugation (4 °C, 15,600×g), supernatants were discarded and 300 μL of isopropanol were used to precipitate the DNA, which was then washed with 70 % ethanol and pelleted by centrifugation. DNA samples were then air-dried and rediluted at 4 °C overnight in 20 μL of nuclease-free H_2O. DNA concentrations and qualities were determined using an ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

DNA sequencing and data treatment

Highly concentrated DNA was required for the direct sequencing of genomic DNA, between 2 and 3 μg/μL. The sequencing reaction was performed with 2 μL of DNA, using the BigDye Terminator v.1.1 Sequencing Kit (Applied Biosystems, Foster City, CA), following the protocol of the manufacturer. Sequencing was performed using an ABI PRISM 3730 DNA Analyzer (Applied Biosystems). Two primers binding in conserved regions of the *rlmH* gene were used for genomic sequencing. The seql_fw primer (CGTTTA GGCCCATACACCAAGATAGAC, starting at position +76 of the *rlmH* gene) was used in the first instance. A second primer, seq2_fw (CGCAGTAACTACGCACTATCATTCAG C, starting at position +357 of the *rlmH* gene), was used when the sequence obtained with the first primer was too short.

Sequencing data were analyzed by Geneious software v7.0.6 (Biomatters Ltd., Auckland, New Zealand). An average read length of 685 bp was obtained, with an average phred40 (99.99 % base call accuracy) quality of 82.8 %.

Nucleotide sequence accession numbers

The DNA sequences surrounding the SCC*mec* insertion sites of one representative strain per sequence group have been deposited in GenBank. The accession numbers are as follows: KJ786840 for strain H26280 (Group 1), KJ786841 for strain H21732 (Group 2), KJ786842 for strain H22446 (Group 3), KJ786843 for strain H25130 (Group 4), KJ786844 for strain H22311 (Group 5), KJ786845 for strain H25652 (Group 6), KJ786846 for strain H24478 (Group 7), KJ786847 for strain H24884 (Group 8), KJ786848 for strain H24314 (Group 9), KJ786849 for strain H23280 (Group 10), KJ786850 for strain H27390 (Group 11), KJ786851 for strain H22887 (Group 12), KJ786852 for strain H27608 (Group 13), KJ786853 for strain H26736 (Group 14), KJ786854 for strain H21876 (Group 15), KJ786855 for strain H25653 (Group 16), KJ786856 for strain H25356 (Group 17), and KJ786857 for strain H24924 (Group 18).

Results

Between July 2010 and June 2013, 848 patients had at least one screening sample detected as being positive by the Xpert MRSA assay. Among them, 108 patients (12.7 %) were colonized by *S. aureus* isolates which did not contain the *mecA* gene.

Sequences downstream of the *rlmH* gene were determined by sequencing directly the genomic DNA and aligned to GenBank/EMBL DNA sequences databases using the BLAST program [23]. Table 1 shows the different groups of sequences and their closest matches obtained with BLAST analysis. Eighteen different groups of sequences were found, highlighting the great diversity of the genetic content downstream of the SCC*mec* insertion site, as previously described

Table 1 Characterization of the staphylococcal cassette chromosome *mec* (SCC*mec*) insertion site of methicillin-susceptible *Staphylococcus aureus* (MSSA) strains misidentified as methicillin-resistant *S. aureus* (MRSA) by the Xpert MRSA polymerase chain reaction (PCR) assay

