Spontaneous Staphylococcal Cassette Chromosome *mec* Element Excision in *Staphylococcus aureus* Nasal Carriers

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Among 23 patients carrying methicillin-resistant *Staphylococcus aureus* (MRSA) in their anterior nares, 6 (26%) also carried methicillin-susceptible *S. aureus* (MSSA) as less prevalent flora. In 4 of the 6 patients, the MSSA was unrelated to prevalent MRSA, as determined by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and staphylococcal protein A (*spa*) typing. However, in two patients, the strains were identical except for the absence of spontaneous staphylococcal cassette chromosome *mec* (SCC*mec*). We consider this evidence of spontaneous SCC*mec* excision *in vivo*.

Staphylococcus aureus is a prominent human pathogen that can cause serious infections (1). It is carried asymptomatically in the anterior nares, and this carriage provides the usual source for subsequent infections. Treatment of infections is made more difficult by the propensity of *S. aureus* to develop antimicrobial resistance, the most important of which is resistance to beta-lactam antibiotics, designated methicillin-resistant *S. aureus* (MRSA). The gene encoding methicillin resistance, *mecA*, is carried in the bacterial chromosome on a genomic island called staphylococcal cassette chromosome *mec* (SCC*mec*), which can vary in size from approximately 20 kb to >60 kb (6). This element is integrated at a specific chromosomal *att* site that is located in the carboxyl terminus of a gene of unknown function named *orfX*, situated near the origin of chromosomal replication. Both excision and integration of SCC*mec* can be mediated by serine recombinases, CcrA and CcrB or CcrC, that are encoded within the element (7, 8, 14). SCC*mec* types, from I to VI, are determined by differences in the DNA sequence around the *mecA* regulators, *mecR1* and *mecI*, and the sequence and/or presence of different *ccr* recombinase genes (*ccrAB* or *ccrC*; 6). Precise excision of SCC*mec* can occur, mediated by Ccr recombinases, or *mecA* itself can be deleted by homologous recombination between repeated insertion sequence (IS) elements (11, 14). Although spontaneous SCC*mec* excision has been observed *in vitro* (7) and strain pairs that appear to be MRSA/methicillin-susceptible *S. aureus* (MSSA) siblings have been recovered from patients in the same geographic location (12), it is unclear if spontaneous SCC*mec* excision occurs within a population of MRSA colonizing an individual patient. We thus undertook the following pilot study to identify excised variants within MRSA in nasal carriers.

All patients admitted to the Hunter Holmes McGuire Veteran Affairs Medical Center in Richmond, VA, undergo routine culturing of the anterior nares to detect the presence of MRSA upon admission to the hospital (active surveillance culturing). This is part of a large prevention initiative designed to reduce the incidence of MRSA among hospitalized patients (9). For the current study, duplicate culture swabs were collected from patients undergoing active surveillance culturing in the hospital. Duplicate culture swabs were numbered for identification purposes during processing, but these numbers were not linked to patient identity. The deidentified swabs were evaluated by quantitative culture for the presence of both MSSA and MRSA. Institutional Review Board (IRB) approval for the current pilot study was not required, as no patient data were collected, and swabs could not be linked to a patient's identity. Swabs were plated on phenol red mannitol salt agar (Becton Dickinson, Sparks, MD), and yellow colonies, indicating *S. aureus*, were transferred by toothpick to brain heart infusion (BHI) agar plates (Becton Dickinson, Sparks, MD) with and without 6 µg/ml of oxacillin. Colonies were confirmed as *S. aureus* by their ability to agglutinate Staphaurex Plus beads (Remel, Le-

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TABLE 1 *S. aureus* nasal carriage isolates from patients with mixed MRSA and MSSA cultures

Patient no.	No. of MRSA ^a	No. of MSSA ^a	MRSA SCC <i>mec</i> type	MRSA MLST	MSSA MLST	MRSA <i>spa</i> type	MSSA <i>spa</i> type	MRSA PFGE type	MSSA vs MRSA PFGE type
3	561	1	II	CC5	CC8	t002	t574	USA100	Different patterns
16	410	3	II	CC5	CC8	t002	t1610	USA100	Different patterns
17	840	5	IV	CC8	CC15	t008	t5334	USA300	Different patterns
18	910	6	II	CC5	CC5	t002	t002	USA100	One band difference
26	55	13	IV	CC8	CC5	t002	t010	USA300	Different patterns
27	68	2	IV	CC8	CC8	t008	t008	USA300	One band difference

