Review

The emergence of *mecC* methicillin-resistant *Staphylococcus aureus*

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The report of methicillin-resistant *Staphylococcus aureus* (MRSA) encoding a divergent *mecA* gene in 2011 was highly significant. This homologue, designated *mecC*, poses diagnostic problems with the potential to be misdiagnosed as methicillin-sensitive *S. aureus*, with important potential consequences for individual patients and for the surveillance of MRSA. *mecC* MRSA have now been reported from 13 European countries and have been isolated from 14 different host species, with evidence of a recent increase in Denmark. The emergence of *mecC* MRSA is a topic of interest to human and veterinary microbiology, and we consider it timely to review here its discovery and subsequent investigation.

Staphylococcus aureus and MRSA in humans and animals

S. aureus is a prominent human pathogen that can cause a diverse range of diseases ranging from relatively minor skin infections to serious and life-threatening infections such as endocarditis, pneumonia, and sepsis. Its impact is enhanced by the development of antibiotic resistance, most notably methicillin-resistant *S. aureus* (MRSA) that is resistant to virtually all β-lactam antibiotics. Although originally regarded as a nosocomial pathogen (hospital-associated MRSA or HA-MRSA), MRSA infections among previously healthy individuals in the community, without links to healthcare settings, emerged in the 1990s and are referred to as community-associated MRSA (CA-MRSA). For the most part HA-MRSA and CA-MRSA involve different lineages, but these distinctions are not absolute, and transfer of strains between these settings is increasingly recognised. In addition to its importance as a human pathogen, *S. aureus* [1], including MRSA [2,3], can colonise and infect a wide range of host species including livestock, wildlife, and companion animals, with bovine mastitis among dairy cattle, lameness in poultry, and severe and lethal infections in farmed rabbits being particularly significant in terms of economic impact. MRSA in animals is not only important from an animal welfare and economic perspective but can act as a reservoir for zoonotic infection of humans. In particular, multilocus sequence type clonal complex (CC)398 is abundant among pigs and other livestock in mainland Europe, and infection of humans in close contact with these animals has led to the recognition of a third epidemiological form of MRSA, livestock-associated MRSA (LA-MRSA) [4].

Mechanism of methicillin-resistance in MRSA and its diagnostic detection

Although methicillin is no longer produced, the name MRSA has persisted and can be regarded as referring to resistance to virtually all β-lactam antibiotics. Susceptibility testing now typically uses oxacillin and/or cefoxitin. β-Lactams bind to the penicillin-binding proteins (PBP) essential for cell wall biosynthesis and inhibit peptidoglycan crosslink formation, leading to bacterial cell lysis. Resistance to β-lactams in MRSA is conferred by the acquisition of a mobile genetic element, the staphylococcal cassette chromosome (SCC*mec*) carrying the *mecA* gene which encodes an altered PBP – PBP2a/PBP2′ – which has reduced affinity for β-lactam antibiotics. As a result, cell wall biosynthesis in MRSA strains continues even in the presence of otherwise inhibitory levels of β-lactam antibiotics. The detection and diagnosis of MRSA in the clinical microbiology setting is very important both for informing the appropriate treatment of individual patients and also for the surveillance of MRSA. The gold standard for confirmation of MRSA is regarded as the molecular detection of either *mecA*, typically by PCR, or of PBP2a/PBP2′, usually by antibody detection with commercially available slide agglutination assays. Crucial to the reliability of these assays is the fact that *mecA* and PBP2a/PBP2′ are both highly conserved among MRSA isolates.

