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A potential diagnostic problem: The Newly Emerging *mecC* Methicillin-Resistant *Staphylococcus aureus* strains

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Abstract:
Background: *Methicillin-resistant S. aureus (MRSA) has been known for just over 50 years, and it poses a serious problem for infection prevention and control and antibiotic treatment globally. In MRSA, resistance against almost all beta-lactam compounds in clinical use is caused by the expression of an alternate penicillin-binding protein (PBP2a) that is encoded by the mecA gene.*
Objective: *The report of homologue, designated mecC gene conferring beta-lactam resistance in Staphylococcus aureus 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.*
Methods: *A wide-ranging search of electronic bibliographic databases was performed on mecC-positive Staphylococcus aureus (ST130-MRSA-XI) and the lesions related to the infection in two diseased free-ranging European hedgehogs (Erinaceus europaeus).*
Results: *This gene, mecC, is situated on a SCCmec XI element that has to date been identified in clonal complexes 49, 130, 425, 599 and 1943. Some of the currently known isolates have been identified from animals. This, and observations of mecA alleles that do not confer beta-lactam resistance, indicate that mec genes might have a reservoir in Staphylococcus species from animals.*
Conclusion: *This recently recognised form of MRSA can colonise and cause disease in humans and a wide range of other host species. Although mecC MRSA are currently rare, and have only been reported in Europe to date, they present a potential diagnostic problem where there is reliance on mecA or PBP2a/2' detection for MRSA diagnosis, and their emergence raises a several opportunities for future research.*
Keywords: *Staphylococcus aureus, MRSA, mecC, zoonosis, antibiotic resistance, sequencing*

I. Introduction

Staphylococcus aureus has emerged as one of the most important human pathogens, and has over the past several decades, been a leading cause of hospital and community acquired infections[1]. It is associated with variety of clinical infections including septicemia, pneumonia, wound sepsis, septic arthritis, osteomylitis and post – surgical toxic shock syndrome with substantial rates of morbidity and mortality[2,3,4].The organism *Staphylococcus aureus* is found on much individual skin and seems to cause no major problems. However, if it gets inside the body, for instance under the skin or into the lungs, it can cause important infections such as boils or pneumonia. 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* [5][1], including MRSA [6,7]. 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) [8][4].

The *mecA* gene is carried within a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*). SCC*mec* elements are highly diverse in their structural organization and genetic

content, and have been classified into types and subtypes from type I to type XI [9-13]. In general, healthcare-associated MRSA carry SCC*mec* types I–IV, whereas type IV is also carried by community-acquired MRSA, as well as type VII. Type V is carried by LA-MRSA ST398, and recently the new SCC*mec*XI has been described in MRSA isolates from cattle and humans carrying a new *mecA* gene variant, $mecA_{LGA251}$, which has been renamed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome (SCC) Elements as *mecC* [13-15]. This novel LA-MRSA carrying the new *mecC* gene was first described in the UK and Denmark from cattle and, although very rarely, as a cause of infections in humans. Recently, the isolation of *mecC*-positive MRSA from animals and humans has also been described in Germany and in other countries in northern Europe, but never from southern Europe [13,15-26]. Moreover, to the best of our knowledge, this LA-MRSA has never been reported as a cause of fatal infections in humans and known virulence factors have been found to be absent in most of the *mecC* MRSA that have been described to date.

II. Research methods and design

A wide-ranging search of electronic bibliographic databases was performed, including Medline and the Directory of Open Access Journals, using the keyword '*mecC'* The majority of the search results was on *mecC*-positive *Staphylococcus aureus* (ST130-MRSA-XI) and the lesions related to the infection in two diseased free-ranging.

III. Results and discussion

Literature search results

I identified published manuscripts focusing on the recently recognised form of MRSA that colonise and cause disease in humans and a wide range of other host species. The main focus was on *mecC*-positive *Staphylococcus aureus* (ST130-MRSA-XI) and the lesions related to the infection in two diseased free-ranging.

