Staphylococcal Cassette Chromosome *mec*: Recent Advances and New Insights

Anna C. Shore[1,2*], David C. Coleman[1]

[1]Microbiology Research Unit, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Dublin 2, Ireland. [2]Department of Clinical Microbiology, School of Medicine, University of Dublin, Trinity College, St. James's Hospital, Dublin 8, Ireland

*Corresponding author. Mailing address: Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Dublin 2, Ireland. Phone: 353 1 6127608. Fax: 353 1 6127295. E-mail: anna.shore@dental.tcd.ie

Summary

Staphylococcal cassette chromosome (SCC) elements are complex mobile genetic elements that often carry antimicrobial resistance and in some cases virulence-associated genes. In addition to SCC*mec*, which harbours the methicillin resistance gene *mec*, many different SCC elements have been identified in staphylococci. Recent findings have significantly enhanced our understanding of the diversity of SCC*mec* elements and their contribution to the evolution of MRSA and are the focus of this short review. This includes the identification of (i) novel *mec* genes and allelic variants, (ii) an extensive array of *ccr* and *mec* complex genes as well as SCC*mec*, SCC and pseudo SCC/SCC*mec* elements and composite islands (CIs) in staphylococci, (iii) potential *mec*, SCC and SCC*mec* precursors among distinct coagulase-negative staphylococcal species, and (iv) SCC encoded virulence-associated genes. Due to their complex nature and increasing diversity, detailed characterisation of SCC and SCC*mec* elements and CIs represents a unique challenge but is vital for effective epidemiological typing and tracking of MRSA and other staphylococci and to enhance our understanding of the origins and evolution of MRSA.

Keywords: SCC*mec*; SCC; MRSA; coagulase-negative staphylococci; composite islands; *mecA*; *mecC*.

Introduction

Staphylococcus is a genus of Gram-positive bacteria comprising more than 40 different species encompassing the coagulase-negative staphylococci (CoNS), such as *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, and coagulase-positive staphylococci, such as *Staphylococcus aureus* and *Staphylococcus pseudintermedius*. Staphylococci form part of the normal flora of human and animal skin and mucous membranes and are commonly associated with opportunistic infections. *Staphylococcus aureus* is the most pathogenic species in humans and capable of causing a spectrum of infections due to its ability to express a diverse range of virulence factors and resistance to multiple antimicrobial agents, often encoded on mobile genetic elements (MGEs) (Malachowa & Deleo, 2010).

Staphylococcal cassette chromosome (SCC) elements are a unique class of MGEs prevalent in staphylococci and include SCC*mec*, which harbours the *mec* genes encoding resistance to methicillin and almost all ß-lactam antibiotics (Ito et al., 2003). Since its first identification in 1961 in the United Kingdom a variety of different methicillin resistant *S. aureus* (MRSA) clones have emerged and spread worldwide, many exhibiting resistance to several classes of antimicrobial agents (Chambers and Deleo, 2009; Jevon, 1961). MRSA are a major nosocomial problem worldwide and have also emerged as a significant cause of infections in the community, and among animals (Deleo et al., 2010; Weese, 2010)

SCC*mec* elements are characterised by several well-defined features (Ito et al., 2001; IWG-SCC, 2009). They integrate into the staphylococcal chromosome at a specific site (*attB* or the integration site sequence ISS) within the 3' end of the *orfX* gene encoding a ribosomal methyltransferase (Boundy et al., 2013). SCC*mec* elements are flanked by direct and inverted repeat sequences (DRs and IRs, respectively). Each SCC*mec* element

carries a cassette chromosome recombinase (*ccr*) and *mec* gene complex. The *ccr* genes encode serine recombinases that mediate site- and orientation-specific integration and excision of SCC*mec*. The *mec* complex genes include the *mec* gene and, when present, its regulatory genes *mecR1*, a sensor inducer, and *mecI,* a repressor. SCC*mec* elements also frequently harbour integrated insertions sequences, plasmids and transposons, often encoding additional resistance determinants. The regions outside of the *ccr* and *mec* gene complexes vary in length and have been designated the "Joining" or "J" regions, namely J1, J2 and J3.

Eleven SCC*mec* types based on complete nucleotide sequence data have been described to date in *S. aureus*, ranging in size from 20-60 kb (Garcia Alvarez et al., 2011; IWG-SCC, 2009; Li et al., 2011l Shore et al., 2011a). Each SCC*mec* type has been designated a Roman numeral based on the order of its description and each has a unique combination of the *mec* and *ccr* gene complex (IWG-SCC, 2009; http://www.sccmec.org). Four classes of the *mec* gene complex and seven *ccr* gene complexes have been described to date in MRSA (http://www.sccmec.org). Many different SCC*mec* subtypes have also been described that harbour the same *ccr* and *mec* gene combination but vary in the J regions (IWG-SCC, 2009).

This review focuses on recent findings that have significantly enhanced our understanding of the origins, evolution, structure, function and diversity of SCC*mec*. An in-depth overview of SCC*mec* typing methodology is beyond the scope of this review, but has been discussed in detail elsewhere (Turlej et al., 2011).

Staphylococcal *mec* genes

mecA. Until 2011, *mecA* was the only known *mec* gene type in staphylococci. *mecA* encodes penicillin binding protein PBP2a or PBP2', and when native PBPs have

been inactivated by ß-lactam antibiotics, PBP2a can continue cell-wall biosynthesis (Hartman and Tomasz, 1984; Ito et al., 1999; Reynold and Brown, 1985; Utsui and Yokota, 1985). The majority of MRSA and other methicillin-resistant staphylococci described to date harbour *mecA* and it has been reported in association with SCC*mec* types I-X and their subtypes (IWG-SCC, 2009; Li et al., 2011).

Using criteria proposed by the International Working Group on Staphylococcal Cassette Chromosome elements (IWG-SCC), *mecA* allelic variants can be differentiated into three *mecA* allotypes, namely *mecA*, *mecA1* and *mecA2* (Ito et al., 2012). The majority of MRSA and other methicillin-resistant staphylococci from animals and humans that harbour the *mecA* allotype can express high-level methicillin resistance. In contrast, *mecA1* and *mecA2* have only been reported in staphylococci of animal origin, mainly *Staphylococcus sciuri* and *Staphylococcus vitulinus,* which are commonly susceptible to ß-lactams (Couto et al., 1996; Monecke et al., 2012a; Wu et al., 1996).

A recent study identified 32 *mecA* allelic variants sharing ≥95% nucleotide similarity within the three *mecA* allotypes (Monecke et al., 2012a). Isolates harbouring different *mecA* alleles exhibited a range of resistance levels to ß-lactams. However, isolates harbouring the same *mecA* allele often exhibited different levels of resistance, even within the same species, indicating that strain-specific factors may be significant in the expression of methicillin resistance. Furthermore, different *mecA* alleles can be present in isolates belonging to the same MRSA clone suggesting either multiple independent acquisitions of the same SCC*mec* type harbouring variant *mecA* alleles or evolution of the *mecA* alleles within these strains over time. Finally, Monecke et al. (2012a) also identified different MRSA clones with the same *mecA* allele suggesting the acquisition of a common *mecA* allele by different SCC/SCC*mec* elements and

subsequently different clones, or possibly convergent evolution of *mecA* alleles in different clones.

mecC. In 2011, a highly divergent *mecA* gene, termed *mecC*, was independently identified in MRSA by two groups of researchers, from two patients in Irish hospitals and from 51 hospitalised patients in England, Scotland and Denmark and 15 bovine milk samples in England (Garcia Alvarez et al., 2011; Ito et al., 2012; Shore et al., 2011a). Two of these isolates underwent whole-genome sequencing, one from a patient in Ireland (M10/0061) and one (LGA251) from milk from a cow with mastitis in England and in both studies *mecC* was subsequently identified and localised to a novel and highly divergent SCC*mec* element, designated type XI (class E *mec* and *ccrA1B3*) (Garcia Alvarez et al., 2011; Shore et al., 2011a). Additional MRSA isolates harbouring *mecC* have subsequently been reported from humans, livestock and domestic and wild animals, but to date only in Europe (Cuny et al., 2011; Laurent et al., 2012; Paterson et al., 2012; Petersen et al., 2013; Pichon et al., 2013; Robb et al., 2012; Sabat et al., 2012; Stegger et al., 2012; Walther et al., 2012). However, the reported prevalence rates among human MRSA remains low, ranging from 0.08-5.9% (Cuny et al., 2011; Petersen et al., 2013; Pichon et al., 2013; Stegger et al., 2012).

The predominantly animal MRSA lineages that *mecC* has been identified in (CC130, CC425, CC1943, CC599, CC49), the absence of lysogenic ß-toxin converting bacteriophages in *mecC*-positive isolates, the identification of *mecC* among many different animal species and among humans with contact with animals, suggest an animal origin for *mecC* (Garcia Alvarez et al., 2011; Paterson et al., 2012; Petersen et al., 2013; Robb et al., 2012; Shore et al., 2011a; Stegger et al., 2012; Walther et al., 2012). However, to date the oldest known *mecC*-positive MRSA isolate was recovered in 1975 from a human in Denmark, while currently the oldest known animal isolate was recovered in

1993 (Garcia Alvarez et al., 2011; Paterson et al., 2012). A possible precursor to *mecC* in MRSA was recently identified in a bovine *Staphylococcus xylosus* isolate (Harrison et al., 2012). This novel *mecC* allotype, designated *mecC1*, exhibited 93.5% DNA sequence identity to *mecC*, and formed part of a class E *mec* complex on what was designated a SCC*mec* XI remnant lacking various components of SCC*mec* XI, in particular the *ccr* genes.

