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The origin of a methicillin-resistant *Staphylococcus aureus* isolate at a neonatal ward in Sweden—possible horizontal transfer of a staphylococcal cassette chromosome *mec* between methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus aureus*

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ABSTRACT

The first methicillin-resistant *Staphylococcus aureus* (MRSA) strain originated when a staphylococcal cassette chromosome *mec* (SCC*mec*) with the gene *mecA* was integrated into the chromosome of a susceptible *S. aureus* cell. The SCC*mec* elements are common among the coagulase-negative staphylococci, e.g. *Staphylococcus haemolyticus*, and these are considered to be potential SCC*mec* donors when new clones of MRSA arise. An outbreak of MRSA occurred at a neonatal intensive-care unit, and the isolates were all of sequence type (ST) 45, as characterized by multilocus sequence typing, but were not typeable with respect to SCC*mec* types I, II, III or IV. During the same time period, methicillin-resistant *S. haemolyticus* (MRSH) isolates identified in blood cultures at the same ward were found to be genotypically homogenous by pulsed-field gel electrophoresis, and did not carry a type I, II, III or IV SCC*mec* either. Thus, the hypothesis was raised that an SCC*mec* of MRSH had been transferred to a methicillin-susceptible *S. aureus* strain and thereby created a new clone of MRSA that caused the outbreak. This study showed that MRSA from the outbreak carried a *ccr*C and a class C *mec* complex that was also found among MRSH isolates. Partial sequencing of the *mec* complexes showed more than 99% homology, indicative of a common type V SCC*mec*. This finding may provide evidence for a recent horizontal transfer of an SCC*mec* from MRSH to an identified potential recipient, an ST45 methicillin-susceptible *S. aureus* strain, thereby creating a new clone of MRSA that caused the outbreak.

Keywords *mecA*, methicillin-resistant *Staphylococcus haemolyticus*, methicillin-resistant *Staphylococcus aureus*, methicillin-susceptible *Staphylococcus aureus*, multilocus sequence typing, staphylococcal cassette chromosome *mec*

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INTRODUCTION

The first methicillin-resistant *Staphylococcus aureus* (MRSA) strain arose when a staphylococcal cassette chromosome *mec* (SCC*mec*) with the gene *mecA* was integrated into the chromosome of a susceptible *S. aureus* strain [1]. Although very few MRSA clones have disseminated internationally [2–4], it is clear that new clones are continuously arising, spreading and adapting to environments predominantly outside the hospital settings [5,6]. The community-acquired MRSA (CA-MRSA) clones seem to have much more diverse origins than the nosocomial strains, and it is believed that these new clones of MRSA arise spontaneously and independently outside the hospital environment [7]. However, an interesting recent finding is that not only the CA-MRSA but also the hospital-acquired MRSA isolates in a low-endemic area, such as Örebro County, Sweden, represent a large variety of clones [8]. This clearly illustrates that the MRSA isolates studied here consist mainly of sporadic and imported cases, and that no clonal spread has taken place in the Örebro University Hospital (ÖUH).

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A possible reservoir and donor of resistance genes is the large group of coagulase-negative staphylococci (CoNS) that are known to carry SCC*mec* elements at a high frequency. *Staphylococcus haemolyticus* is well known for its multidrug resistance. It is considered to be one of the most virulent CoNS, and sequencing has been performed in order to characterize and understand this species in detail [9].

So far, six structurally different types of SCC*mec* (I, II, III, IV, V and VI) have been identified in MRSA, and these contain a characteristic combination of two essential genetic components, the *mec* gene complex (class A, B or C) and the cassette chromosome recombinase (*ccr*) gene complex (type 1, 2, 3, 4 or 5) [1,10–12]. The SCC*mec* types IV and V have been associated with the emerging MRSA strains that are disseminating in the community and, in addition, several unknown types of SCC*mec* have been found among the CA-MRSA isolates [13,14]. Essential for transfer of an SCC*mec* element between two bacterial species is the action of the cassette chromosome recombinases, which are capable of excising and integrating the element into the bacterial chromosome at a specific site located at the 3′-end of the open reading frame X (*orfX*) [15]. It is likely that a smaller size of SCC*mec*, as in the case of type IV and type V, may provide an advantage when the element is transferred among bacteria, thereby creating new clones of MRSA.