Mechanism of methicillin-resistance in MRSA

Three distinctly different mechanisms of methicillin resistance have been described in *Staphylococcus aureus.* The best documented and probably most important mechanism is production of a unique, low affinity penicillin binding protein, PBP 2a. Strains possessing PBP2a are resistant to methicillin, oxacillin, and probably all other currently available ß-lactam antibiotics. Two additional mechanisms of reduced susceptibility to methicillin have been described. Borderline resistance to the semi-synthetic penicillns (BROSA) has been attributed to the hyper production of normal staphylococcal ß- lactamase. A third mechanism has recently been advanced that describes an intermediate level of resistance to methicillin due to production of modified, normal PBPs with reduced affinity for ß- lactams (MODSA) [27]. The mechanism of resistance in *S. aureus* is based on the production of an additional low affinity penicillin – binding protein (PBP' or PBP2a), which is encoded by the *mec*A gene [28-30]. ß-lactam antibiotics have a low affinity for PBP2a therefore cellwall synthesis is able to proceed in their presence (Figure 1). Many strains are heterogeneous in their phenotypic expression of methicillin resistance, despite their genetic homogeneity. Several factors are known to influence phenotypic expression of methicillin resistance. Resistance arises by acquisition of *Staphylococcus aureus* cassette chromosome SCC*mec a*nd is horizontally transferred [31]. Expression of this gene yields PBP2a, penicillin binding protein with affinity for β lactam rings (the primary active site of the β lactam antibiotics) [32]. In addition to the *mec*A gene, SCC*mec* contain transposon and integrated copies of plasmid that carry various resistance genes against non-beta lactam [33].

The basis of most methicillin resistance is the production of an additional penicillin binding protein. PBP2' or PBP2a mediated by the *mec*A gene. *Mec*A is an additional gene found in methicillin resistant Staphylococci and with no allelic equivalent in methicillin – susceptible Staphylococci. There are several additional genes that effect the expression of methicillin resistance in *S. aureus,* but these are found in susceptible as well as resistant strains [34-36]. Unlike plasmid-encoded penicillinase, the methicillin resistance determinant *mec* is chromosomally encoded. Horizontal transfer of *mec* is thought to be relatively rare; only a handful of ancestral strains account for all clinical isolates worldwide [37]. Ribotyping (a genotyping scheme that uses Southern blot analysis to identify DNA restriction enzyme polymorphisms of the five to six ribosomal RNA genes distributed throughout the *S. aureus* chromosome) and cluster analysis indicate that *mec* has integrated into at least three distinct methicillin-susceptible chromosomal backgrounds; A B, and C [38,39]. *Mec* itself is polymorphic; three types have been identified: l, II, and III. These polymorphs differ in number of base pairs, genetic organization, number of insertion sequences and resistance determinants. All three *mec* types have been found integrated into ribotype cluster A. Type II *mec* has also integrated into cluster B and C ribotype backgrounds. Thus, five distinct clones of MRSA have been identified worldwide since the first strain was isolated in the United Kingdom in 1961 ; even if more clones were identified, the relatively low number pales in comparison to the large number of distinct clones of methicillin-susceptible clones.

Unlike the mechanisms responsible for horizontal transfer of penicillinase resistance, the mechanism by which *mec* might be mobilized and transferred had not been understood until recently, Hiramatsu and co-workers have identified two genes, *ccr* A and B (cassette chromosome recombinase genes A and B), which are homologous to DNA recombinases of the invertase-resolvase family and can mobilize *mec* [33]. The proteins encoded by these genes catalyze precise excision and precise site-specific and orientation-specific integration of *mec* into the *S aureus* chromosome. Thus, *mec* is somewhat analogous to the pathogenicity islands found in gram-negative bacilli except that this locus encodes resistance determinants instead of virulence factors. How an element as large as *mec* is transferred from donor to recipient is not known. Nevertheless, as the prevalence of MRSA strains has increased so has the abundance of *mec* DNA[33]. Eventhough transfer of *mec* occurs rarely, the chances that it might occur have correspondingly increased.