Discovery of *mecC* MRSA: genome sequencing to identify a novel resistance gene

An epidemiological study of bovine mastitis [5] led to the isolation in 2007 of a *S. aureus* isolate, LGA251, from a bulk tank milk sample in southwest England which was phenotypically MRSA (i.e., resistant to oxacillin and cefoxitin). At that time this in itself was immediately significant because it represented the first detection of MRSA in the UK dairy herd. However, confirmatory tests for the *mecA*

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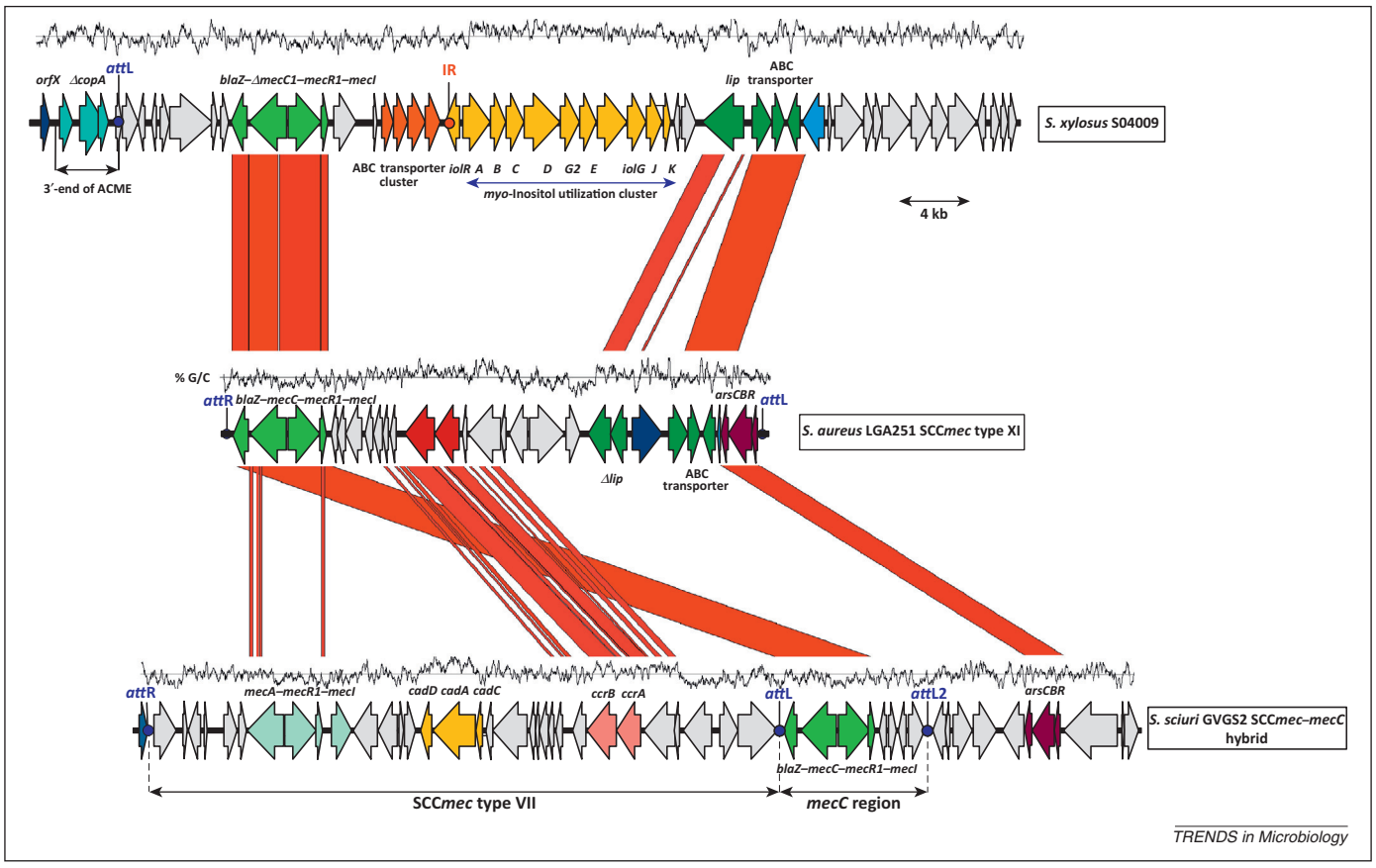