The relatively low numbers of *mecC*-positive MRSA reported to date may be due to difficulties with detection of this novel *mec* gene. While many isolates harbouring *mecC* are phenotypically resistant to oxacillin and cefoxitin, resistance levels can be low and isolates can even appear susceptible, particularly to oxacillin (Paterson et al., 2012; Sabat et al., 2012; Shore et al., 2011a; Walther et al., 2012). Commercially available chromogenic MRSA detection agar has also been reported to be unreliable for the detection of *mecC*-positive MRSA (Cuny et al., 2011). While both *mecC* and *mecA* have been shown to encode a PBP2a, they share just 62% amino acid identity and differences have been observed in the properties of the PBP2a proteins encoded by *mecC* and *mecA* (Kim et al., 2012). Some *mecC*-positive isolates test negative for PBP2a with commercial slide latex agglutination assays commonly used to confirm methicillin resistance in MRSA and are not detected as MRSA by conventional *mecA* PCRs, the GenXpert real-time PCR assay or by DNA microarray profiling using the StaphyType kit (Alere, Germany) (Shore et al., 2011a). An upgraded version of the Alere DNA microarray system that includes detection of *mecC* and other SCC*mec* XI-associated genes will soon be available and real-time and endpoint PCRs have been developed to detect *mecC* (Cuny et al., 2011; Pichon et al., 2012; Stegger et al., 2012). These developments will promote the accurate detection of *mecC*-positive MRSA.

Extensive diversity in *mec* and *ccr* genes and SCC*mec* elements in *S. aureus* and other staphylococci

The *mec* gene complex. Five classes of the *mec* gene complex have been described in staphylococci (http://www.sccmec.org) (IWG-SCC, 2009; Katayama et al., 2001; Shore et al., 2011a). A third *mec* regulatory gene *mecR2*, encoding an anti-repressor that was previously designated a xylose repressor homologue (*xylR*), was recently identified in the class A *mec* complex that is required for continuous expression of ß-lactam resistance in strains encoding *mecR1* and *mecI* by inactivation MecI by proteolytic cleavage (Arede et al., 2012).

Extensive work has been undertaken to rationalise the nomenclature of SCC and SCC*mec* elements (IWG-SCC, 2009), however, challenges remain particularly in relation to the nomenclature of *ccr* and *mec* complexes. The *mec* complex designations used to date in various publications have involved different approaches and a consensus approach is required. For example, five variants of the class A *mec* complex and four each of the B and C *mec* complexes, with truncations of varying length in the *mec* regulatory genes *mecI* and *mecR1* and/or the presence of insertion sequences or transposons, have been reported to date but they have been designated class A, A, A1, A.3 and A.4, class B, B_1, B_2/B2 and B(L), and class C1, C2, C1-like and C4, respectively (Heusser et al., 2007; Katayama et al., 2001; Kinnevey et al., 2012; Li et al., 2011; Lim et al., 2002; Shore et al., 2005; Shukla et al., 2004). We recently proposed that the class C1-like *mec* complex be redesignated class C3 *mec* (Kinnevey et al., 2013) and similarly the class A and B *mec* complexes could be redesignated classes A1-A5 and B1-B4 *mec*, respectively, reflecting the chronological order of their description.

The *ccr* gene complex. Integration and excision of SCC*mec* into *orfX* is catalysed by *ccrAB* and *ccrC*, which, similar to bacteriophage integrases (Carroll et al., 1995), are

responsible for catalysing DNA cleavage, strand exchange and recombination between the two attachment sites, one on the SCC element (*att*SCC) and the other on the bacterial chromosome (*attB*) (Wang and Archer, 2010). The inverted repeats flanking SCC*mec* also appear to play a role in the excision but not integration of SCC*mec* and 100-200 bp sequences upstream and downstream of the *attB* sequence determine the frequency and efficiency of SCC*mec* insertion and may determine why SCC*mec* acquisition by *S. aureus* is limited and does not appear to occur in all lineages (Wang et al., 2012).

Guidelines and criteria were established by the IWG-SCC for naming *ccr* genes, allotypes and alleles, with different *ccr* genes sharing <50% DNA sequence identity, allotypes sharing 50-85% DNA sequence identity and alleles sharing >85% DNA sequence identity (IWG-SCC, 2009). Based on this nomenclature, there are three well-defined *ccr* gene types designated *ccrA*, *ccrB* and *ccrC*. A fourth type of *ccr* gene located directly upstream of *ccrC*, termed *ccrAA*, has been identified that shares 35-41% DNA sequence similarity to *ccrA*, *ccrB* and *ccrC* (Monecke et al., 2011). However, a functional role for *ccrAA* has yet to be demonstrated.

Several allotypes of *ccrA*, *ccrB,* and *ccrC* have been described in staphylococci including *ccrA1-A7, ccrB1-B7* and *ccrC1* (Descloux et al., 2008; IWG-SCC, 2009; Urushibara et al., 2011). Based on the different combinations of the *ccr* allotypes eight *ccr* complexes or *ccr* types have been defined in staphylococci (1, *ccrAB1*; 2, *ccrAB2*; 3, *ccrAB3;* 4, *ccrAB4*; 5, *ccrC1*; 6, *ccrA5B3*; 7, *ccrA1B6*; 8, *ccrA1B3*) (http://www.sccmec.org). So far, the type 6 *ccr* genes have been found exclusively in non-*S. aureus* staphylococci and types 7 and 8 exclusively in MRSA (Garza-Gonzalez et al., 2010; IWG-SCC, 2009; Lebeaux et al., 2012; Zong and Lu, 2010; Zong et al., 2011).

At least two novel combinations of *ccr* allotypes have recently been described in staphylococci but have not yet been designated *ccr* type numbers including *ccrA1B4* from

Staphylococcus saprophyticus and *ccrA7B3* from *S. sciuri* (Urushibara et al., 2011; Zong et al., 2011). Using the IWG-SCC MRSA nomenclature system, these two novel *ccr* complexes could be designated *ccr* types 9 and 10, respectively. Also a large number of non-typeable *ccr* complexes have been reported in non-*S. aureus* staphylococci (Bouchami et al., 2011a; Bouchami et al., 2011b; Lebeaux et al., 2011; Ruppe et al., 2009; Zong et al., 2011). Lastly, *ccr* allotypes with >85% DNA identity to more than one allotype have also been described and it has been proposed that in this situation the *ccr* allotype should be assigned to the closest match (Zong and Lu, 2010).

Numerous alleles of each *ccr* allotype have been reported in staphylococci but not all alleles have been designated allele numbers (IWG-SCC, 2009). We recently identified *ccrC1* allele 11 in a SCC_{CRISPR} element that formed part of a composite island (CI) in ST779 MRSA (Kinnevey et al., 2013). We also identified the sixth *ccrA4* and *ccrB4* alleles in the same CI as part of a SCC element and recommended designating those previously identified as *ccrA4* and *ccrB4* alleles 1-5 in the chronological order of their description (Kinnevey et al., 2013). Further complicating *ccr* nomenclature is the fact that two distinct groups of *ccrC* complexes have been identified (Chebowicz et al., 2011). An improved *ccrC* classification scheme has been proposed to aid SCC*mec* typing where those *ccrC* complexes that are located between *orfX* and the *mec* gene complex are designated Group I *ccr* gene complex while those located after *mec* be designated Group II *ccr* gene complex (Chebowicz et al., 2011).

SCC*mec* types in MRSA. The most recently described SCC*mec* elements, IX, X and XI share features that provide insights into the possible origin of SCC*mec* (Li et al., 2011; Shore et al., 2011a). Firstly, all three elements have been found in MRSA strains in humans but are considered to be of animal origin. Secondly, each of these elements carries at least one operon encoding resistance to heavy metals such as arsenic and copper,

which appears to be an attribute of SCC*mec* elements originating in animals. Additionally, the significant sequence divergence evident in SCC*mec* XI suggests that it may have evolved separately to SCC*mec* in human MRSA clones and may have originated in other staphylococcal species or other genera. For example, the *mec* complex of SCC*mec* XI shared its genetic organisation with that of a *mec* complex identified on a transposon integrated in *orfX* in *Macrococcus caseolyticus*, and may represent an ancestral form of the *mec* complex in MRSA. Other unusual features of SCC*mec* IX and X include the type and location of their *ccr* complexes and the orientation of transcription of *mecA* in SCC*mec* X (Li et al., 2011).

