

# A *Staphylococcus xylosus* Isolate with a New *mecC* Allotype

Ewan M. Harrison,<sup>a</sup> Gavin K. Paterson,<sup>a</sup> Matthew T. G. Holden,<sup>c</sup> Fiona J. E. Morgan,<sup>a</sup> Anders Rhod Larsen,<sup>d</sup> Andreas Petersen,<sup>d</sup> Sabine Leroy,<sup>e</sup> Sarne De Vlieghe,<sup>f</sup> Vincent Perreten,<sup>g</sup> Lawrence K. Fox,<sup>h</sup> Theo J. G. M. Lam,<sup>i</sup> Otlis C. Sampimon,<sup>1\*</sup> Ruth N. Zadoks,<sup>j</sup> Sharon J. Peacock,<sup>b,c</sup> Julian Parkhill,<sup>c</sup> Mark A. Holmes<sup>a</sup>

University of Cambridge, Department of Veterinary Medicine, United Kingdom<sup>a</sup>; University of Cambridge, Department of Clinical Medicine, Cambridge, United Kingdom<sup>b</sup>; Wellcome Trust Sanger Institute, Hinxton, United Kingdom<sup>c</sup>; Statens Serum Institute, Department of Microbiology and Infection Control, Copenhagen, Denmark<sup>d</sup>; INRA, UR454 Microbiologie, Saint-Genès Champanelle, France<sup>e</sup>; M-team and Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium<sup>f</sup>; Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland<sup>g</sup>; College of Veterinary Medicine, Washington State University, Pullman, Washington<sup>h</sup>; GD Animal Health Service Deventer, Deventer, The Netherlands<sup>i</sup>; Moredun Research Institute, Penicuik, United Kingdom<sup>j</sup>

**Recently, a novel variant of *mecA* known as *mecC* (*mecA*<sub>LGA251</sub>) was identified in *Staphylococcus aureus* isolates from both humans and animals. In this study, we identified a *Staphylococcus xylosus* isolate that harbors a new allotype of the *mecC* gene, *mecC1*. Whole-genome sequencing revealed that *mecC1* forms part of a class E *mec* complex (*mecI-mecR1-mecC1-blaZ*) located at the *orfX* locus as part of a likely staphylococcal cassette chromosome *mec* element (SCC*mec*) remnant, which also contains a number of other genes present on the type XI SCC*mec*.**

Methicillin resistance in staphylococci is encoded by *mecA*, encoding the penicillin-binding protein 2a (PBP2a), which has a low affinity for beta-lactam antibiotics (1). As a result, the transpeptidase activity of PBP2a is functional at normally inhibitory concentrations of beta-lactam antibiotics, allowing cell wall synthesis to occur (2–4). Recently, a novel variant of *mecA* was identified in *Staphylococcus aureus* from cattle (5), humans, and a range of other animal species (6) in Denmark, France, The Netherlands, Ireland, Germany, Belgium, and the United Kingdom (5, 7–11). This subtype was originally designated *mecA*<sub>LGA251</sub> but has since been renamed *mecC* and shares 70% nucleotide identity with the conventional *mecA* gene. The *mecC* gene is present with its cognate regulators *mecI-mecR1*, as part of a class E *mec* complex that shares structural similarity (*mecI-mecR1-mecC-blaZ*) with a *mec* gene complex found in *Macrococcus caseolyticus* (12). The class E complex is present as part of a larger, 29.4-kb, type XI staphylococcal cassette chromosome *mec* element (SCC*mec*) inserted at *orfX*; this element also includes the recombinase genes *ccrAB* and arsenic resistance genes. In this work, we describe a highly related *mecC* homolog present in the *orfX* locus in a *Staphylococcus xylosus*.