Group	ST	CC	Number of isolates	Accession number	BLAST result	Sequence identity	Notes
SCC-unrelated sequences: 53.7 %							
1	ST1, ST3, ST81	CC15	40	KJ786840	*S. aureus* strain 15575, SCC*mec* insertion site genomic sequence	100 %	Genomic sequence downstream *rlmH* encoding a putative transposase and enterotoxin [24]
2	ST59, ST97, ST425	CC59, CC97	6	KJ786841	*S. aureus* LGA251, complete genome sequence	99 %	Genomic sequence downstream type XI SCC*mec* containing a putative restriction-modification system
3	ST59, ST718	CC59	4	KJ786842	*S. aureus* strain 15585, SCC*mec* insertion site genomic sequence	100 %	Genomic sequence downstream *rlmH* encoding unknown proteins [24]
4	ST22	CC22	4	KJ786843	*S. aureus* isolate CMFT503, SCC*mec* region	100 %	Genomic sequence downstream type IV SCC*mec* containing a putative restriction-modification system
5	ST1278	CC15	2	KJ786844	*S. aureus* isolate CMFT503, SCC*mec* region	89 %	Genomic sequence downstream type IV SCC*mec* containing a putative restriction-modification system
6	ST188	CC15	1	KJ786845	*S. aureus* strain 15682, SCC*mec* insertion site genomic sequence	100 %	Genomic sequence downstream *rlmH* encoding a putative restriction-modification system [24]
7	ST1094		1	KJ786846	*S. aureus* ECT-R complete genome sequence	99 %	Genomic sequence encoding IS1181 transposase
SCC-related sequences: 33.3 %							
8	ST8	CC8	18	KJ786847	*S. aureus* isolate CMFT535, SCC*mec* region	99 %	SCC element carrying type 1 *ccrAB* genes encoding a putative restriction-modification system
9	ST78, ST88, ST129	CC88	11	KJ786848	*S. haemolyticus* JCSC1435, complete genome sequence	93 %	ΨSCC encoding unknown proteins
10	ST30, ST852	CC22, CC30	3	KJ786849	*S. aureus* strain 15580, SCC*mec* insertion site genomic sequence	99 %	ΨSCC encoding putative transposases
11	ST1, ST5	CC5, CC15	2	KJ786850	*S. aureus* strain WAMRSA40 SCC*mec* genomic sequence	100 %	ΨSCC containing *pls* gene
12	ST5	CC5	2	KJ786851	*S. aureus* strain USA300_R114 SCC*mec* IVa and ACME sequences	97 %	Identity to parts of the ACME element in strain USA300_R114
SCC*mec*-related sequences: 10.2 %							
13	ST5, ST8, ST45, ST125	CC5, CC8, CC45	8	KJ786852	Several MRSA strains	100 %	Several strains carrying type II or type IV SCC*mec*
14	ST45	CC45	1	KJ786853	Several MRSA strains	100 %	Several strains carrying type III or type V SCC*mec*
15	ST1	CC15	1	KJ786854	*S. aureus* strain LG1-053 genomic sequence	100 %	SCC*mec*N1
16	ST7		1	KJ786855	*S. aureus* strain RN7170	95 %	J1 region of type II.4 SCC*mec*

Table 1 (continued)

Group	ST	CC	Number of isolates	Accession number	BLAST result	Sequence identity	Notes
Unknown sequences: 2.9 %							
17	ST45	ST45	2	KJ786856	Unknown type 1	-	No matches with GenBank/EMBL DNA sequences databases
18	ST34	CC30	1	KJ786857	Unknown type 2	-	No matches with GenBank/EMBL DNA sequences databases

[24]. More than half of all sequences (53.7 %) were not related to SCC elements in general (i.e., presence of sequences in which *ccr* recombinase genes are absent) and SCC*mec* in particular (i.e., absence of *mecA* and *ccr* genes). Thirty-seven percent of the strains belonged to the 15575 type (Group 1, Table 1), which is a previously described MSSA associated with a positive Xpert MRSA assay [14, 24]. Even with the absence of an SCC cassette, these strains showed high sequence identity with the 5′ ends of SCC*mec* types II or IV, thus explaining the positive result of the Xpert MRSA assay [14]. All strains of the 15575 group belonged to the same clonal complex (CC15, Table 1), indicating clonal dissemination, rather than the presence of a hypothetical mobile genetic element at this chromosomal site. Four other groups (Groups 2, 4, 5, and 6, Table 1) of SCC-unrelated sequences suggested the presence of putative restriction-modification systems, whose presence was previously described in this region of the chromosome [24].