SCC*mec* subtypes in MRSA. While many different subtypes of SCC*mec* have been described in MRSA, most have been assigned to SCC*mec* II and IV (IWG-SCC, 2009; Shore et al., 2005). Subtypes essentially harbour the same *ccr* and *mec* complex genes as previously described SCC*mec* elements but differ in the J regions. In 2009, the IWG-SCC recommended that, similar to reporting novel SCC*mec* types, novel SCC*mec* subtypes should be characterised based on nucleotide sequencing of the entire element and that subtypes should be assigned upper or lower case alphabetic suffixes depending on the variation from previously described SCC*mec* elements (IWG-SCC, 2009). An uppercase suffix should indicate variation due to the presence or absence of MGEs, while a lowercase should be used to indicate DNA sequence variation in the J regions (IWG-SCC, 2009).

In Ireland extensive diversity was detected in SCC*mec* II elements from ST8-MRSA isolates (mainly *spa* type t190) that predominated in Irish hospitals in the 1990s (Shore et al., 2005). Five SCC*mec* II subtypes, designated SCC*mec* IIA, IIB, IIC, IID and IIE, were identified that differed in the *mec* complex and J regions, possibly due to recombination within the J1 region and the presence or absence of MGEs in the J2 and J3

regions and within the *mec* complex. However, the nomenclature for these subtypes was assigned prior to the IWG-SCC criteria. Since SCC*mec* IIA-IIE, mainly differ from SCC*mec* II due to sequence variation in the J1 region it may be appropriate to reclassify these with lower case suffixes i.e. IIa-IIe. However, other SCC*mec* elements have been designated these SCC*mec* subtypes. Since SCC*mec* types IIB, IID and IIE also differ from SCC*mec* II due to the absence of pUB110 in the latter two subtypes and absence of Tn*554* in IIB, the use of the uppercase suffixes may, in these instances, be appropriate.

The current IWG-SCC guidelines provide valuable guidance on many aspects of SCC*mec* nomenclature, but as outlined above, there are still ambiguities that need to be resolved. One difficulty associated with the current SCC*mec* nomenclature is that there are only a limited number of alphabetic designations that can be used for SCC*mec* subtype designation. An alternative nomenclature for SCC*mec* subtype designation was proposed which involved designating each of the J regions an Arabic number in the order of discovery, but this has not been widely adopted. According to the IWG-SCC (2009) a novel computer-based binary system that will assign SCC*mec* subtypes in an unambiguous manner based on the presence or absence of specific DNA sequences is under development.

SCC*mec* in non-*S. aureus* staphylococci. SCC*mec* is even more prevalent and diverse in non-*S. aureus* staphylococci than MRSA, and CoNS in particular are considered to be a reservoir for SCC*mec* in *S. aureus* (Barbier et al., 2010; Bouchami et al., 2011b; Garza-Gonzalez et al., 2010; Hanssen and Ericson Sollid, 2006; Ruppe et al., 2009; Zong et al., 2011). SCC*mec* is less well defined in non-*S. aureus* staphylococci and they are often non-typeable due to (i) no identified *ccr* and/or *mec* gene complex, (ii) detection of more than one *ccr* complex, or (iii) detection of novel combinations of *ccr* and *mec* complex genes that cannot be assigned to previously described SCC*mec* types.

When SCC*mec* types are reported in non-*S. aureus* staphylococci that have previously been reported in MRSA then the MRSA SCC*mec* designations are used. However, while the nomenclature for *ccr* and *mec* complexes used for MRSA in some cases includes those found exclusively in non-*S. aureus* staphylococci, SCC*mec* elements to date found exclusively in non-*S. aureus* staphylococci with novel combinations of *ccr* and *mec* gene complexes are not assigned SCC*mec* type numbers, and if they are assigned numeral names they are different to those used for MRSA. In most cases this is because these SCC*mec* elements have predominantly been characterised using PCR-based methods and not by complete nucleotide sequencing. For example, in one study 10 SCC*mec* elements with novel combinations of *mec* and *ccr* complexes in a variety of non-*S. aureus* staphylococci from humans were designated untypeable 1-10 (UT1-UT10) (Zong et al., 2011). Another study described novel SCC*mec* types in *Staphylococcus hominis* and designated these NT1-4 or new1 (Bouchami et al., 2011a). While these temporary designations are useful, all such novel types should ideally be defined on the basis of complete nucleotide sequencing. However, entire nucleotide sequence data of novel SCC*mec* elements in non-*S. aureus* staphylococci are scarce. Among those that have been sequenced are an SCC*mec* element with class A *mec* and type 6 *ccr* (*ccrA5B3*) in *Staphylococcus cohnii* (Zong and Lu, 2010). Since non-*S. aureus* staphylococci appear to be a reservoir for SCC*mec* a rational and combined approach to nomenclature of SCC*mec* types is required, ideally by extending the MRSA scheme.

Specific SCC*mec* types have been found to predominate among particular non-*S. aureus* staphylococcal species. For example SCC*mec* types II-III and type V predominate among *S. pseudointermedius* isolates in Europe and the USA, respectively, but SCC*mec* types IV and VII (*S. pseudointermedius* VII, differs from VII in MRSA) as well as non-typeable and novel types have also been identified (Perreten et al., 2010). Among CoNS,

the most diversity in SCC*mec* has been detected in *S. epidermidis, S. heamolyticus* and *S. hominis*. Several studies have shown that SCC*mec* type IV is common among *S. epidermidis* but SCC*mec* types I, II, III, V, VI and novel types have also been identified (Lebeaux et al., 2012; Rolo et al., 2012; Smyth et al., 2010; Wisplinghoff et al., 2003; Zong et al., 2011). In *S. haemolyticus*, the SCC*mec* V *ccr* and *mec* complex genes *ccrC* and class C *mec* have been found to predominate but commonly not in association with SCC*mec* V, but with multiple possible novel SCC*mec* types (Bouchami et al., 2012; Lebeaux et al., 2012; Ruppe et al., 2009). Among *S. hominis* isolates common SCC*mec* types include a novel SCC*mec* type (class A *mec* and *ccrAB1*) as well as SCC*mec* type VI, VIII and III and non-typeable elements have also been identified (Bouchami et al., 2011a; Lebeaux et al., 2012; Zong et al., 2011). The large numbers of non-typeable SCC*mec* elements identified in these studies by PCR highlight the need for sequencing of entire SCC*mec* elements from CoNS.

SCC and atypical SCC/SCC*mec* elements

A range of SCCs and atypical SCC/SCC*mec* elements have been described in staphylococci and examples of fully-sequenced elements are listed in Table 1. While these elements share the common features of integration into the ISS within *orfX* and are flanked by DRs and IRs, each has unique characteristics and appear to represent various stages in the evolution of SCC and SCC*mec* elements. The variety of these elements in staphylococci demonstrates the role that SCC plays in allowing staphylococci to adapt to its environment through the acquisition of additional antimicrobial resistance and virulence genes.

SCC elements. These elements harbour a *ccr* complex but lack *mecA* and often carry other antimicrobial resistance or virulence-associated genes and can form part of larger

CIs e.g. the SCC*fus* element carrying the fusidic acid resistant in MSSA476 (Holden et al., 2004) (Table 1). The IWG-SCC has recommended that SCC elements should be designated by adding a suffix after SCC describing the characteristic gene name or function, or when no specific gene has been identified, using the strain name (IWG-SCC, 2009). While the only currently fully sequenced SCC element from *S. hominis* are SCC*cap1* and SCC_{12263} (Table 1), a recent study has shown that *S. hominis* isolates may harbour many potential SCC elements based on the detection of *ccr* but no *mec* complex genes (Bouchami et al., 2011a). Thus, *S. hominis* may constitute a reservoir for SCC elements and for the assembly of SCC*mec* elements (Bouchami et al., 2011a).

Pseudo (ψ) SCC*mec* elements. These elements carry *mecA* but lack the *ccr* complex. To date five ψSCC*mec* elements have been described in staphylococci (Table 1). Due to their similarity to previously described SCC*mec* elements, three of these ψSCC*mec* elements appear to be the result of deletion events or may represent precursors of known SCC*mec* elements. For example, in ψSCC*mec* II.5 and ψ$SCC_{mec16691}$, the regions extending from within or just beyond the *mec* complex to the chromosomal junction are absent (Chen et al., 2010; Han et al., 2009). In ψSCC*mec* II.5, this missing region is replaced by Tn*6012*, while in ψ$SCC_{mec16691}$ the truncated element lacks the J1 and J2 regions as well as *ccr*. The *S. xylosus* ψ$SCC_{mecS04009}$ is similar to SCC*mec* XI in that it carries class E *mec*, but lacks the *ccr* genes and the J1 and J2 regions associated with SCC*mec* XI (Harrison et al., 2012). However, many of the genes encoded in J1 and J2 regions of SCC*mec* XI, including the arsenic resistance genes, are located either adjacent to ψ$SCC_{mecS04009}$ or elsewhere on the chromosome of the *S. xylosus* element indicating that this may represent an ancestral form of SCC*mec* XI (Harrison et al., 2012).

