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Mini review

Staphylococcal cassette chromosome *mec*: Recent advances and new insights

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ABSTRACT

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.

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 and 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 β-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; Jevons, 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 insertion 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 to 60 kb (García-Álvarez et al., 2011; IWG-SCC, 2009; Li et al., 2011; 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

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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 β-lactam antibiotics, PBP2a can continue cell-wall biosynthesis (Hartman and Tomasz, 1984; Ito et al., 1999; Reynolds 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 β-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 β-lactams. However, isolates harbouring the same *mecA* allele often exhibited different levels of resistance, even within the same species, indicating that strain-specific factors maybe 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 (García-Álvarez 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*) (García-Álvarez 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., 2012; 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 to 5.9% (Cuny et al., 2011; Petersen et al., 2013; Pichon et al., 2012; Stegger et al., 2012).

The predominantly animal MRSA lineages that *mecC* has been identified in (CC130, CC425, CC1943, CC599, CC49), the absence of lysogenic β-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* (García-Álvarez 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 (García-Álvarez 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., 2013). 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 β-lactam resistance in strains encoding *mecR1* and *mecI* by inactivation MecI by proteolytic cleavage (Arêde 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