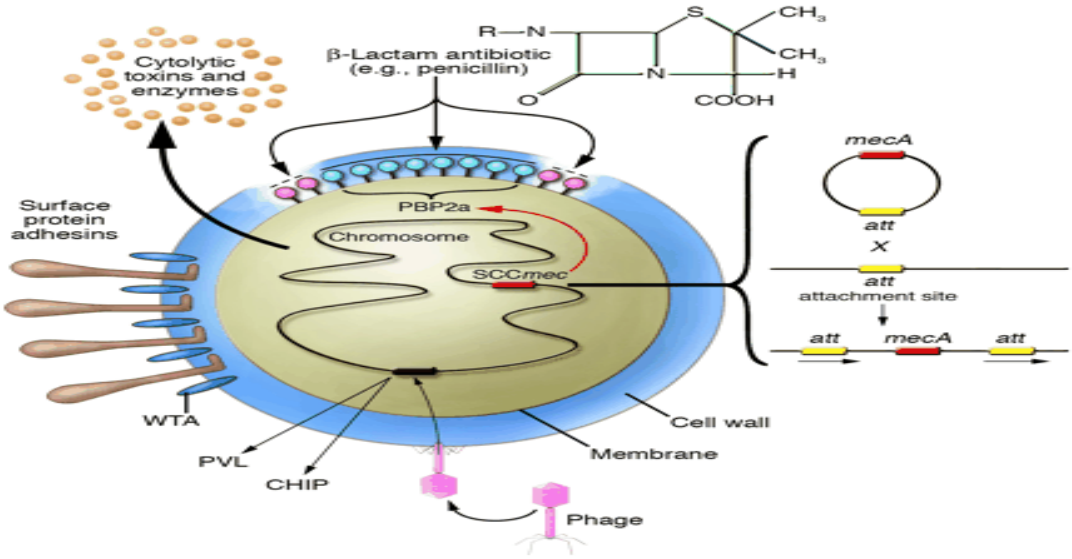

Figure I: The schematic diagram illustrating the mechanism of resistance of MRSA

Diagnostic Detection of MRSA in the Clinical Microbiology

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[40]. 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[40].

Discovery of *mecC* MRSA

An epidemiological study of bovine mastitis [41] 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* gene and PBP2a/2′ were repeatedly negative [42]. 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 [42]. 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 [42]. 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 [42]. Similarly to conventional *mecA*, *mec*A$_{LGA251}$ is located within a SCC*mec* element inserted into the 3′ region of *orfX* (Figure 2).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 [42]. 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 [43]. *mecC* was chosen because an additional divergent homologue of *mecA*, distinct from *mecA*$_{LGA251}$, had already been described in *Macrococcus caseolyticus* [44] and was designated *mecB* [43]. Published at the same time as the UK and Danish report [42], 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 [45].