One-third (36) of the isolates was associated to SCC-related elements. Among them, 18 strains (16.7 %) showed 100 % identity with an SCC element carrying *ccrAB1* genes (Table S1) and a putative restriction-modification system from strain CMFT535. Eleven strains (10.2 %) carried a DNA segment closely related to a ΨSCC (SCC without *ccr* genes) from *Staphylococcus haemolyticus* strain JCSC1435 encoding unknown proteins. The remaining strains in this group were associated to other ΨSCC or parts of an ACME element (Table 1).

Only 10.2 % of the strains carried sequences that were associated with the presence of SCC*mec* (Table 1). Seven strains had the same genotype of local MRSA clones known to carry type IV SCC*mec* (DLST 3-3, ST 8-IV; DLST 1-52, ST 45-IV; DLST 4-36, ST 125-IV) and had sequences that matched with several type IV SCC*mec* present in the databases. This was also true for one strain carrying type II SCC*mec* (DLST 2-23, ST 5-II). Two strains had infrequent genotypes and carried sequences related to type II and type III/V SCC*mec*, respectively. One strain had 100 % identity sequence with that of SCC*mec*N1 from *S. aureus* LG1-053.

The remaining three strains (2.8 %) showed no identity to sequences present in the GenBank/EMBL DNA sequences databases.

Discussion

We characterized the SCC*mec* insertion site in 108 isolates that were misidentified as MRSA by the Xpert MRSA assay. Our findings indicate that this chromosomal region is highly diverse among isolates. Surprisingly, more than half of the strains were not associated to SCC or SCC*mec* sequences and one-third carried non-*mec* SCC-related sequences. Our results suggests that dropout of the *mecA* gene could have occurred in only 10.2 % of the strains. This observation is in disagreement with the general idea that MSSA which had a positive result with the Xpert MRSA assay are essentially strains that lost the *mecA* gene.

Although the clonal spread of some STs could account for these false-positive results (Group 1, ST1; Group 8, ST 8), the fact that different DLST types were observed strongly suggest that this dissemination is not solely local.

The genomic structure of SSCs is complex and, despite many recent next-generation sequencing studies on MRSA, we are still not able to have a clear picture of these mobile genetic elements. Moreover, little is known on SCCs that do not contain the *mecA* gene. Our results suggest that they are genetically diverse and more frequent than previously thought.