A search of the EMBL nucleotide database identified submission of sequences from *S. xylosus* strain S04009 (13) with a high degree of similarity (>90%) to *mecC* (5, 8). However, antimicrobial susceptibility testing of strain S04009 by disk diffusion with oxacillin and cefoxitin showed it to be susceptible to both antibiotics but resistant to penicillin using British Society for Antimicrobial Chemotherapy (BSAC) criteria (version 10.2) (data not shown). Therefore, we submitted the *mecC*-positive strain S04009 and a *mecC*-negative *S. xylosus* isolate, S040010, for whole-genome sequencing to further characterize the *mecC*-containing region. Illumina library preparation was carried out as described by Quail et al. (14), and HiSeq sequencing was carried out following the manufacturer's standard protocols (Illumina, Inc.). Genome sequencing confirmed the presence of a *mecC* homolog in S04009 located downstream of the *S. xylosus orfX* homolog, a region associated with horizontally transferred elements (Fig. 1). Immediately downstream of *orfX* in S04009 is a 3.3-kb region that shows a

high degree of similarity (>95% nucleotide identity) to the 3' end of the arginine catabolic mobile element (ACME) in the *S. aureus* USA300 strain FPR3757 (EMBL accession no. CP000255) and contains a truncated version of *copA*, an ATPase copper transporter (15). Next to this region is the *mec* complex. The *mecC* gene in S04009 shares 93.5% nucleotide identity to *mecC* in *S. aureus* LGA251 and 69.9% to *mecA* from *S. aureus* strain MRSA252. Based on the current guidelines for reporting *mecA* homologs, the S04009 *mecC* gene is a new allotype of the LGA251 *mecC*, herein referred to as *mecC1* (16). Sequence analysis of the *mecC1* gene identified a frameshift mutation close to the 5' end of the gene, resulting in a truncated 64-amino-acid (aa) product, providing a molecular basis for the oxacillin and cefoxitin susceptibility of strain S04009. *mecC1* is found in a homologous class E *mec* gene complex (*mecI-mecR1-mecC1-blaZ*) which has been previously reported in *S. aureus* (5, 8). The presence of the *blaZ* gene is likely to account for the observed penicillin resistance of S04009 despite *mecC1* being inactivated. *mecI*, *mecR1*, and *blaZ* in S04009 share 91.1%, 90.0%, and 90.9% nucleotide identity, respectively, with their homologs in LGA251. Downstream of the *mec* gene complex is a hypothetical protein conserved in a number of coagulase-negative *Staphylococcus* (CoNS) species, followed by a tandem pair of ATP-binding cassette transporters (ABC transporters). After the final ABC transporter gene, there is an imperfect 53-bp inverted repeat (IR), which suggests that this region was once part of a separate mobile element or has undergone deletion mediated by this repeat. Immediately upstream of this is a *myo*-inositol (MI)