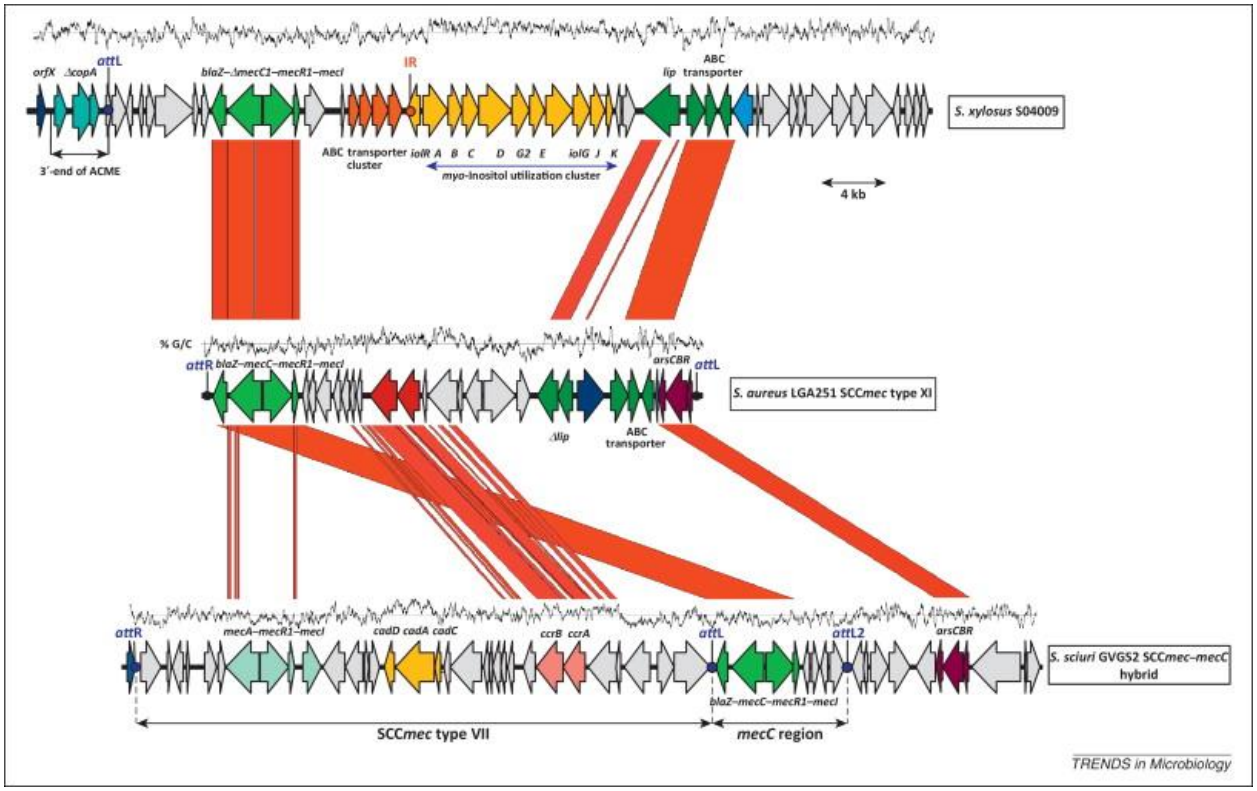

Figure 2: Comparison of the *mecC1* region in *Staphylococcus xylosus* strain S04009 [74] (EMBL accession number HE993884), SCC*mec* type XI in *Staphylococcus aureus* LGA251 [42] (EMBL FR821779), and a hybrid SCC*mec*–*mecC* in *S. sciuri* strain GVGS2 [75] (EMBL HG515014). Areas in red show regions 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.

Detection of *mecC* MRSA

Although there are obviously differences in biochemistry between *mecA* and *mecC*-encoded PBP2a, *mecC* nonetheless confers methicillin resistance, and such strains need to be identified correctly as MRSA in diagnostic laboratories. Where laboratories are performing antimicrobial susceptibility testing, *mecC* MRSA will likely be correctly identified as MRSA. Importantly, cefoxitin has been found to be more reliable than oxacillin in disc diffusion, broth microdilution, and agar dilution assays [47]. However, significant differences in the reliability of agars from different manufacturers have been described [47].

Similarly, *mecC* MRSA produce a distinctive antibiotic susceptibility profile compared to *mecA* MRSA when assayed using the automated Vitek 2 system from BioMérieux [48]. Where both oxacillin and cefoxitin are included, *mecA* MRSA, as might be expected, typically display resistance to both. By contrast, the majority of

mecC MRSA show resistance to cefoxitin, and are therefore reported as MRSA, but however show susceptibility to oxacillin. Testing of a panel of 896 *S. aureus* isolates (comprising *mecA* MRSA, *mecC* MRSA, and *mec*-negative MSSA) found that this oxacillin-sensitive/cefoxitin-resistant profile had a sensitivity of 88.7% and a specificity of 99.5% for the identification of *mecC* MRSA isolates from MSSA and *mecA* MRSA [48]. This profile therefore provides a zero-cost screening method for identification of *mecC*-positive MRSA strains in the many clinical laboratories already using Vitek 2, although subsequent PCR would be needed to confirm *mecC* status. The performance of other automated systems for the detection of *mecC* MRSA resistance has not been fully tested and reported.

The differences in oxacillin and cefoxitin sensitivities displayed by *mecC* MRSA isolates are consistent with the findings of Kim *et al*. discussed above, demonstrating that the *mecC*-encoded PBP2a, unlike the *mecA*-encoded counterpart, has a higher relative affinity for oxacillin than for cefoxitin, leading to higher levels of resistance to cefoxitin than to oxacillin [46]. *mecC* MRSA appear to grow reliably on commercial chromogenic agar plates designed to identify MRSA, although there are indications that some MRSA agars may perform better than others for the recovery of *mecC* MRSA [49]. *mecC* MRSA typically have lower MICs to oxacillin and cefoxitin than their *mecA* counterparts, and this may affect their recovery on selective agars.