^a Number of MRSA and MSSA colonies on agar recovered from initial screening swab.

nexa, KS). Each colony not growing on oxacillin agar was confirmed as MSSA by spreading 0.1 ml of an overnight culture on an oxacillin plate. The absence of colonies after 72 h of incubation confirmed susceptibility. Staphylococcal protein A (*spa*) typing and pulsed-field gel electrophoresis (PFGE) were performed as previously described (4, 13). The clonal complexes (CC) were inferred from *spa* type.

A total of 23 patients were confirmed as being colonized with MRSA, with the number of *S. aureus* colonies from individual patient culture swabs growing on initial screening plates as follows: 1 to 70 (15 patients), 100 to 1,000 (5 patients), 10^3 to 10^4 (3 patients). Six patients had one or more MSSA subpopulations, and the characteristics of these patient isolates are shown in Table 1. The number of MSSA isolates in these six patients varied widely from as few as a single isolate (patient 3) to as many as 13 MSSA out of 55 MRSA (24%). Four patients had an MSSA population that was completely different from the cocolonizing MRSA, and the MSSA isolates from each patient were different from those of the other patients. It is tempting to speculate that the MSSA represented the patients' preceding colonizing isolate that was replaced by the MRSA that was acquired in the hospital. The MRSA strains colonizing these six patients were SCCmec type II/multilocus sequence type (MLST) CC5/PFGE USA100, the usual health care-associated strain type, in three patients and type IV/CC8/USA300, the epidemic community-associated strain that is increasingly being seen in nosocomial infections (10), in the remaining three patients. The MSSA from two patients had the same MLST and *spa* type as the cocolonizing MRSA, and the PFGE patterns were identical except for a single band, the presumed chromosomal location of SCCmec (Fig. 1a). In order to find out if SCCmec or *mecA* had been deleted from the MSSA in these two patients, PCR was performed using primers anchored in *orfX*, genes flanking SCCmec in published genomes and genes at the extremities of SCCmec types II and IV (Table 2 and Fig. 1). The DNA sequence of each PCR amplicon was determined at the Virginia Commonwealth University nucleic acid research facility. In patient 18, all MSSA colonies had the same PFGE pattern and the DNA sequence of the PCR amplicon in isolate 18-1-2 indicated that SCCmec was absent, precisely at the *attB* insertion site (Fig. 1b; 14). The two MSSA isolates from patient 27 were found to have different PFGE patterns, each with single band differences from the MRSA isolate, but the bands were of different sizes (Fig. 1a). The DNA sequence of the PCR amplicon from MSSA isolate 27-1-1 with the smaller band compared to the MRSA isolate (27-1C in Fig. 1) indicated that the entire SCCmec type IV and the flanking arginine catabolic mobile element (ACME) island (2) were missing, while in isolate 27-1-2 with the smaller deletion, only SCCmec was missing and the ACME region remained (Fig. 1b). It has been previously shown that SCCmec type IV from USA300 isolates and ACME can excise independently of one another (2). We did not investigate whether any MRSA colonies from patient 27 had excised ACME alone.

On the basis of the PCR and typing results, we consider the MRSA/MSSA pairs from patients 18 and 27 to be isogenic strains in which SCCmec was deleted in the MSSA derivative. The sizes of the band shifts are consistent with the loss of type II SCCmec in patient 18 (~50 kb) and of type IV SCCmec plus ACME (~55 kb) and type IV SCCmec alone (~25 kb). The