Received 5 October 2012 Returned for modification 9 November 2012

Accepted 11 December 2012

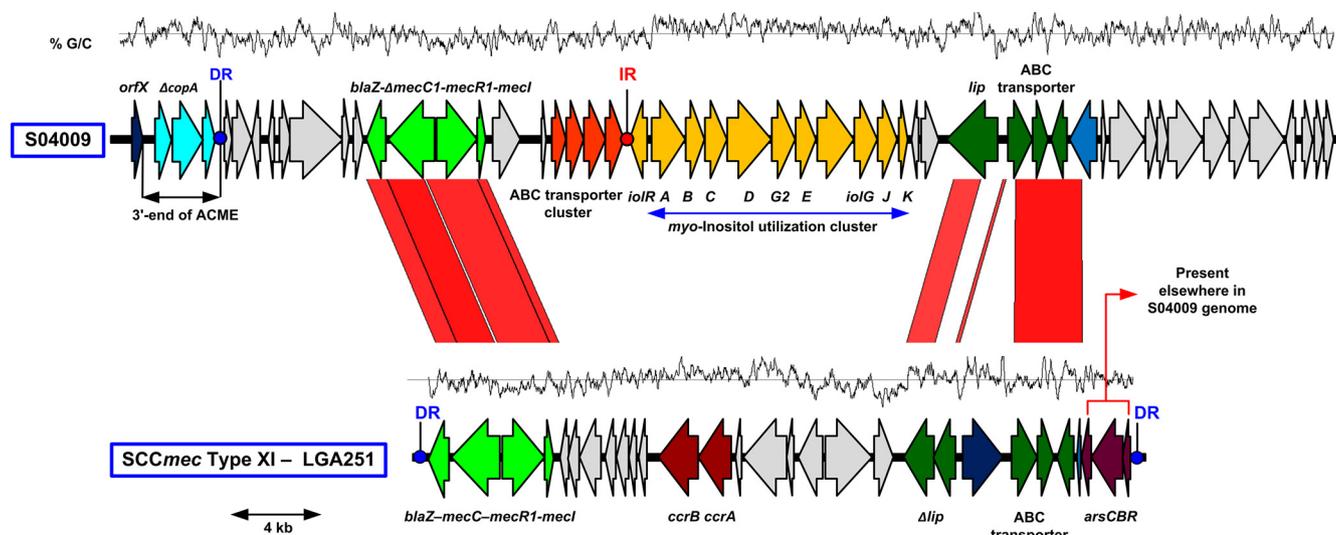
Published ahead of print 28 December 2012

Address correspondence to Mark A. Holmes, mah1@cam.ac.uk.

\* Present address: Otlis C. Sampimon, Pfizer Animal Health, Capelle aan den IJssel, The Netherlands.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.01882-12



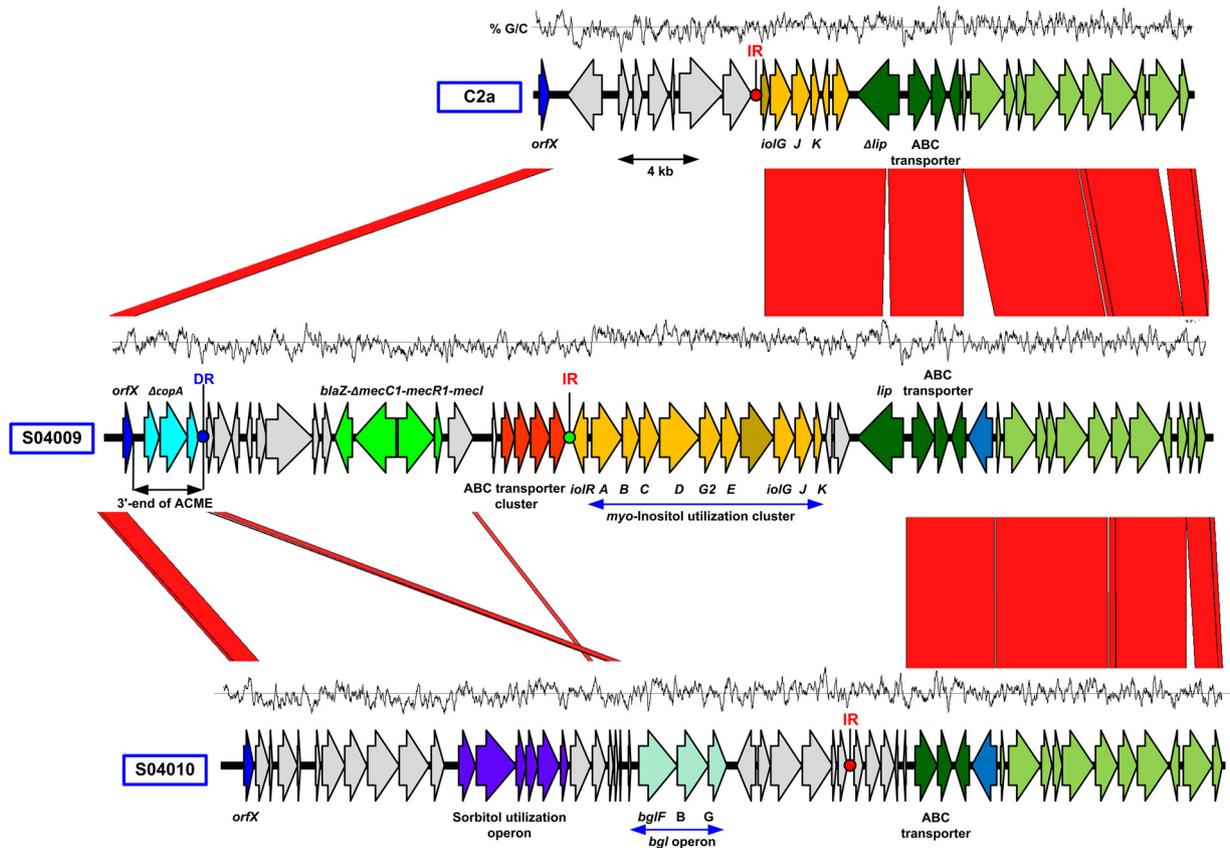
**FIG 1** Comparison of the *orfX* region in *S. xylosus* S04009 (EMBL accession no. HE993884) and the SCC*mec* type XI in LGA251 (EMBL accession no. FR821779). Red areas are regions conserved between the two sequences, and homologous coding sequences (CDSs) are in bright and dark green. Other CDSs of interest discussed in the text are highlighted in color. Blue and red dots indicate direct repeats (DR) and inverted repeats (IR), respectively. The GC content is shown above each region.