Pseudo (ψ) SCC elements. These elements lack *ccr* and *mec* genes, commonly form part of CIs (Table 1) and can be differentiated into three groups (i) arginine catabolic

mobile elements (ACMEs), (ii) SCC-like elements, chromosome cassettes or SCC*mec* insertion site genomic sequences, and (iii) SCC*mec* remnants that lack *mecA* and *ccr* genes but have a genomic organisation almost identical to a previously described SCC*mec* element apart from the absence of a contiguous region of *ccr* and *mec* and intervening genes (Table 1). ACMEs have been identified in complete and truncated forms and have been reported in different staphylococcal species within many different CIs (Bartels et al., 2011; Diep et al., 2006; Miragaia et al., 2009; Pi et al., 2009; Shore et al., 2011b). Complete ACMEs can contain *arc* and *opp* gene clusters, both of which are homologues of genes that are recognised bacterial virulence factors (Diep et al., 2006). SCC-like elements, chromosome cassettes or SCC*mec* insertion site genomic sequences have very different nucleotide sequences to those found in other SCC/SCC*mec* elements and form part of CIs but generally do not encode any known virulence or resistance genes (Table 1). Finally, SCC*mec* remnants have been described that lack *mecA* and *ccr* genes but have similarity to previously described SCC*mec* suggesting that they are either derived from SCC*mec* elements with the loss of *mecA* and sections of SCC*mec* or represent SCC*mec* precursors prior to the acquisition of *mecA* (Table 1). Another fully sequenced remnant (SCC*mec* IID remnant) that harbours *ccr* and *mec* complex genes but without *mecA* is therefore not classified as a SCC, ψSCC*mec* or ψSCC element (Shore et al., 2008).

Composite islands (CIs). Wang et al. (2012) reported multiple *att* sites on the staphylococcal chromosome and on SCC*mec,* which can lead to the accumulation of complex CIs differentiated by the presence of multiple DRs flanking individual element components. Several CIs, ranging in size from 41-91 kb have been fully sequenced in staphylococci (Table 1). These are distinct from individual or mosaic SCC*mec* elements that have two or more *ccr* genes on one element (Heusser et al., 2007; IWG-SCC, 2009). They often harbour a number of *ccr* and antimicrobial resistance genes and in some cases

virulence-associated genes (Table 1). For example, a 51 kb CI in ST779 MRSA consisted of three distinct elements, each flanked by DRs, including a ψSCC*mec* with *mecA*, *fusC* and two copper resistance genes, a SCC element with *ccrAB4* and a SCC_{CRISPR} element with *ccrC* and a clustered regularly interspaced short palindromic repeat (CRISPR) region that encodes a prokaryotic defence mechanism against foreign DNA (Kinnevey et al., 2013). In non-*S. aureus* staphylococci reports of two or more *ccr* genes in an isolate are common but the element(s) on which they are located are often not well characterised (Bouchami et al., 2011b; Ruppe et al., 2009; Zong et al., 2011). However, two large CIs have been described in *S. haemolyticus* and *S. epidermidis* (Table 1).

The origin and evolution of SCC*mec*

Several studies have shown that SCC and *mecA* existed as individual genetic components that originated and evolved in different animal commensals, prior to the emergence of SCC*mec* in staphylococci. While the mechanism(s) of transfer and the precise steps in the evolution of *mec* and SCC have not yet been elucidated, evidence to date indicates that *S. sciuri*, *Staphylococcus fleuretti*, *S. xylosus*, *M. caseolyticus* and *S. hominis* all carry SCC and/or *mec* genes that represent ancestral forms and different steps in the evolution of SCC*mec* (Couto et al., 1996; Tsubakishita et al., 2010a; Tsubakishita et al., 2010b; Wu et al., 1998).

All *S. sciuri* isolates investigated to date carry *mecA* (predominantly allotype *mecA1*) and most isolates do not exhibit methicillin resistance indicating that its original physiological function may not have been to confer ß-lactam resistance (Couto et al., 1996; Couto et al., 2000; Monecke et al., 2012a). A xylose gene cluster, that includes *xylR*, a homologue of *mecR2*, has been linked to *mecA1* in *S. sciuri*, suggesting that the accumulation of *mecA* and at least some of its regulatory genes, may have originally

occurred here (Tsubakishita et al., 2010b). Interestingly, *mecA* the *mec* regulatory genes *mecI* and *mecR1* have been identified in *S. fleuretti* as well as a xylose operon with *xylR*, located immediately downstream of the *mec* complex genes and may represent a putative precursor of the class A *mec* complex (Tsubakishita et al., 2010b).

A theory was proposed in the 1980s that a recombination event linking *mecA* to the ß-lactamase regulatory genes may have occurred due to the similarity between *mecR1* and *mecI* and the *blaZ* regulatory genes *blaR1* and *blaI* (Song et al., 1987). The finding of *mecB* with a *mec* complex also harbouring *blaZ* in *M. caseolyticus* as well as the finding of *mecC* and *mecC1* on a *mec* complex also carrying *blaZ* in MRSA with SCC*mec* XI and in *S. xylosus*, respectively, further supports this theory (Harrison et al., 2013; Shore et al., 2011; Tsubakishita et al., 2010b). While no *ccr* genes were identified in *S. xylosus*, in *M. caseolyticus*, a SCC element (SCC_{7096}) with *ccr* was identified adjacent to the *mec* complex on a separate element but on the same transposon within a plasmid integrated downstream of *orfX* in the chromosome and the *mec* complex was flanked by DRs specifically recognised by *ccr* genes (Harrison et al., 2013). In staphylococci, the numerous putative SCC elements that have recently been identified in *S. hominis* suggests that the assembly of the *mec* complex and SCC may have occurred here (Bouchami et al., 2011a).

Recent studies have revealed that specific staphylococcal species may be sources of particular SCC*mec* elements or specific *ccr* and *mec* complex genes in MRSA. In particular *S. epidermidis* appears to be a reservoir for SCC*mec* IV, *S. haemolyticus* for *ccrC* and class C *mec* and *S. hominis* for *ccrAB1*, *ccrAB4* and class A *mec* (Bouchami et al., 2011a; Bouchami et al., 2011b; Lebeaux et al., 2012). However, additional detailed studies of SCC*mec* in individual CoNS species are needed in order to determine the precise role that each played in the emergence of SCC*mec* in MRSA.

Virulence genes in SCC elements

A number of virulence-associated genes have been identified on SCC elements and their presence on SCC/SCC*mec* elements and CIs also harbouring antimicrobial resistance genes may promote their spread among staphylococci. The best known example is the ACME-*arc* pseudo-SCC element, which is more abundant among CoNS, especially *S. epidermidis* and S. *haemolyticus*, than *S. aureus* (Diep et al., 2006; Miragaia et al., 2009; Pi et al. 2009; Shore et al. 2011). Evidence to date suggests that the ACME-*arc* operon, encoding an arginine deaminase pathway, contributes to host colonisation and transmission (Diep et al. 2006).

Although rare, there have been a number of reports of CRISPR (clustered randomly interspersed palindromic repeat region) in SCC/SCC*mec* elements in CoNS and MRSA (Holt et al., 2011; Kinnevey et al., 2013). CRISPR consists of multiple short nucleotide repeat sequences which constitutes a prokaryotic defence mechanism that protects the bacterial genome against foreign invading DNA i.e. bacteriophage and plasmid DNA.

Phenol-soluble modulin (PSM), a proinflammatory cytolytic toxin, is the only toxin gene identified to date in SCC*mec* (Queck et al., 2009). It has been identified in a variety of animal and human staphylococci and is located between *mecI* and *mecR2* in SCC*mec* types II, III and VIII and some SCC*mec* II subtypes as well as some irregular or truncated SCC*mec* elements (Monecke et al., 2012b). PSM-*mec* has been shown to supress colony spreading and exotoxin production, in particular the core genome toxin PSMα, but enhances biofilm formation (Kaito et al., 2011).

A putative novel adhesion encoded by a the *spj* gene was recently identified in a novel SCC*mec* IV subtype (IV1) in CA-MRSA in Japan. This cell wall anchored surface

protein with a conserved Leu-Pro-X-Thr-Gly (LPXTG) pentapeptide sequence at the C-terminus may play a role in attachment and host tissue colonisation (Iwao et al., 2012).

Lastly, in addition to *mec*, genes encoding several other cell wall synthesis enzymes have been identified in SCC elements and it has been suggested that SCC elements may be specialised for the transfer of such genes (Luong et al., 2002; Mongkolrattanothai et al., 2004). Examples include the type 1 capsule polysaccharide gene *cap1* on a SCC element in *S. hominis* and a homolog of *pbp4* and a teichoic acid biosynthesis protein, *tagF*, on a SCC element that forms part of a CI in *S. epidermidis* (Table 1).

Conclusions

Studies undertaken over the last decade to elucidate the mechanisms underlying the emergence and evolution of MRSA strains have revealed an ever-increasing complexity among SCC*mec* and SCC elements in staphylococci and have provided new insights into the likely origins of SCC*mec*. The increasing diversity identified in SCC*mec* and SCC elements has complicated typing methodology currently used for epidemiological investigations but has also highlighted difficulties with current SCC*mec* and SCC nomenclature, especially among non-*S. aureus* staphylococci. Investigating the role of specific CoNS and other bacterial genera in the evolution of SCC*mec* in MRSA, in particular using high-throughput whole-genome sequencing platforms to accurately determine the DNA sequence of entire elements, will provide fruitful avenues for future research.