Figure 1. Comparison of the *mecC1* region in *Staphylococcus xylosus* strain S04009 [38] (EMBL accession number HE993884), SCC*mec* type XI in *Staphylococcus aureus* LGA251 [6] (EMBL FR821779), and a hybrid SCC*mec–mecC* in *S. sciuri* strain GVGS2 [39] (EMBL HG515014). Areas in red show regions conserved between the two sequences; homologous coding sequences are marked in the same colour. Blue and red dots indicate the SCC*mec* attachment sites (*att*L and *att*R) and inverted repeats (IR), respectively. The %G/C content of the region is shown above each genome schematic. Abbreviations: ABC, ATP-binding cassette; ACME, arginine catabolic mobile element; SCC*mec*, staphylococcal cassette chromosome.

gene and PBP2a/2′ were repeatedly negative [6]. Genome sequencing of LGA251 at the Wellcome Trust Sanger Institute revealed that the strain carried a novel *mecA* homologue, initially termed $mecA_{LGA251}$, which was only ~69% identical to conventional *mecA* at the DNA level, and the encoded PBP2a/2′ was ~63% identical at the amino acid level [6]. This explained the resistance of LGA251 and why it produced negative results by *mecA* PCR and PBP2a/2′ slide agglutination. A retrospective search of isolate collections in the UK and Denmark identified a further 65 isolates positive for $mecA_{LGA251}$ isolated not only from dairy cattle but also from humans, including the earliest known isolate, a Danish blood isolate from 1975 [6]. In consequence, although $mecA_{LGA251}$ MRSA has only recently been recognised, it may have been causing human infections for over 35 years. These $mecA_{LGA251}$ MRSA isolates belonged predominantly to CC130 and ST425 [6]. Similarly to conventional *mecA*, $mecA_{LGA251}$ is located within a SCC*mec* element inserted into the 3′ region of *orfX* (Figure 1). The LGA251 SCC*mec* was also novel; in other words, it had divergent *ccrA* and *ccrB* recombinases (belonging to the *ccrA1* and *ccrB3* groups and representing a novel combination of recombinase groups designated type 8 *ccr*), divergent *mecA* regulatory genes (*mecI*/*mecR*), and the absence of one of the three joining regions (J3) that are normally present [6]. The SCC*mec* sequence from LGA251 was submitted to the Working Group on the Classification of SCC and given the designation type XI SCC*mec* in November 2009. $mecA_{LGA251}$ was itself subsequently renamed *mecC* in 2012 [7]. *mecC* was chosen because an additional divergent homologue of *mecA*, distinct from $mecA_{LGA251}$, had already been described in *Macrococcus caseolyticus* [8] and was designated *mecB* [7]. Published at the same time as the UK and Danish report [6], work in the Republic of Ireland independently described *mecC* and type XI SCC*mec* in human MRSA strains isolated in 2010 and belonging to CC130 [9].

Functional characterisation of *mecC*-encoded PBP2a

The function of the *mecC*-encoded PBP2a/2′ and its role in β-lactam resistance was formally demonstrated by the work of Kim *et al*. which also highlighted noteworthy differences in the properties of the *mecA* and *mecC*-encoded proteins [10]. Although the detection of *mecC*-encoded PBP2a in LGA251 was problematic, most likely due to low expression levels resulting from *mecI*/*mecR*, inducible expression of *mecC* in a methicillin-sensitive *S. aureus* (MSSA) strain conferred high minimum inhibitory concentration (MIC) values against a range of β-lactams [10]. Recombinant $PBP2a_{mecC}$ protein was bound by β-lactams but showed higher affinity for oxacillin compared to cefoxitin, whereas $PBP2a_{mecA}$ showed less preference. The two proteins also displayed differences in their thermostabilty and temperature optima, with $PBP2a_{mecC}$ appearing to be