utilization cluster, which was previously identified in strain S04009 by subtractive hybridization (13). Downstream from the MI utilization cluster are more genes present in joining region 1 (J1) in the type XI SCC*mec* in LGA251. The lipase gene, which is present as two truncated pseudogenes (SARLGA251\_00420 and SARLGA251\_00430) in LGA251, is intact in S04009. Adjacent to these are genes for an ABC transporter permease, an ABC transporter ATPase, and a conserved hypothetical protein (SARLGA251\_00470-490) with 96%, 97%, and 98% nucleotide identity, respectively, to those in LGA251. Downstream of the conserved ABC transporter genes in S04009 is a gene for a major facilitator superfamily (MFS) protein that is absent from SCC*mec* type XI, ending the region of homology. Interestingly, *arsR*, *arsB*, and *arsC* are present in S04009 and share 83%, 88%, and 91% nucleotide identity, respectively, with their homologs in LGA251. However, they are not found proximal to the *orfX* region but are instead associated with a Tn554-like transposon and are inserted at a different location in the S04009 genome (data not shown).

In order to further understand the evolutionary history of the *mecC1*-containing element in *S. xylosus*, we compared the *orfX* locus of strain *S. xylosus* S04009 with those of two other *S. xylosus* strains, S040010, and a third *S. xylosus* strain, C2a (*S. Leroy*, unpublished data). Immediately downstream of the *orfX* in C2a is an ~9-kb region absent from S04009, which shares blocks of homology to *Enterococcus faecalis* D32 (EMBL accession no. CP003726) at the 5' end and to *Staphylococcus haemolyticus* JCS1435 (EMBL accession no. AP006716) at the 3' end. This region contains a number of genes associated with mobile elements, including a truncated abortive phage infection protein (AIPR), a type I restriction modification system restriction subunit, and two genes that likely encode an McrBC 5-methylcytosine restriction system. Immediately flanking this region is a truncated copy of the putative Na<sup>+</sup>/*myo*-inositol cotransporter, which is interrupted by a 55-bp imperfect inverted repeat (Fig. 2). In S04010, downstream of *orfX* is an ~30 kb region which is absent from both S04009 and C2a. This region displays short regions of homology to corre-