Acknowledgments

Research undertaken in the authors' laboratory was supported by the Dublin Dental University Hospital Microbiology Research Unit. The authors' wish to thank Dr. Stefan Monecke (Alere Technologies GmbH, Jena, Germany; Institute for Medical Microbiology and Hygiene, Faculty of Medicine "Carl Gustav Carus", Technical University of Dresden, Germany) and Dr. Peter Kinnevey (Division of Oral Biosciences, Dublin Dental University Hospital) for critical reading of the manuscript.

References

Arakere, G., Nadig, S., Ito T., Ma, X.X., Hiramatsu, K. 2009. A novel type-III staphylococcal cassette chromosome *mec* (SCC*mec*) variant among Indian isolates of methicillin-resistant Staphylococcus aureus. FEMS Microbiol. Lett. 292(1):141-148.

Arêde, P., Milheiriço, C., de Lencastre, H., Oliveira, D.C. 2012. The anti-repressor MecR2 promotes the proteolysis of the *mecA* repressor and enables optimal expression of beta-lactam resistance in MRSA. PLoS Pathog. 8(7):e1002816.

Barbier, F, Ruppé, E., Hernandez, D., Lebeaux, D., Francois, P., Felix, B., Desprez, A,, Maiga, A., Woerther, P.L., Gaillard, K., Jeanrot, C., Wolff, M., Schrenzel, J., Andremont, A., Ruimy, R. 2010. Methicillin-resistant coagulase-negative staphylococci in the community: high homology of SCC*mec* IVa between *Staphylococcus epidermidis* and major clones of methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis. 202(2):270-281.

Bartels, M. D., Hansen, L.H., Boye, K, Sørensen, S.J., Westh, H. 2011. An unexpected location of the arginine catabolic mobile element (ACME) in a USA300-related MRSA strain. PLoS One 6(1):e16193.

Berglund, C., Ito, T., Ikeda, M., Ma, X.X., Söderquist, B., Hiramatsu, K. 2008. Novel type of staphylococcal cassette chromosome mec in a methicillin-resistant *Staphylococcus aureus strain isolated in Sweden.* Antimicrob. Agents Chemother. Oct;52(10):3512-3516.

Bouchami, O, Ben Hassen, A., de Lencastre, H., Miragaia, M. 2011a. Molecular epidemiology of methicillin-resistant *Staphylococcus hominis* (MRSHo): low clonality and reservoirs of SCC*mec* structural elements. PLoS One 6(7):e21940.

Bouchami, O., Ben Hassen, A., de Lencastre, H., Miragaia, M. 2011b. High prevalence of *mec* complex C and *ccrC* is independent of SCC*mec* type V in *Staphylococcus haemolyticus*. Eur. J. Clin. Microbiol. Infect. Dis. 31(4):605-614.

Boundy, S., Safo, M.K., Wang, L., Musayev, F.N., O'Farrell, H.C., Rife, J.P., Archer, G.L. 2013. Characterization of the *Staphylococcus aureus* rRNA Methyltransferase Encoded by *orfX*, the Gene Containing the Staphylococcal Chromosome Cassette *mec* (SCC*mec*)Insertion Site. J. Biol. Chem. 288(1):132-140.

Carroll, D., M. A. Kehoe, D. Cavanagh, and D. C. Coleman. 1995. Novel organization of the site-specific integration and excision recombination functions of the *Staphylococcus aureus* serotype F virulence-converting phages phi 13 and phi 42. Mol. Microbiol. 16:877-893.

Chambers, H. F., Deleo, F.R. 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat. Rev. Microbiol. 7:629-641.

Chen, L., Mediavilla, J.R., Smyth, D.S., Chavda, K.D., Ionescu, R., Roberts, B.R., Robinson, D.A., Kreiswirth, B.N. 2010. Identification of a novel transposon (Tn6072) and a truncated staphylococcal cassette chromosome *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 54: 3347-3354.

1 Chlebowicz, M. A., van Dijl, J. M., Buist, G. 2011. Considerations for the distinction of
2 *ccrC*-containing staphylococcal cassette chromosome *mec* elements. Antimicrob.
3 Agents Chemother. 55(4):1823-1824.
4 Couto, I., de Lencastre, H., Severina, E., Kloos, W., Webster, J.A., Hubner, R.J., Sanches,
5 I.S., Tomasz, A. 1996. Ubiquitous presence of a *mecA* homologue in natural
6 isolates of *Staphylococcus sciuri*. Microb. Drug Resist. 2(4):377-291.
7 Couto, I., Sanches, I.S., Sá-Leão, R., de Lencastre, H. 2000. Molecular characterization of
8 *Staphylococcus sciuri* strains isolated from humans. J. Clin. Microbiol.
9 38(3):1136-1143.
10 Cuny, C., Layer, F., Strommenger, B., Witte, W. 2011. Rare occurrence of methicillin-
11 resistant *Staphylococcus aureus* CC130 with a novel *mecA* homologue in humans
12 in Germany. PLoS One 6(9):e24360.
13 DeLeo, F.R., Otto, M., Kreiswirth, B.N., Chambers, H.F. 2010. Community-associated
14 meticillin-resistant *Staphylococcus aureus*. Lancet 375(9725):1557-1568.
15 Descloux, S., Rossano A., Perreten, V. 2008. Characterization of new staphylococcal
16 cassette chromosome *mec* (SCC*mec*) and topoisomerase genes in fluoroquinolone-
17 and methicillin-resistant *Staphylococcus pseudintermedius*. J. Clin. Microbiol.
18 46(5):1818-1823.
19 Diep, B.A., Gill, S.R., Chang, R.F., Phan, T.H., Chen, J.H., Davidson, M.G., Lin, F., Lin,
20 J., Carleton, H.A., Mongodin, E.F., Sensabaugh, G.F., Perdreau-Remington, F. 2006.
21 Complete genome sequence of USA300, an epidemic clone of community-
22 acquired meticillin-resistant *Staphylococcus aureus*. Lancet 367(9512):731-739.
23 García-Álvarez, L., Holden, M.T., Lindsay, H., Webb, C.R., Brown, D.F., Curran, M.D.,
24 Walpole, E., Brooks, K., Pickard, D.J., Teale, C., Parkhill, J., Bentley, S.D.,
25 Edwards, G.F., Girvan, E.K,. Kearns, A.M., Pichon, B., Hill, R.L., Larsen, A.R.,

Skov, R.L., Peacock, S.J, Maskell, D.J., Holmes M.A. 2011. MRSA harbouring a novel *mecA* homologue emerging in human and bovine populations in the UK and Denmark. LACET Infect. Dis. Aug;11(8):595-603.

Garza-González, E., López, D., Pezina, C., Muruet ,W., Bocanegra-García, V., Muñoz, I., Ramírez, C., Llaca-Díaz, J.M. 2010. Diversity of staphylococcal cassette chromosome *mec* structures in coagulase-negative staphylococci and relationship to drug resistance. J. Med. Microbiol. 59(3):323-329.

Han, X., Ito, T., Takeuchi, F, Ma, X.X., Takasu, M., Uehara, Y., Oliveira, D.C., de Lencastre, H., Hiramatsu, K. 2009. Identification of a novel variant of staphylococcal cassette chromosome *mec*, type II.5, and Its truncated form by insertion of putative conjugative transposon Tn6012. Antimicrob. Agents Chemother. 53(6):2616-2619.

Hanssen, A. M., Ericson Sollid, J.U. 2006. SCC*mec* in staphylococci: genes on the move. FEMS Immunol. Med. Microbiol. 46(1):8-20.

Harrison, E.M., Paterson, G.K., Holden, M.T., Morgan, F.J., Larsen, A.R., Petersen, A., Leroy, S., De Vliegher, S., Perreten, V., Fox, L.K., Lam, T.J., Sampimon, O.C., Zadoks, R.N., Peacock, S.J., Parkhill, J., Holmes, M.A. 2012. A *Staphyloccocus xylosus* isolate with a new *mecC* allotype. Antimicrob. Agents Chemother. epub ahead of print. PMID: 23274660

Hartman, B. J., Tomasz, A. 1984. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. J. Bacteriol. 158(2):513-6.

Heusser R, Ender M, Berger-Bächi B, McCallum N. 2007. Mosaic staphylococcal cassette chromosome *mec* containing two recombinase loci and a new *mec* complex, B2. Antimicrob. Agents Chemother. 51(1):390-393.

Holden, M.T., Feil, E.J., Lindsay, J.A., Peacock, S.J., Day, N.P., Enright, M.C., Foster, TJ, Moore, C.E., Hurst, L., Atkin, R., Barron, A., Bason, N.., Bentley, S.D., Chillingworth, C., Chillingworth, T., Churcher, C., Clark, L., Corton, C., Cronin, A., Doggett, J., Dowd, L., Feltwell, T., Hance, Z., Harris, B., Hauser, H., Holroyd, S., Jagels, K., James, K.D., Lennard, N., Line, A., Mayes, R., Moule, S., Mungall K., Ormond, D., Quail, M.A., Rabbinowitsch, E., Rutherford, K., Sanders, M., Sharp, S., Simmonds, M., Stevens, K., Whitehead, S., Barrell, B.G., Spratt, B.G., Parkhill, J. 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc. Natl. Acad. Sci. USA. Jun 29;101(26):9786-9791.