One major problem is where molecular detection of *mecA* is used to identify or confirm MRSA. Laboratories using this approach, most often PCR, will need to consider incorporating universal *mec* gene primers able to amplify both *mecA* and *mecC* or the addition of *mecC*-specific primers. This latter option has the benefit of differentiating *mecC* MRSA, thereby facilitating their surveillance and the isolation of strains for further characterisation. Various modified PCR assays have been developed to detect and/or differentiate *mecC* MRSA [50–52], and many commercial PCR-based assays are being, or have been, modified to include *mecC* detection [50,53]. Commercial slide agglutination assays for *mecA*-encoded PBP2a will also misidentify *mecC* MRSA as being methicillin-susceptible. These tests may be modified in due course to detect *mecC* MRSA, but currently the use of commercial slide agglutination assays alone will produce false-negative results for these strains. Strains found to be phenotypically resistant but *mecA* and/or PBP2a-negative are potentially *mecC* MRSA, and *mecC* PCR would be warranted to confirm this. *mec* gene-negative MRSA have also been reported [54].

mecC MRSA pose a potential diagnostic loophole which clinical microbiology laboratories should be aware of and which will require validation of testing approaches to ensure that *mecC* MRSA are correctly identified as MRSA. Statistically robust, formal studies are needed to validate the diverse MRSA susceptibility testing regimes for their correct identification of *mecC* MRSA as methicillin-resistant, even if only to confirm that current methods are sufficient.

Epidemiology of *mecC* MRSA

Following the original discoveries of *mecC* MRSA in the UK, Denmark, and the Republic of Ireland such strains, both human and animal origin, were rapidly identified in a further 10 Western European countries (Table 1). In many cases these reports represent small numbers of isolates identified by opportunistic sampling; for example, retrospective testing of previously identified atypical MRSA isolates. From these data it is unclear how common *mecC* MRSA truly are. In Denmark, however, where reporting of human MRSA is mandatory and extensive strain collections are maintained, the prevalence of *mecC* MRSA among all MRSA was found to be 1.9% in 2010, increasing to 2.8% in 2011 [55]. Further evidence supporting a recent increase is that very few Danish *S. aureus* isolates collected prior to 2003 were found to be *mecC* MRSA [55]. By comparison, large-scale collection and characterisation of human MRSA in Germany found only two *mecC* MRSA isolates among 3207 MRSA isolates (prevalence 0.06%), with no indication of a change in prevalence between 2004/05 and 2010/11 [56]. In the UK, a study in England during 2011–2012 surveyed 335 sequential MRSA isolates from individual patients collected from each of six clinical microbiological laboratories, and found a prevalence rate for *mecC* MRSA of 0.45% (nine *mecC* MRSA isolates from a total of 2010 MRSA isolates collected) [57]. The screening of 565 *S. aureus* isolates collected between 2005 and 2011 in western Switzerland did not identify any *mecC* MRSA isolates, suggesting that these are also rare in that country [58]. *mecC* MRSA has yet to be reported from outside Western Europe, and a small survey of US service personnel injured during deployment in Iraq and Afghanistan and transitioned through Germany *en route* to the USA found no *mecC* MRSA among 102 MRSA isolates [59]. *mecC* MRSA currently appears to be uncommon in humans, but there are interesting geographical differences in prevalence; the recent increase in Denmark highlights the need to monitor *mecC* MRSA.