sponding regions in *E. faecalis*, *Staphylococcus carnosus* subsp. *carnosus*, *S. aureus*, and a number of other Gram-positive species. The region proximal to *orfX* contains a number of hypothetical proteins and, like C2a, a putative restriction modification system. Downstream of this is a sorbitol utilization operon which is found next to a type IV SCC*mec* in *S. aureus* strain VRS3a (17) and part of a SCC*mec*WAMRSA40 composite island (EMBL accession no. JQ746621) which is found on the chromosome in *S. carnosus* strain TM300 (17). The sorbitol operon is also present in *E. faecalis* strain D32. Further downstream from this are three genes that make up a *bgl* (ary1-β,<sub>D</sub>-glucoside) operon. Downstream from this is an ~200-bp region that shares 91% nucleotide identity with the IR-containing region in C2a (Fig. 2), the IR itself being identical in 50 of 55 nucleotides. Further small regions of homology exist between S04009 and S04010, consisting of an ~750 bp region immediately downstream of the ACME DR in S04009 and a region just before the *bgl* operon in S04010. In order to ascertain the prevalence of *mecC1* in *S. xylosus* strains, we screened a total of 114 *S. xylosus* isolates from a wide range of sources, though with a deliberate bias toward isolates from bovine milk, as this was the original source of the strain S04009 (Table 1). (Additional information about *S. xylosus* strains screened for *mecC* is presented in Table 2.) We screened the strains by PCR using primers for *mecC*/*mecC1*, *blaZ*, and *mecA* and universal staphylococcal 16S primers (Table 3). Neither *mecA*, *mecC*, nor *blaZ* was detected in any of these isolates.

The finding that multiple components of the type XI SCC*mec* are present in contiguous blocks in the chromosome of *S. xylosus* S04009 suggests that this element may represent the remnants of an ancestral SCC*mec* element. Given the lack of any SCC*mec* flanking repeats in S04009 and the change in the GC content after the inverted repeat between the MI utilization cluster region and the *mecC1*-containing region, it is not clear if these two regions represent a single larger element or multiple independent acquisitions by an ancestral strain. The presence of the truncated MI cluster in C2a does suggest that the MI utilization cluster was part



**FIG 2** Comparison of the *orfX* regions in *S. xylois* strains C2a (EMBL accession no. HE993886), S04009 (EMBL accession no. HE993884), and S04010 (EMBL accession no. HE993885). Red areas show regions conserved between the two sequences, and homologous CDSs are in light and dark green. Other CDSs of interest discussed in the text are highlighted in color. Blue dots indicate direct repeats (DR), and green and red dots show inverted repeats (IR) (the inverted repeats in C2a and S04010 are virtually identical). The GC content of the region is shown above each genome schematic. The eighth gene in the *myo*-inositol cluster that is truncated in C2a and intact in S04009 is indicated with shading.

of a single contiguous block with the lipase and the ABC transporters in both S04009 and C2a. The finding that the arsenic resistance genes are also present in S04009 in association with a transposon further highlights a potential mechanism for the acquisition of these genes into the type XI SCC*mec*. Therefore, based on the available evidence, we suggest that the class E *mec* complex in *S. xylois* was part of a larger ancestral SCC*mec* element which probably included the MI cluster and the lipase and ABC transporters and that this element has undergone gradual deletion and acquisition (of the arsenic resistance genes) to the type XI SCC*mec* identified in *S. aureus* LGA251 (5). The fact that we found no other *S. xylois* strains harboring *mecC* or *blaZ* suggests that *mecC1* might be present in only a minor subset of *S. xylois* isolates. It is noteworthy that *S. xylois* is present in fermented foods such as sausage (18, 19) and cheese (20), highlighting another potential route for the transmission of antibiotic resistance genes from the

environment to human flora (21). Given the recent discovery of *mecR2*, a third regulator of *mecA* expression, it would also be interesting to see if the expression of *mecC1* is positively regulated in the same way by *xylR*, encoding the xylose operon repressor (present in *S. xylois* S04009), which is a close homolog of the *mecR2* regulator (22). In *S. xylois*, as in other staphylococci, the *orfX* locus is a site for the integration of multiple SCC-like elements. The strains analyzed in this study have metabolic utilization clusters present at *orfX*, which may reflect the biological niche occu-