Holt, D.C., Holden, M.T., Tong, S.Y., Castillo-Ramirez, S., Clarke, L., Quail, M.A., Currie, B.J., Parkhill, J., Bentley, S.D., Feil, E.J., Giffard, P.M. 2011. A very early-branching *Staphylococcus aureus* lineage lacking the carotenoid pigment staphyloxanthin. Genome Biol. Evol. 3:881-889.

International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). 2009. Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. Antimicrob. Agents Chemother. 53:4961-4967.

Ito, T., Hiramatsu, K., Tomasz, A., de Lencastre, H., Perreten, V,. Holden, M.T., Coleman, D.C., Goering, R., Giffard, P.M., Skov, R.L., Zhang, K., Westh, H., O'Brien, F., Tenover, F.C., Oliveira, D.C., Boyle-Vavra, S., Laurent, F., Kearns, A.M., Kreiswirth, B., Ko, K.S., Grundmann, H., Sollid, J.E., John, J.F. Jr, Daum, R., Soderquist, B., Buist, G.; International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). 2012. Guidelines for

reporting novel *mecA* gene homologues. Antimicrob. Agents Chemother. 56(10):4997-9.

Ito, T., Katayama, Y., Asada, K., Mori, N., Tsutsumimoto, K., Tiensasitorn, C., Hiramatsu, K. 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 45(5):1323-1336.

Ito, T., Katayama, Y., Hiramatsu K. 1999. Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents Chemother. 43(6):1449-1458.

Ito, T., Okuma, K., Ma, X.X., Yuzawa, H., Hiramatsu, K. 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist. Updat. 6(1):41-52.

Iwao, Y., Takano, T., Higuchi, W., Yamamoto, T. 2012. A new staphylococcal cassette chromosome *mec* IV encoding a novel cell-wall-anchored surface protein in a major ST8 community-acquired methicillin-resistant *Staphylococcus aureus* clone in Japan. J. Infect. Chemother. 18(1):96-104.

Jansen, W.T., Beitsma M.M., Koeman, C.J., van Wamel, W.J., Verhoef, J., Fluit, A.C. 2006. Novel mobile variants of staphylococcal cassette chromosome *mec* in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 50(6):2072-2078.

Jevons, M.P. 1961. Celbenin-resistant staphylococci. Br. Med. J. i:124-125.

Kaito, C., Saito, Y., Nagano, G., Ikuo, M., Omae, Y., Hanada, Y., Han, X., Kuwahara-Arai, K., Hishinuma, T., Baba, T., Ito, T., Hiramatsu, K., Sekimizu, K. 2011. Transcription and translation products of the cytolysin gene *psm-mec* on the mobile genetic element SCCmec regulate *Staphylococcus aureus* virulence. PLoS Pathog. 7(2):e1001267.

Katayama, Y., Ito, T., Hiramatsu, K. 2001. Genetic organization of the chromosome region surrounding *mecA* in clinical staphylococcal strains: role of IS431-mediated *mecI* deletion in expression of resistance in *mecA*-carrying, low-level methicillin-resistant Staphylococcus haemolyticus. Antimicrob. Agents Chemother. 45(7):1955-1963.

Katayama, Y., Takeuchi, F., Ito, T., Ma, X.X., Ui-Mizutani, Y., Kobayashi, I., Hiramatsu, K. 2003. Identification in methicillin-susceptible *Staphylococcus hominis* of an active primordial mobile genetic element for the staphylococcal cassette chromosome *mec* of methicillin-resistant *Staphylococcus aureus*. J. Bacteriol. 185(9):2711-22.

Kim, C., Milheiriço, C., Gardete, S., Holmes, M.A., Holden, M.T., de Lencastre, H., Tomasz, A. 2012. Properties of a novel PBP2A protein homolog from Staphylococcus aureus strain LGA251 and its contribution to the beta-lactam-resistant phenotype. J. Biol. Chem. 287(44):36854-3663.

Kinnevey, P.M., Shore, A.C., Brennan, G.I., Sullivan, D.J., Ehricht, R., Monecke, S., Slickers, P., Coleman, D.C. 2013. Emergence of Sequence Type 779 Methicillin-Resistant *Staphylococcus aureus* Harboring a Novel Pseudo Staphylococcal Cassette Chromosome *mec* (SCC*mec*)-SCC-SCCCRISPR Composite Element in Irish Hospitals. Antimicrob Agents Chemother 57(1):524-531.

Laurent, F., Chardon, H., Haenni, M., Bes, M., Reverdy, M.E., Madec, J.Y., Lagier, E., Vandenesch, F., Tristan, A. 2012. MRSA harboring *mecA* variant gene *mecC*, France. Emerg. Infect. Dis. 18(9):1465-1467.

Lebeaux, D., Barbier, F., Angebault, C., Benmahdi, L., Ruppé, E., Felix, B., Gaillard, K., Djossou, F., Epelboin, L., Dupont, C., Renard, M., Peroz, G., Vandenesch, F., Wolff, M., Andremont, A., Ruimy, R. 2012. Evolution of nasal carriage of

methicillin-resistant coagulase-negative staphylococci in a remote population. Antimicrob. Agents Chemother. 56(1):315-323.

Li, S., Skov, R.L., Han, X., Larsen, A.R., Larsen, J., Sørum, M., Wulf, M., Voss, A., Hiramatsu, K., Ito, T. 2011. Novel types of staphylococcal cassette chromosome *mec* elements identified in clonal complex 398 methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. 55(6):3046-3050.

Lim, T. T., Coombs, G.W., Grubb, W.B. 2002. Genetic organization of *mecA* and *mecA*-regulatory genes in epidemic methicillin-resistant *Staphylococcus aureus* from Australia and England. J. Antimicrob. Chemother. 50(6):819-824.

Lindqvist, M., Isaksson, B., Grub, C., Jonassen, T.Ø., Hällgren, A. 2012. Detection and characterisation of SCC*mec* remnants in multiresistant methicillin-susceptible *Staphylococcus aureus* causing a clonal outbreak in a Swedish county. Eur. J. Clin. Microbiol. Infect. Dis. 2012. 31(2):141-147

Luong, T.T., Ouyang, S., Bush, K., Lee, C.Y. 2002. Type 1 capsule genes of *Staphylococcus aureus* are carried in a staphylococcal cassette chromosome genetic element. J. Bacteriol. 184(13):3623-3629.

Malachowa, N., Deleo, F.R. 2010. Mobile genetic elements of *Staphylococcus aureus*. Cell. Mol. Life Sci. 67:3057-3071.

Miragaia, M., de Lencastre, H., Perdreau-Remington, F., Chambers, H.F., Higashi, J., Sullam, P.M., Lin, J., Wong, K.I., King, K.A., Otto, M., Sensabaugh, G.F., Diep, B.A. 2009. Genetic diversity of arginine catabolic mobile element in *Staphylococcus epidermidis*. PLoS One 4(11):e7722.

Monecke, S., Coombs, G., Shore, A.C., Coleman, D.C., Akpaka, P., Borg, M., Chow, H., Ip, M., Jatzwauk, L., Jonas, D., Kadlec, K., Kearns, A., Laurent, F., O'Brien, F.G., Pearson, J., Ruppelt, A., Schwarz, S., Scicluna, E., Slickers, P., Tan, H.L., Weber,

S., Ehricht, R. 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS One 6(4):e17936.

Monecke, S., Müller, E., Schwarz, S., Hotzel, H., Ehricht, R. 2012a. Rapid microarray-based identification of different *mecA* alleles in Staphylococci. Antimicrob. Agents Chemother. 56(11):5547-5554.

Monecke, S., Engelmann, I., Archambault, M., Coleman, D.C., Coombs, G.W., Cortez, de Jäckel, S., Pelletier-Jacques, G., Schwarz, S., Shore, A.C., Slickers, P., Ehricht, R. 2012b. Distribution of SCC*mec*-associated phenol-soluble modulin in staphylococci. Mol. Cell. Probes 26(2):99-103.

Mongkolrattanothai K, Boyle S, Murphy TV, Daum RS. 2004. 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 Chemother. 48(5):1823-36.

Paterson, GK, Larsen, AR, Robb, A, Edwards, GE, Pennycott, TW, Foster, G, Mot, D, Hermans, K, Baert, K, Peacock, SJ, Parkhill, J, Zadoks, RN, Holmes, MA. 2012. The newly described *mecA* homologue, *mecALGA251*, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species. J. Antimicrob. Chemother. 67(12):2809-2813.

Perreten, V., Kadlec, K., Schwarz, S., Grönlund Andersson, U., Finn, M., Greko, C., Moodley, A., Kania, S.A., Frank, L.A., Bemis, D.A, Franco, A., Iurescia, M., Battisti, A., Duim, B., Wagenaar, J.A., van Duijkeren, E., Weese, J.S., Fitzgerald, J.R., Rossano, A., Guardabassi, L. 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J. Antimicrob. Chemother. 65(6):1145-1154.