In order to improve rapid PCR-based assays for MRSA screening, further studies are needed in order to investigate the diversity, dissemination, and prevalence of such elements. In the meantime, the results from rapid PCR-based tests should be confirmed by conventional culture methods.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y (2005) The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect Control Hosp Epidemiol 26(2):166–174
2. Filice GA, Nyman JA, Lexau C, Lees CH, Bockstedt LA, Como-Sabetti K, Lesher LJ, Lynfield R (2010) Excess costs and utilization associated with methicillin resistance for patients with *Staphylococcus aureus* infection. Infect Control Hosp Epidemiol 31(4):365–373
3. Macedo-Viñas M, De Angelis G, Rohner P, Safran E, Stewardson A, Fankhauser C, Schrenzel J, Pittet D, Harbarth S (2013) Burden of meticillin-resistant *Staphylococcus aureus* infections at a Swiss University hospital: excess length of stay and costs. J Hosp Infect 84(2):132–137
4. Ott E, Bange FC, Reichardt C, Graf K, Eckstein M, Schwab F, Chaberny IF (2010) Costs of nosocomial pneumonia caused by meticillin-resistant *Staphylococcus aureus*. J Hosp Infect 76(4): 300–303
5. Katayama Y, Ito T, Hiramatsu K (2000) A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 44(6):1549–1555
6. Tacconelli E, De Angelis G, de Waure C, Cataldo MA, La Torre G, Cauda R (2009) Rapid screening tests for meticillin-resistant *Staphylococcus aureus* at hospital admission: systematic review and meta-analysis. Lancet Infect Dis 9(9):546–554
7. Wassenberg M, Kluytmans J, Erdkamp S, Bosboom R, Buiting A, van Elzakker E, Melchers W, Thijsen S, Troelstra A, Vandenbroucke-Grauls C, Visser C, Voss A, Wolffs P, Wulf M, van Zwet T, de Wit A, Bonten M (2012) Costs and benefits of rapid screening of methicillin-resistant *Staphylococcus aureus* carriage in intensive care units: a prospective multicenter study. Crit Care 16(1):R22
8. Blanc DS, Basset P, Nahimana-Tessemo I, Jaton K, Greub G, Zanetti G (2011) High proportion of wrongly identified methicillin-resistant *Staphylococcus aureus carriers* by use of a rapid commercial PCR assay due to presence of staphylococcal cassette chromosome element lacking the *mecA* gene. J Clin Microbiol 49(2):722–724
9. Boyle-Vavra S, Daum RS (2010) Reliability of the BD GeneOhm methicillin-resistant *Staphylococcus aureus* (MRSA) assay in detecting MRSA isolates with a variety of genotypes from the United States and Taiwan. J Clin Microbiol 48(12):4546–4551
10. Ciardo DE, Burger S, Payer M, Lee C, McCallum N (2010) GeneXpert captures unstable methicillin-resistant *Staphylococcus aureus* prone to rapidly losing the *mecA* gene. J Clin Microbiol 48(8):3030–3032
11. Donnio PY, Oliveira DC, Faria NA, Wilhelm N, Le Coustumier A, de Lencastre H (2005) Partial excision of the chromosomal cassette containing the methicillin resistance determinant results in methicillin-susceptible *Staphylococcus aureus*. J Clin Microbiol 43(8):4191–4193
12. Roisin S, Laurent C, Nonhoff C, Deplano A, Hallin M, Byl B, Struelens MJ, Denis O (2012) Positive predictive value of the Xpert MRSA assay diagnostic for universal patient screening at hospital admission: influence of the local ecology. Eur J Clin Microbiol Infect Dis 31(5):873–880
13. Stamper PD, Louie L, Wong H, Simor AE, Farley JE, Carroll KC (2011) Genotypic and phenotypic characterization of methicillin-susceptible *Staphylococcus aureus* isolates misidentified as methicillin-resistant *Staphylococcus aureus* by the BD GeneOhm MRSA assay. J Clin Microbiol 49(4): 1240–1244
14. Wong H, Louie L, Lo RY, Simor AE (2010) Characterization of *Staphylococcus aureus* isolates with a partial or complete absence of staphylococcal cassette chromosome elements. J Clin Microbiol 48(10):3525–3531
15. 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 (2004) New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. J Clin Microbiol 42(5):1875–1884
16. Arbefeville SS, Zhang K, Kroeger JS, Howard WJ, Diekema DJ, Richter SS (2011) Prevalence and genetic relatedness of methicillin-susceptible *Staphylococcus aureus* isolates detected by the Xpert MRSA nasal assay. J Clin Microbiol 49(8):2996–2999
17. Blanc DS, Nahimana I, Zanetti G, Greub G (2013) MRSA screening by the Xpert MRSA PCR assay: pooling samples of the nose, throat, and groin increases the sensitivity of detection without increasing the laboratory costs. Eur J Clin Microbiol Infect Dis 32(4):565–568
18. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K (2007) Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 51(1):264–274
19. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38(3):1008–1015
20. Basset P, Senn L, Prod'hom G, Bille J, Francioli P, Zanetti G, Blanc DS (2010) Usefulness of double locus sequence typing (DLST) for regional and international epidemiological surveillance of methicilin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 16(8):1289–1296
21. Kuhn G, Francioli P, Blanc DS (2007) Double-locus sequence typing using *clfB* and *spa*, a fast and simple method for epidemiological typing of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 45(1):54–62
22. Stojanov M, Sakwinska O, Moreillon P (2013) Expression of SCC*mec* cassette chromosome recombinases in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. J Antimicrob Chemother 68(4):749–757
23. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3):403–410
24. Noto MJ, Kreiswirth BN, Monk AB, Archer GL (2008) Gene acquisition at the insertion site for SCC*mec*, the genomic island conferring methicillin resistance in *Staphylococcus aureus*. J Bacteriol 190(4): 1276–1283