FIG 1 (a) PFGE pattern of the MRSA and MSSA found in patients where both MRSA and MSSA were present. The molecular typing results are above each lane: MR, methicillin resistant; MS, methicillin susceptible; Ridom is *spa* type, Ridom classification; *mec* is the SCCmec type. Isolate 27-1C shows the standard USA300 PFGE pattern, and additional typing confirmed it to be a USA300 strain with type IV SCCmec presumed to be located on the chromosomal fragment indicated by the white arrow. Isolates 27-1-1 and 27-1-2 differ from the pattern of 27-1C by a single band (white arrows) which are approximately 50 and 25 kb smaller, consistent with the deletion of SCCmec and ACME (50 kb) in 27-1-1 and SCCmec (25kb) in 27-1-2. Isolate 18-1-2C shows a PFGE pattern similar to those characteristic of USA100 isolates. The white arrow indicates the chromosomal fragment presumed to contain type II SCCmec. Isolate 18-1-2 differs from 18-1-2C by a single band (white arrow) which is approximately 55 kb smaller, consistent with the excision of SCCmec. (b) Excision of SCCmec type IV and ACME from the USA300 strain from patient 27 and SCCmec type II from the parent USA100 strain from patient 18. Black arrows indicate the location of primers used to identify the excision of either SCCmec alone or both SCCmec and ACME simultaneously. The primers in Table 2 correspond to the numbers in parentheses next to the gene numbers.

association of the change in PFGE band size with the loss of SCCmec was further investigated by inducing the excision of SCCmec *in vitro*. Plasmid pWA46, containing *ccrAB* under the control of a cadmium-induced promoter, was introduced into the parent MRSA strains from patients 18 and 27 by transduction as previously described (14). Induction of *ccrAB* followed by selective plating to BHI agar plates with and without oxacillin identified several colonies from patient 18 and 27 that

TABLE 2 Primers used in characterization of SCCmec deletions

No. ^a	Primer	Sequence
1	OrfX_F	ACGTTTAGGCCCATACACCA
2	USA300_0025_R	TAGCCATGACCGTGTGAGA
3	USA300_0046_F	GAGAAGACTTGCTGGGTGCT
4	USA300_0047_R	AATCCCGTAATTGCACATC
5	USA300_CopA_F	TCGAAGTAAAAGCCGGAGAA
6	USA300_0083_R	TCGGTGGGTTCATATCTGGT
7	N315_0077_F	TACTGTGCACCGGAACAGAT
8	N315_0078_R	TGAGGATTCTGTGGATGTCCT

^a See Fig. 1b for the position of each numbered primer in the cartoon of the PCR strategy for identifying SCCmec deletions.

showed susceptibility to oxacillin. These strains were analyzed with PFGE, and their pulsed-field patterns were shown to be identical to the MSSA strains isolated from the nasal swabs of patients 18 and 27 (data not shown). PCR amplification of sequences at the *attB* insertion site confirmed that SCCmec had been excised from both strains. These data strongly suggest that the band patterns seen in strains 18-1-2 and 27-1-1 are those of their parent strains, 18-1-2C and 27-1C, following the excision of SCCmec.

It has been noted in other studies that MSSA and MRSA of similar types and PFGE patterns can be found in the same geographic region (5, 12), but it is not clear from those studies if the MSSA strains were the progenitors of the MRSA that has acquired SCCmec or the result of SCCmec or *mecA* excision. In the current study, the MSSA variant of each isogenic MRSA/MSSA strain pair was far less prevalent than the MRSA, suggesting that SCCmec excision had occurred rather than acquisition. It is also interesting that spontaneous SCCmec excision could be detected at all *in vivo*. Spontaneous excision is a rare event *in vitro*. We were unable to detect spontaneous excision of SCCmec from the isolates from patients 18 and 27 after sampling more than 1,000 colonies of each that had been passaged multiple times in drug-free media. Excision only occurred after overproduction of CcrAB as described above. It has been shown in competition experiments that isogenic *mecA*/SCCmec-negative variants are more fit than parents containing *mecA* (3, 11). Thus, in the absence of selective pressure, *mecA*-negative or SCCmec-excised derivatives could arise *in vivo*, where fitness and competition play a role, but not *in vitro*, where fitness is less favored. In addition, the passage of MRSA in certain antibiotics such as vancomycin leads to spontaneous excision of SCCmec or deletion of *mecA* (11). This suggests that SCCmec excision could be driven *in vivo* by exposure of patients to specific drugs, leading to infections that can be treated with beta-lactam antibiotics. These hypotheses should be tested in future studies.

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