An outbreak of MRSA occurred at the neonatal intensive-care unit (NICU) at ÖUH in 1998, and these isolates were all sequence type (ST) 45, as determined by multilocus sequence typing (MLST), but were not typeable with respect to SCC*mec* types I, II, III or IV [8,16]. During the same period, methicillin-resistant *S. haemolyticus* (MRSH) isolates identified in blood cultures obtained from patients at the same NICU were found to be genotypically homogenous as determined by pulsed-field gel electrophoresis (PFGE) [17] and did not carry type I, II, III or IV SCC*mec* elements either. Thus, the hypothesis was raised that an endemic MRSH strain (or strains) has transferred an SCC*mec* element to a methicillin-susceptible *S. aureus* (MSSA) strain and thereby created a new clone of MRSA that caused the present outbreak in the NICU.

The aim of this study was to examine the SCC*mec* elements of the MRSA and the MRSH isolates from the NICU, to investigate the homology among them, and to compare isolates with other origins, in order to evaluate possible horizontal acquisition of an SCC*mec* element.

MATERIALS AND METHODS

Bacterial isolates

The material analysed consisted of all MRSA ($n = 35$) and MRSH ($n = 16$) isolates from Örebro County that did not carry SCC*mec* type I, II, III or IV elements. Twenty-five of the MRSA isolates had a direct or indirect association with an outbreak in the NICU at ÖUH in 1998. Of the remaining isolates, nine were regarded as CA-MRSA and one as hospital-acquired MRSA with a possible origin in China [8]. All MRSH isolates were from the hospital setting, and all but one isolate originated from the NICU [17]. The exception was an MRSH isolate from a patient at the general intensive-care unit at ÖUH. All MRSH isolates were cultured from blood. Also, all MSSA isolates detected in blood cultures at the NICU from 1990 to 1998 were screened. Those with the same toxin profile, determined by SET-RPLA (reverse passive latex agglutination), TST-RPLA and EXT-RPLA (Denka Seiken, Tokyo, Japan), similar to the MRSA isolated from the NICU, i.e. negative for staphylococcal enterotoxins A, B, C, D and toxic shock syndrome toxin-1, as well as exfoliative toxins A and B, were selected ($n = 28$). The 15 MRSH isolates from the NICU were previously investigated genotypically by PFGE and phenotypically by the Phene Plate system, and constituted one major clone and one additional single isolate [17]. The MRSH isolate from the general intensive-care unit had a unique PFGE pattern in comparison with the major clone of MRSH originating from the NICU (unpublished data), but was possibly related to the additional single isolate identified at the NICU.

S. aureus isolates were identified by routine diagnostic procedures, including DNase and coagulase tests. The species *S. haemolyticus* was determined by using the ID32 Staph system (BioMérieux, Marcy l'Etoile, France). Antibiotic susceptibility testing was performed using disk diffusion and the Etest (AB Biodisk, Solna, Sweden), as recommended by the Swedish Reference Group for Antibiotics (SRGA) and the SRGA subcommittee on methodology (http://www.srga.org). The following antibiotics were tested: oxacillin–cefoxitin, fusidic acid, clindamycin, ciprofloxacin, erythromycin, gentamicin, rifampin and vancomycin. Resistance to oxacillin (MIC >1.0 mg/L according to the SRGA guidelines) was measured using the Etest. Methicillin resistance was confirmed by detecting the presence of *mecA*. The *nuc* gene, which is specific for *S. aureus*, was detected to verify MRSA [16].

DNA isolation and preparation

All isolates were cultured on blood agar (Columbia II agar (BD Diagnostic Systems, Sparks, MD, USA)) 4.25% w/v and defibrinated horse blood (SVA, Uppsala, Sweden) 6% (w/v) and incubated overnight at 37°C. The *S. haemolyticus* isolates were prelysed with lysozyme (25 mg/mL) and lysostaphin (2 mg/mL) (Bio-Rad Laboratories AB, Sundbyberg, Sweden) before automated DNA isolation from all isolates using the MagNa Pure LC DNA Isolation Kit III and the MagNa Pure LC Instrument (Roche Diagnostics, Mannheim, Germany).