**TABLE 1** Bacterial strains used in this study

| Species          | Strain  | Relevant characteristics       | Reference |
|------------------|---------|--------------------------------|-----------|
| <i>S. xylois</i> | S04009  | <i>mecC1</i> , bovine mastitis | 13        |
| <i>S. xylois</i> | S040010 | Bovine mastitis                | 13        |
| <i>S. xylois</i> | C2a     | Human skin commensal           | 13        |
| <i>S. aureus</i> | LGA251  | <i>mecC</i> , ST425            | 5         |

**TABLE 2** Overview of *Staphylococcus xylois* isolates screened for *mecC*

| No. of isolates screened | Relevant characteristics   | Reference        |
|--------------------------|--|------------------|
| 15                       | The Netherlands, bovine milk; oxacillin MIC $\geq$ 0.5 $\mu$ g/ml            | 26               |
| 20                       | Switzerland, bovine milk; oxacillin MIC $\geq$ 0.5 $\mu$ g/ml                | This work        |
| 5                        | France, various sources  | 13               |
| 3                        | Switzerland, horse skin; 2 isolates with oxacillin MIC $\geq$ 0.5 $\mu$ g/ml | 27               |
| 70                       | United States, bovine milk and streak canals                                 | 28 and this work |
| 1                        | United States, human skin; ATCC 29971  | 29               |

TABLE 3 Oligonucleotide primers used in this study

| Primer name | Sequence (5'→3')                  | Target      | Reference |
|-------------|-----------------------------------|-------------|-----------|
| mecC1 + 2_F | 5'-AAGTTAATCAAAAATGGGTTTCAGC-3'   | <i>mecC</i> | This work |
| mecC1 + 2_R | 5'GGTTGTAATGCTGTACCAGATCC-3'      | <i>mecC</i> | This work |
| blaZ_XI_F   | 5'-CGTTTTGCWATGCTTCCAC-3'         | <i>blaZ</i> | This work |
| blaZ_XI_R   | 5'-CKGGTCTTTCTAGATGGATG-3'        | <i>blaZ</i> | This work |
| MecA1       | GTA GAA ATG ACT GAA CGT CCG ATA A | <i>mecA</i> | 30        |
| MecA2       | CCA ATT CCA CAT TGT TTC GGT CTA A | <i>mecA</i> | 30        |
| 16SF        | CCTATAAGACTGGGATAAAGCTCGGG        | 16S rDNA    | 31        |
| 16SR        | CTTTGAGTTTCAACCTTGGCGTGCC         | 16S rDNA    | 31        |

pied by the *S. xyloso* isolates included in this study. In addition, regions of DNA are present in both C2a and S40010 with close homology to *E. faecalis* strain D32, an isolate from a pig (23). This indicates that horizontal gene transfer between enterococci and staphylococci is a relatively common occurrence (24, 25), an important observation in relation to the transfer of vancomycin resistance to *S. aureus*. In conclusion, this study further highlights the fact that CoNS from both humans and animals are an important reservoir of resistance genes that have the potential to be transferred into more pathogenic staphylococcal species.

**Nucleotide sequence accession numbers.** The nucleotide sequences determined for the *orfX* region of S04009, S04010, and C2a have been deposited in the EMBL database under accession numbers HE993884, HE993885, and HE993886, respectively.

#### ACKNOWLEDGMENTS

This work was supported by a Medical Research Council Partnership grant (G1001787/1) held between the Department of Veterinary Medicine, University of Cambridge (M.A.H.), the School of Clinical Medicine, University of Cambridge (S.J.P.), the Moredun Research Institute (R.N.Z.) and the Wellcome Trust Sanger Institute (J.P. and S.J.P.).