Although a number of multilocus sequence types have been found among *mecC* MRSA isolates, two major lineages are responsible for the vast majority of isolates to date: CC130, which seems to predominate, and ST425. Among these lineages a large number of *spa*-types are represented (Table 1), with t843, associated with CC130, being the most common. *mecC*-negative ST425 have also been reported [60], as have CC130 MSSA, although the *mec* gene status of the latter was not confirmed [61]. *mecC* MRSA have been found in a wide range of other host species encompassing livestock, wildlife, and companion animals from many European countries

(Table 1). As with human isolates, these isolates predominantly belong to CC130 and to a lesser degree ST425. These lineages therefore appear to have a very broad host tropism. There are few data on the prevalence among animals, although a British study of bovine bulk tank milk found that 2.67% of dairy farms in England were positive for *mecC* MRSA but, interestingly, no positive farms were found in Scotland during the same survey [62]. Assessing the prevalence of *mecC* MRSA among different livestock species, understanding their role in veterinary disease, and the risk of zoonotic transmission are important topics for future research.

mecC-positive *Staphylococcus aureus* (ST130-MRSA-XI) and the lesions related to the infection in two diseased free-ranging European hedgehogs (*Erinaceus europaeus*) have been described. One was found dead in 2003 in central Sweden, and suffered from *S. aureus* septicaemia. The other one, found on the island of Gotland in the Baltic Sea in 2011, showed a severe dermatitis and was euthanised. ST130-MRSA-XI isolates were isolated from lesions from both hedgehogs and were essentially identical to previously described isolates from humans. Both isolates carried the complete SCC*mec* XI element. They lacked the *lukF-PV/lukS-PV* and *lukM/lukF-P83* genes, but harboured a gene for an exfoliative toxin homologue previously described from *Staphylococcus hyicus*, *Staphylococcus pseudintermedius* and other *S. aureus* of the CC130 lineage. To the best of our knowledge, these are the first reported cases of CC130-MRSA-XI in hedgehogs. Given that one of the samples was taken as early as 2003, this was the earliest detection of this strain and of *mecC* in Sweden. This and several other recent observations suggest that CC130 might be a zoonotic lineage of *S. aureus* and that SCC*mec* XI/*mecC* may have originated from animal pathogens[62].

Table 1: Distribution and characteristics of reported *mecC* MRSA

Country	Host species	Earliest reported isolate	*spa*-types	Multilocus sequence types (clonal complex)	References
UK	Human, dairy cattle, wild common seal, wild chaffinch, domestic dog	1993	t6300, t6292, t6220, t843, t6293, t1736, t1535, t7947, t7485, t7946, t7945, t6383, t742, t7734, t978, t6594, t7914, t9376, t6386, t9605, t8833, t11702, t11706, t9280	ST425 (CC425), ST130 (CC130), ST1245 (CC130), ST1526 (CC130), ST1944 (CC130), ST1764 (CC130), 1943 (CC1943/1946), ST1945 (CC130), ST1946 (CC1943/1946), ST2179 (CC599)	[42,51,57,62,69]
Denmark	Human, cattle, sheep	1975	t373, t528, t6220, t9397, t978, t2345, t3391, t8835, t9395, t843, t1535, t528, t1773, t1048, t3256, t1532, t1736, t3218, t3570, t5970, t9397, t5930 and t7603.	ST130 (CC130), ST1943 (CC130)	[42,69,78,79]
Republic of Ireland	Human	2010	t843 and t373	ST130 (CC130), ST1764 (CC130)	[45]
Germany	Human, wild hare, sheep, domestic dog, domestic cat, domestic guinea pig	2004	t843, t10513, t1736, 1773, t978, t7189, t1535, t10033, t10006, t1694, t278, t10009	ST130 (CC130), ST1945 (CC130), ST599 (CC599), ST2361 (CC1943/1946)	[49,56,73, 80,81]
France	Human, dairy cattle	2007	t9280, t843	ST130 (CC130), ST1945 (CC130)	[67,82]
The Netherland	Human	Not provided	Not provided	Not provided	[71]
Belgium	Wild brown rat, farmed rabbit, dairy cattle, beef cattle	1995	t208, t742, t9925, t1736	ST2273 (CC49), ST425 (CC425), ST2508 (CC599), ST130 (CC130)	[69,83]
Sweden	Dairy cattle, wild hedgehog	2003	t524, t9111	ST130 (CC130), ST425 (CC425)	[72,84]
Norway	Domestic cat	2012	t6902	ST2497 (CC1943/1946)	[68]
Austria	Wild European otter and wild European hedgehog	Winter 2012/13	t4335 and t3256	ST2620 (CC130), ST130 (CC130)	[73]
Spain	Human	2008	t843 and t6220	ST130 (CC130), ST1945 (CC130)	[66,85]