Petersen, A., Stegger, M., Heltberg, O., Christensen, J., Zeuthen, A., Knudsen, L.K., Urth, T., Sorum, M., Schouls, L., Larsen, J., Skov, R., Larsen, A.R. Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel *mecC* gene in Denmark corroborates a zoonotic reservoir with transmission to humans. 2013. Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel *mecC* gene in Denmark corroborates a zoonotic reservoir with transmission to humans. Clin. Microbiol. Infect. 19(1):E16-22.

Pi, B., Yu, M., Chen, Y., Yu, Y., Li, L. 2009. Distribution of the ACME-*arcA* gene among meticillin-resistant *Staphylococcus haemolyticus* and identification of a novel *ccr* allotype in ACME-*arcA*-positive isolates. J. Med. Microbiol. 58(Pt 6):731-736.

Pichon, B., Hill, R., Laurent, F., Larsen, A.R., Skov, R.L., Holmes, M., Edwards, GF, Teale, C., Kearns, A.M. 2012. Development of a real-time quadruplex PCR assay for simultaneous detection of *nuc*, Panton-Valentine leucocidin (PVL), *mecA* and homologue *mecALGA251*. J. Antimicrob. Chemother. 67(10):2338-2341.

Queck, S.Y., Khan, B.A., Wang, R., Bach, T.H., Kretschmer, D., Chen L., Kreiswirth, B.N., Peschel, A., Deleo, F.R., Otto, M. 2009. Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA. PLoS Pathog. 5(7):e1000533.

Reynolds, P. E., Brown, D.F. 1985. Penicillin-binding proteins of beta-lactam-resistant strains of *Staphylococcus aureus*. Effect of growth conditions. FEBS Lett. 192(1):28-32.

Robb, A., Pennycott, T., Duncan, G., Foster, G. 2012. *Staphylococcus aureus* carrying divergent *mecA* homologue (*mecA*(LGA251)) isolated from a free-ranging wild bird. Vet. Microbiol. 162(1): 300-301.

Rolo, J., de Lencastre, H., Miragaia, M. 2012. Strategies of adaptation of *Staphylococcus epidermidis* to hospital and community: amplification and diversification of SCC*mec*. J. Antimicrob. Chemother. 67(6):1333-1341.

Ruppé E, Barbier F, Mesli Y, Maiga A, Cojocaru R, Benkhalfat M, Benchouk S, Hassaine H, Maiga I, Diallo A, Koumaré AK, Ouattara K, Soumaré S, Dufourcq JB, Nareth C, Sarthou JL, Andremont A, Ruimy R. 2009. Diversity of staphylococcal cassette chromosome *mec* structures in methicillin-resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* strains among outpatients from four countries. Antimicrob. Agents Chemother. 53(2):442-449.

Sabat, A.J., Koksal, M., Akkerboom, V., Monecke, S., Kriegeskorte, A., Hendrix, R., Ehricht, R., Köck, R., Becker, K., Friedrich, A.W. 2012. Detection of new methicillin-resistant *Staphylococcus aureus* strains that carry a novel genetic homologue and important virulence determinants. J. Clin. Microbiol. 50(10):3374-3377.

Shore, A.C., Deasy, E.C., Slickers, P., Brennan, G., O'Connell, B., Monecke, S., Ehricht, R., Coleman, D.C. 2011a. Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA, mecI, mecR1, blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 55(8):3765-3773.

Shore, A.C., Rossney, A.S., Brennan, O.M., Kinnevey, P.M., Humphreys, H., Sullivan, D.J., Goering, R.V., Ehricht, R., Monecke, S., Coleman, D.C. 2011b. Characterization of a Novel Arginine Catabolic Mobile Element (ACME) and Staphylococcal Chromosomal Cassette *mec* Composite Island with Significant Homology to Staphylococcus epidermidis ACME type II in Methicillin-Resistant

Staphylococcus aureus Genotype ST22-MRSA-IV. Antimicrob Agents Chemother. 55(5):1896-1905.

Shore, A.C., Rossney, A.S., O'Connell, B., Herra, C.M., Sullivan, D.J., Humphreys, H, Coleman, D.C. 2008. Detection of staphylococcal cassette chromosome *mec*-associated DNA segments in multiresistant methicillin-susceptible *Staphylococcus aureus* (MSSA) and identification of *Staphylococcus epidermidis ccrAB4* in both methicillin-resistant *S. aureus* and MSSA. Antimicrob. Agents Chemother. 52(12):4407-4419.

Shore, A., Rossney, A.S., Keane, C.T., Enright, M.C., Coleman, D.C. 2005. Seven novel variants of the staphylococcal chromosomal cassette mec in methicillin-resistant *Staphylococcus aureus* isolates from Ireland. Antimicrob. Agents Chemother. 49(5):2070-2083.

Shukla, S.K., Ramaswamy, S.V., Conradt, J., Stemper, M.E., Reich, R., Reed, K.D., Graviss, E.A. 2004. Novel polymorphisms in mec genes and a new *mec* complex type in methicillin-resistant *Staphylococcus aureus* isolates obtained in rural Wisconsin. Antimicrob. Agents Chemother. 48(8):3080-3085.

Smyth, D. S., Wong, A., Robinson, D.A. 2010. Cross-species spread of SCC*mec* IV subtypes in staphylococci. Infect Genet Evol. 11(2):446-453

Song, M.D., Wachi, M., Doi, M., Ishino, F., Matsuhashi, M. 1987. Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. FEBS Lett. 221(1):167-171.

Stegger, M., Andersen, P.S., Kearns, A., Pichon, B., Holmes, M.A., Edwards, G., Laurent, F., Teale, C., Skov, R., Larsen, A.R. 2012. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecA*(LGA251). Clin. Microbiol. Infect. 18(4):395-400.

Takeuchi, F., Watanabe, S., Baba, T., Yuzawa, H., Ito, T., Morimoto, Y., Kuroda, M., Cui, L., Takahashi, M., Ankai, A., Baba, S., Fukui, S., Lee, J.C., Hiramatsu, K. 2005. Whole-genome sequencing of *staphylococcus haemolyticus* uncovers the extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. 187(21):7292-7308.

Tsubakishita, S., Kuwahara-Arai, K., Baba, T., Hiramatsu, K. 2010a. Staphylococcal cassette chromosome *mec*-like element in *Macrococcus caseolyticus*. Antimicrob. Agents Chemother. 54(4):1469-1475.

Tsubakishita, S., Kuwahara-Arai, K., Sasaki, T., Hiramatsu, K. 2010b. Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. Antimicrob. Agents Chemother. 54(10):4352-4359.

Turlej, A., Hryniewicz, W., Empel, J. 2011. Staphylococcal cassette chromosome *mec* (SCC*mec*) classification and typing methods: an overview. Pol. J. Microbiol. 60(2):95-103.

Urushibara, N., Paul, S.K., Hossain, M.A., Kawaguchiya, M., Kobayashi, N. 2011. Analysis of Staphylococcal cassette chromosome *mec* in Staphylococcus haemolyticus and *Staphylococcus sciuri*: identification of a novel ccr gene complex with a newly identified *ccrA* allotype (*ccrA7*). Microb. Drug Resist. 17(2):291-297.

Utsui, Y., Yokota, T. 1985. Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 28(3):397-403.

Walther, B., Wieler, L.H., Vincze, S., Antão, E.M,. Brandenburg, A., Stamm, I., Kopp, P.A., Kohn, B., Semmler, T., Lübke-Becker, A. 2012. MRSA variant in companion animals. Emerg. Infect. Dis. 18(12):2017-2020.

Wang, L., Archer, G.L. 2010. Roles of CcrA and CcrB in excision and integration of staphylococcal cassette chromosome *mec*, a *Staphylococcus aureus* genomic island. J. Bacteriol. 192(12):3204-3212.

Wang, L., Safo, M., Archer, G.L. 2012. Characterization of DNA sequences required for the CcrAB-mediated integration of staphylococcal cassette chromosome *mec*, a *Staphylococcus aureus* genomic island. J. Bacteriol. 194(2):486-498.

Weese, J. S. 2010. Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J. 51(3):233-244.

Wisplinghoff, H., Rosato, A.E., Enright, M.C., Noto, M., Craig, W., Archer, G.L. 2003. Related clones containing SCC*mec* type IV predominate among clinically significant *Staphylococcus epidermidis* isolates. Antimicrob. Agents Chemother. 47(11):3574-3579.

Wu, S., de Lencastre, H., Tomasz, A. 1998. Genetic organization of the *mecA* region in methicillin-susceptible and methicillin-resistant strains of *Staphylococcus sciuri*. J. Bacteriol. 180(2):236-242.

Wu, S., Piscitelli, C., de Lencastre, H., Tomasz, A.. 1996. Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologue of *mecA* from a methicillin susceptible strain of *Staphylococcus sciuri*. Microb. Drug Resist. 2(4):435-441.

Zong, Z., Lu, X. 2010. Characterization of a new SCC*mec* element in *Staphylococcus cohnii*. PLoS One 5(11):e14016.