We are grateful to Alexander Tomasz and Hermínia de Lencastre for their comments on the manuscript.

#### REFERENCES

- Hartman BJ, Tomasz A. 1984. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. *J. Bacteriol.* 158:513–516.
- Lim D, Strynadka NC. 2002. Structural basis for the beta lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. *Nat. Struct. Biol.* 9:870–876.
- Ubukata K, Nonoguchi R, Matsuhashi M, Konno M. 1989. Expression and inducibility in *Staphylococcus aureus* of the *mecA* gene, which encodes a methicillin-resistant *S. aureus*-specific penicillin-binding protein. *J. Bacteriol.* 171:2882–2885.
- Fuda C, Suvorov M, Vakulenko SB, Mobashery S. 2004. The basis for resistance to beta-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. *J. Biol. Chem.* 279:40802–40806.
- García-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA. 2011. Methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect. Dis.* 11:595–603.
- 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.* 67:2809–2813.
- Cuny C, Layer F, Strommenger B, Witte W. 2011. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mecA* homologue in humans in Germany. *PLoS One* 6:e24360. doi:10.1371/journal.pone.0024360.
- Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, Ehrlich R, Coleman DC. 2011. 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.* 55:3765–3773.
- Kriegeskorte A, Ballhausen B, Idelevich EA, Kock R, Friedrich AW, Karch H, Peters G, Becker K. 2012. Human MRSA isolates with novel genetic homolog, Germany. *Emerg. Infect. Dis.* 18:1016–1018.
- Sabat AJ, Koksall M, Akkerboom V, Monecke S, Kriegeskorte A, Hendrix R, Ehrlich R, Kock R, Becker K, Friedrich AW. 2012. Detection of new methicillin-resistant *Staphylococcus aureus* that carry novel genetic homologue and important virulence determinants. *J. Clin. Microbiol.* 50:3374–3377.
- Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. 2012. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecA(LGA251)*. *Clin. Microbiol. Infect.* 18:395–400.
- Tsubakishita S, Kuwahara-Arai K, Baba T, Hiramatsu K. 2010. Staphylococcal cassette chromosome *mec*-like element in *Macrocooccus caseolyticus*. *Antimicrob. Agents Chemother.* 54:1469–1475.
- Dordet-Frisoni E, Dorchies G, De Araujo C, Talon R, Leroy S. 2007. Genomic diversity in *Staphylococcus xyloso*. *Appl. Environ. Microbiol.* 73:7199–7209.
- Quail MA, Zozarewa I, Smith F, Scally A, Stephens PJ, Durbin R, Sverdlow H, Turner DJ. 2008. A large genome center's improvements to the Illumina sequencing system. *Nat. Methods* 5:1005–1010.
- Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, Lin F, Lin J, Carleton HA, Mongodin EF, Sensabaugh GF, Perdreau-Remington F. 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet* 367:731–739.
- Ito T, Hiramatsu K, Tomasz A, de Lencastre H, Perreten V, Holden MTG, Coleman DC, Goering R, Giffard PM, Skov RL, Zhang K, Westh H, O'Brien F, Tenover FC, Oliveira DC, Boyle-Vavra S, Laurent F, Kearns AM, Kreiswirth B, Ko KS, Grundmann H, Sollid JE, John JF, Daum R, Soderquist B, Buist G. 2012. Guidelines for reporting novel *mecA* gene homologues. *Antimicrob. Agents Chemother.* 56:4997–4999.
- Kos VN, Desjardins CA, Griggs A, Cerqueira G, Van Tonder A, Holden MTG, Godfrey P, Palmer KL, Bodi K, Mongodin EF, Wortman J, Feldgarden M, Lawley T, Gill SR, Haas BJ, Birren B, Gilmore MS. 2012. Comparative genomics of vancomycin-resistant *Staphylococcus aureus* strains and their positions within the clade most commonly associated with methicillin-resistant *S. aureus* hospital-acquired infection in the United States. *mBio* 3:e00112–12. doi:10.1128/mBio.00112-12.
- Even S, Leroy S, Charlier C, Zakour NB, Chacornac JP, Lebert I, Jamet E, Desmonts MH, Coton E, Pochet S, Donnio PY, Gautier M, Talon R, Le Loir Y. 2010. Low occurrence of safety hazards in coagulase negative staphylococci isolated from fermented foodstuffs. *Int. J. Food Microbiol.* 139:87–95.
- Perreten V, Giampa N, Schuler-Schmid U, Teuber M. 1998. Antibiotic resistance genes in coagulase-negative staphylococci isolated from food. *Syst. Appl. Microbiol.* 21:113–120.
- Coton E, Desmonts MH, Leroy S, Coton M, Jamet E, Christieans S, Donnio PY, Lebert I, Talon R. 2010. Biodiversity of coagulase-negative