Switzerlad	Human	2011	t11150	ST130 (CC130)	[58]
Finland	Dairy cattle	2006	t3256	ST130 (CC130)	[86]

Diseases cause by *mecC* MRSA

mecC MRSA have been isolated from carriage and a range of infections in humans (list of references are shown in Table 1). These are predominantly skin and soft-tissue infections but include severe bone infections [67], nosocomial pneumonia [49] and fatal bacteraemia [66]. *mecC* MRSA can also cause disease in veterinary species; for example, mastitis in dairy cattle has been noted in several countries, and other examples include chronic conjunctivitis in a domestic cat [68] and a rabbit isolate of *mecC* MRSA from Belgium belonging to a highly virulent clone among farmed rabbits [6,70]. It appears that, similarly to conventional MRSA lineages, *mecC* MRSA strains are highly-versatile pathogens able to cause a wide range of infections in a range of host species, including severe and fatal infections. In agreement with these epidemiological observations, microarray analysis and genome sequencing reveal that *mecC* MRSA isolates encode several known or putative *S. aureus* virulence factors, including several adhesins, superantigens, and toxins [49,64,71]. In addition, a novel allele of *etd* (encoding exfoliative toxin D) with only 59% identify to the previously described *etd* gene was identified in CC130 isolates, and was putatively named *etd2* [64,72]. However, where tested, *mecC* MRSA strains have been negative for Panton–Valentine leukocidin [51,55], a prominent virulence factor among CA-MRSA, and they have been negative for the human immune evasion genes *sak*, *chp*, and *scn* [49,71], consistent with a possible origin for these strains in an animal reservoir. Resistance to non-β-lactam antibiotics is currently uncommon among *mecC* MRSA isolates, and MICs for oxacillin and cefoxitin are generally low compared to those that can be seen among *mecA* MRSA. It will be of interest and of potential importance to monitor if these features change in the future.

Distribution of *mecC* among other Staphylococci

The origins of *mecC* MRSA and SCC*mec* type XI are unclear, but *mecC* has also been detected by PCR in *Staphylococcus stepanovicii* from a wild European lynx in Austria [73], and a homologue, *mecC1*, located within a SCC*mec* IX-like element has been described using genome sequencing of *Staphylococcus xylosus* isolated from bovine milk in France [74]. This latter gene has 93.5% sequence identity to *mecC* in MRSA and is therefore classed as an allotype of *mecC* (≥70% but <95% nucleotide sequence identify) [43,74]. Finally, *mecC* has been found in *Staphylcoccus scirui* within a novel hybrid SCC*mec–mecC* element in isolates from caesarean incision wounds in Belgian Blue cattle [75]. As suggested for *mecA* [76,77], it is possible that *mecC* has its origin among coagulase-negative staphylococci; further investigations, including whole-genome sequencing of *mecC* staphylococci, may offer clues to the origin and evolution of this resistance determinant. These data also mean that clinical microbiology laboratories should be aware not only of *mecC* MRSA but also of the possible occurrence of *mecC* in other pathogenic species of methicillin-resistant staphylococci.

IV. Conclusion

mecC MRSA represent a recently recognised form of MRSA, encoding a divergent *mec* gene, which can colonise and cause disease in humans and a wide range of other host species. Thus it is important also to screen wildlife isolates for *mec* genes. Although *mecC* MRSA are currently rare, and have only been reported in Europe to date, they present a potential diagnostic problem where there is reliance on *mecA* or PBP2a/2′ detection for MRSA diagnosis, and their emergence raises a several questions for future research. Due to the wide geographical distribution of novel MRSA strains and their rapid spread into different lineages, measures to detect and prevent dissemination of these strains are needed. Clinicians should be aware that patients living in rural areas could be colonized or infected with these strains, and clinical microbiology laboratories should perform traditional culture-based techniques in order to detect them and, if appropriate, include routine PCR analysis for the detection of the novel *mecC* gene.

Competing interests

I declare that I have no financial or personal relationship(s) that may have inappropriately influenced me in writing this article.

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