Zong, Z., Peng, C. Lu, X. 2011. Diversity of SCC*mec* elements in methicillin-resistant coagulase-negative staphylococci clinical isolates. PLoS One 6(5):e20191.

Table 1. Examples of fully sequenced SCC elements, pseudo SCC*mec* and SCC elements and composite islands (CIs) identified in staphylococci

Element type (definition)[a]	Examples (size)	Associated staphylococcal species	Characteristic genes			Other elements in CI[b]	Reference
			***ccr* type**	***mec* complex genes**	**Additional antibiotic resistance or virulence genes identified**		
SCC (*ccr* but no *mec* complex)	SCC_{M1} (14 kb)	*S. aureus*	*ccrAB4*	none	None	SCC*mec* IIE or ψSCC*arc* & SCC*mec* IVa	Shore et al., 2012; Bartels et al., 2011
	SCC476 or SCC*fus* (23 kb)	*S. aureus*	*ccrAB1*	none	*fusC*	SCC*mec* I	Holden et al., 2004
	SCC*Hg* (32 kb)	*S. aureus*	*ccrC*	none	*merA* & *merB*	SCC*mec* III	Ito et al., 2001
	$SCC_{M06/0171}$ (18 kb)	*S. aureus*	*ccrAB4*	none	None	SCC_{CRISPR} & ψSCC$mec_{M06/0171}$	Kinnevey et al., 2013
	SCC_{CRISPR} (17 kb)	*S. aureus*	*ccrC*	none	CRISPR	$SCC_{M06/0171}$ & ψSCC$mec_{M06/0171}$	Kinnevey et al., 2013
	SCC*cap1* (27 kb)	*S. hominis*	Ψ*ccr*	none	None	Not part of CI	Luong et al. 2002
	SCC*h1435* (22 kb)	*S. haemolyticus*	*ccrC*	none	None	ψSCC*h1,* ψSCC*h2,* ψSCC*h3*, SCC*mec*(h1435)& ψSCC*h4*	Takeuchi et al., 2005
	SCC_{pbp4} (19 kb)	*S. epidermidis*	*ccrAB2*	none	*pbp4 & tagF*	ACME II & $SCC_{ATCC12228}$	Mongkolrattanothai et al., 2004
	$SCC_{ATCC12228}$ (38 kb)	*S. epidermidis*	*ccrAB4*	none	*copA*, mercury & cadmium resistance gene clusters	ACME II & SCC_{pbp4}	Mongkolrattanothai et al., 2004
	SCC_{12263} (ca. 21 kb)	*S. hominis*	*ccrAB1*	none	None	Not part of CI	Katayama et al., 2003
Pseudo (ψ) SCC*mec* (*mec* complex but no *ccr*)	ψSCC*mec* II.5 (28 kb)	*S. aureus*	none	*mecA* & *ΔmecR1*	*ant*(4')	Not part of CI	Han et al., 2009
	ψSCCmec_{16691} (11 kb)	*S. aureus*	None	IS431, *mecA*, *mecR1* and *mecI* (Class A *mec*)	None identified	Not part of CI	Chen et al., 2010
	ψSCC$mec_{M06/0171}$ (16 kb)	*S. aureus*	None	IS431, *mecA* & *ΔmecR1* & IS431 (class C4 *mec*)	*copB*, *copC* & *fusC*	SCC_{CRISPR} & $SCC_{M06/0171}$	Kinnevey et al., 2013
	ψSCC*mec*(*h1435*)[c]	*S. haemolyticus*	None	*mecA* & *ΔmecR1*	*cadD*, *cadX*, *arsC*, *arsB*	ψSCC*h1,* ψSCC*h2,*	Takeuchi et al., 2005

Element type (definition)[a]	Examples (size)	Associated staphylococcal species	Characteristic genes			Other elements in CI[b]	Reference
			ccr type	*mec* complex genes	Additional antibiotic resistance or virulence genes identified		
					& *arsR*	SCC*h1435*, ψSCC*h3* & ψSCC*h4*	
	ΨSCC*mec*$_{S04009}$ (18 kb)	*S. xylosus*	None	*blaZ, mecC1, mecR1* & *mecI* (class E *mec*)	None identified	3.3 kb of 3'-end ACME I	Harrison et al., 2012
Pseudo (Ψ) SCC (Lack *ccr* and *mec* genes)	ACME, ΔACME or ΨSCC*arc* (12-34 kb)	Various staphylococcal species	None	None	*arc* & *opp* operons (*opp* not present in ΔACME/ΨSCC*arc*)	Various SCC*mecs* & SCCs	Diep et al., 2006; Shore et al., 2011b; Bartels et al., 2011;
	ψSCC*h1*, ψSCC*h2,* ψSCC*h3* & ψSCC*h4*[c]	*S. haemolyticus*	None	None	*kdp* (SCC*h1*)	SCC*h1435* & Ψ SCC*mec*$_{h14}$	Takeuchi et al., 2005
	ΨSCC$_{M10/0061}$ (3 kb)	*S. aureus*	None	None	None	SCC*mec* XI	Shore et al., 2011a
	SCC-like or IE25923 (5.9 kb)	*S. aureus*	None	None	None	With SCC*mec* II or IV or not part of CI	Ito et al., 2001; Ito et al., 2003; Jansen et al., 2006
			None	None	None		
	CC$_{V14}$ (ca. 5kb)	*S. aureus*	None	None	None	SCC*mec* III	Arakere et al., 2009
	CC6082 (5.5kb)	*S. aureus*	None	None	None	SCC*mec* VII	Berglund et al., 2008
	SCC*mec* II remnant (ψSCC$_{ECT-R2}$) (12 kb)	*S. aureus*	None	None	*ant*(4')	Not part of CI	Lindqvist et al., 2012
Composite islands (2 or more SCC, SCC*mec* or Ψ elements)	Ψ SCC*mec*$_{M06/0171,}$ SCC$_{M06/0171\ \&}$ SCC$_{CRISPR}$ (51 kb)	*S. aureus*	*ccrAB4* & *ccrC*	IS431, *mecA* & *ΔmecR1* & IS431 (class C4 *mec*)	CRISPR, *fusC, copB, copC*	n/a	Kinnevey et al., 2013
	ΔACME II (ψSCC*arc*), ΔJ1 SCC*mec* I & SCC*mec* IVh (46 kb)	*S. aureus*	*ccrAB2*	IS431, *mecA*, *ΔmecR1* & IS1272 (Class B *mec*)	*arc* operon	n/a	Shore et al., 2011b
	ψSCC*h1*, ψSCC*h2,* SCC*h1435* & Ψ ψSCC*h3*, SCC*mec*$_{h14}$, *&* ψSCC*h4* (91 kb)	*S. haemolyticus*	*ccrC*	*mecA* & *ΔmecR1*	*kdp*	n/a	Takeuchi et al., 2005
	SCC$_{pbp4,}$ ACME II & SCC$_{ATCC12228}$ (91 kb)	*S. epidermidis*	*ccrAB4* & *ccrAB2*	none	*pbp4, tagF*, *copA*, mercury & cadmium resistance gene clusters	n/a	Mongkolrattanothai et al., 2004

Element type (definition)[a]	Examples (size)	Associated staphylococcal species	Characteristic genes			Other elements in CI[b]	Reference
			***ccr* type**	***mec* complex genes**	**Additional antibiotic resistance or virulence genes identified**		
	SCC*mec* III & SCC*Hg* (67 kb)	*S. aureus*	*ccrAB3* & *ccrC*	IS431, *mecA*, *mecR1* and *mecI* (Class A *mec*)	*cadA*, *cadC, tet*(K), *merA* & *merB*	n/a	Ito et al., 2001
	SCC_{M1}, ψSCC*arc* & SCC*mec* IVa (54 kb)	*S. aureus*	*ccrAB4*& *ccrAB2*	IS431, *mecA*, Δ*mecR1* & IS1272 (Class B *mec*)	None	n/a	Bartels et al., 2011
	SCC_{M1} & SCC*mec* IIE (41 kb)	*S. aureus*	*ccrAB4 & ccrAB2*	IS431, *mecA*, *mecR1*, Δ*mecI*, IS1182 (Class A.3 *mec*)	*spc* & *erm*(A)	n/a	Shore et al., 2012
	SCC*mec* XI & $ψSCC_{M10/0061}$	*S. aureus*	*ccrA1B3*	*blaZ*, *mecC*, *mecR1*, *mecI* (Class E *mec*)	Arsenic resistance operon	n/a	Shore et al., 2011a

[a] Each of these elements shares the common feature of integration into the ISS within *orfX* and are flanked by direct and inverted repeats but each also has unique characteristics which are indicated in parenthesis after the element type.

[b] n/a, no applicable

[c] It was not possible to determine the size of the individual elements of the *S. heamolyticus* CI (ψSCC*mec*(*h1435*), ψSCC*h1*, ψSCC*h2,* ψSCC*h3* & ψSCC*h4*), as the size of each element is not stated in the reference and the direct repeats flanking each element are not shown in the Genbank entry (accession number AP006716).