- Staphylococci in French cheeses, dry fermented sausages, processing environments and clinical samples. *Int. J. Food Microbiol.* 137:221–229.
21. Resch M, Nagel V, Hertel C. 2008. Antibiotic resistance of coagulase-negative staphylococci associated with food and used in starter cultures. *Int. J. Food Microbiol.* 127:99–104.
  22. Arêde P, Milheiriço C, de Lencastre H, Oliveira DC. 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:e1002816. doi:10.1371/journal.ppat.1002816.
  23. Larsen J, Schonheyder HC, Singh KV, Lester CH, Olsen SS, Porsbo LJ, Garcia-Migura L, Jensen LB, Bisgaard M, Murray BE, Hammerum AM. 2011. Porcine and human community reservoirs of *Enterococcus faecalis*, Denmark. *Emerg. Infect. Dis.* 17:2395–2397.
  24. Noble WC, Virani Z, Cree RG. 1992. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol. Lett.* 72:195–198.
  25. Weigel LM, Clewell DB, Gill SR, Clark NC, McDougal LK, Flannagan SE, Kolonay JF, Shetty J, Killgore GE, Tenover FC. 2003. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science* 302:1569–1571.
  26. Sampimon OC, Lam TJ, Mevius DJ, Schukken YH, Zadoks RN. 2011. Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine milk samples. *Vet. Microbiol.* 150:173–179.
  27. Schnellmann C, Gerber V, Rossano A, Jaquier V, Panchaud Y, Doherr MG, Thomann A, Straub R, Perreten V. 2006. Presence of new *mecA* and *mph(C)* variants conferring antibiotic resistance in *Staphylococcus* spp. isolated from the skin of horses before and after clinic admission. *J. Clin. Microbiol.* 44:4444–4454.
  28. Park JY, Fox LK, Seo KS, McGuire MA, Park YH, Rurangirwa FR, Sischo WM, Bohach GA. 2011. Detection of classical and newly described staphylococcal superantigen genes in coagulase-negative staphylococci isolated from bovine intramammary infections. *Vet. Microbiol.* 147:149–154.
  29. Schleifer KH, Meyer SA, Rupprecht M. 1979. Relatedness among coagulase-negative staphylococci: deoxyribonucleic acid reassociation and comparative immunological studies. *Arch. Microbiol.* 122:93–101.
  30. Perez-Roth E, Claverie-Martin F, Villar J, Mendez-Alvarez S. 2001. Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. *J. Clin. Microbiol.* 39:4037–4041.
  31. Mason WJ, Blevins JS, Beenken K, Wibowo N, Ojha N, Smeltzer MS. 2001. Multiplex PCR protocol for the diagnosis of staphylococcal infection. *J. Clin. Microbiol.* 39:3332–3338.