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The *bla* and *mec* families of β-lactam resistance genes in the genera *Macrococcus*, *Mammaliicoccus* and *Staphylococcus*: an in-depth analysis with emphasis on *Macrococcus*

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β-Lactamases (Bla) and low-affinity penicillin-binding proteins (PBP2A) are responsible for β-lactam resistance in the genera *Macrococcus*, *Mammaliicoccus* and *Staphylococcus*. These resistance mechanisms are in most species acquired through mobile genetic elements that carry a *blaZ*-like β-lactamase gene for penicillin resistance and/or a *mec* gene (*mecA*, *mecB*, *mecC*, *mecD*) encoding a PBP2A for resistance to virtually all classes of β-lactams. The *mecA* and *mecC* genes can be acquired through staphylococcal cassette chromosome *mec* (SCC*mec*) elements in *Staphylococcus* and *Mammaliicoccus*. The *mecB* and *mecD* genes are found in *Macrococcus* on SCC*mec* elements, as well as on unrelated *mecD*-carrying *Macrococcus* resistance islands (McRI$_{mecD}$) and large *mecB*-carrying plasmids. This review provides a phylogenetic overview of *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* species and an in-depth analysis of the genetic structures carrying *bla* and *mec* genes in these genera. Native *bla* genes were detected in species belonging to the novobiocin-resistant *Staphylococcus saprophyticus* group and *Mammaliicoccus*. The evolutionary relatedness between *Macrococcus* and *Mammaliicoccus* is illustrated on the basis of a similar set of intrinsic PBPs, especially, the presence of a second class A PBP. The review further focuses on macrococcal elements carrying *mecB* and *mecD*, and compares them with structures present in *Staphylococcus* and *Mammaliicoccus*. It also discusses the different recombinases (*ccr* of SCC*mec*) and integrases (*int* of McRI) that contribute to the mobility of methicillin resistance genes, revealing *Macrococcus* as an important source for mobilization of antibiotic resistance genes within the family of *Staphylococcaceae*.

Introduction

Macrococcus, *Mammaliicoccus* and *Staphylococcus* are phylogenetically related Gram-positive coccoid bacteria. *Macrococcus* spp were originally included in the genus *Staphylococcus* and assigned to a separate genus in 1998 due to different genetic and phenotypic features.[1,2] *Mammaliicoccus* were classified until 2020 in the *Staphylococcus sciuri* group and reassigned to a separate genus based on a recent genome-wide phylogenetic analysis of the *Staphylococcaceae* family.[3] Nevertheless, *Macrococcus* and *Mammaliicoccus* share several molecular pathways and antimicrobial resistance mechanisms with *Staphylococcus*.[4,5] For instance, acquired resistance to β-lactam antibiotics is mediated in *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* by the production of either an extra penicillin-binding protein with low affinity for β-lactams (PBP2A) or a β-lactamase (Bla). The β-lactamase, which is encoded by a *blaZ* gene, hydrolyses only penicillin-rings, hence conferring narrow-spectrum β-lactam resistance.[6,7] The PBP2A is an alternative transpeptidase encoded by a *mec* gene which contributes to cell wall synthesis and permits peptidoglycan crosslinking in the presence of β-lactam concentrations that are inhibitory for the other intrinsic PBP.[8] PBP2A confers broad-range resistance to virtually all classes of β-lactam with the exception of a few cephalosporins such as ceftaroline and ceftobiprole, which are still effective against MRSA.[9] PBP2A and BlaZ-like β-lactamases are structurally related and contain a similar transpeptidase domain.[10] Expression of the *mec* and *blaZ* genes is regulated through related systems consisting of a sensor/transducer (MecR1 or BlaR1) and transcriptional repressors (MecI or BlaI).[8] Currently, four *mec* genes (*mecA*, *mecB*, *mecC* and *mecD*) are known; they are organized in operons with cognate regulatory genes (*mecA* or *mecD* with *mecR1* and *mecI*) or with regulatory genes and a *blaZ* gene (*mecB* or *mecC* with *blaZ*, *mecR1* and *mecI*). In addition, operons containing only a β-lactamase gene exist (*blaZ* or *bla*$_{ARL}$ with *blaR1* and *blaI*) in *Staphylococcus*.[11,12]

Infections caused by *Staphylococcus aureus* have been of the greatest concern due to the higher virulence potential of *S. aureus* compared with the other species of the *Staphylococcus*, *Mammaliicoccus* and *Macrococcus* genera. Therefore, resistance to the clinically relevant β-lactam antibiotics has been intensely studied with a focus on *S. aureus* for decades. A few years after introduction of penicillin in 1942 and methicillin in 1959 for clinical application, penicillin- and methicillin-resistant *S. aureus* started to spread in healthcare settings and later in community

settings.[13] The penicillin-resistant strains had acquired the *blaZ* gene on a transposon or plasmid, and the MRSA strains acquired the *mecA* gene through a staphylococcal cassette chromosome *mec* (SCC*mec*) element. So far, up to 14 SCC*mec* types have been described in MRSA.[14,15] With progress and increasing use of WGS, the distribution and complexity of SCC*mec* structures in *Staphylococcus* became more visible and non-*S. aureus* staphylococci and *Mammaliicoccus* were noticed as possible reservoirs of SCC*mec* elements.[16] Since 2011, *mecC* has been identified as a second determinant for methicillin resistance in *Staphylococcus* species.[17,18] In contrast, methicillin resistance in *Macrococcus* species has been less studied and is mediated by *mecB* and *mecD*, both genes were first detected in *M. caseolyticus* in 2009 and 2017, respectively.[4,19] Distribution of *mecA* and *mecC* in *Staphylococcus* and *Mammaliicoccus* and *mecB* and *mecD* in *Macrococcus* seemed to be genus-specific until 2018, when an MRSA containing the *mecB* gene was isolated from a human carrier in Germany.[20] This MRSA strain carried *mecB* on a plasmid typically found in *Macrococcus* species, suggesting that exchange of the mobile genetic element occurred between the genera.[20,21]

This review provides an in-depth overview of the different β-lactam resistance mechanisms present in *Macrococcus*, *Mammaliicoccus* and *Staphylococcus*. An important task was to illustrate the evolutionary relationship between different β-lactam resistance systems by comparing the *mec*- and/or *bla*-containing operons across the three genera. Data from the literature and from recent sequences available in the NCBI GenBank database were used in combination with bioinformatics tools to cluster orthologous genes. This approach allowed visualization of the relationship between members of *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* based on several hundreds of genes. A cross-species comparison of the different intrinsic proteins that could be involved in final peptidoglycan assembly, such as PBPs and glycosyltransferases (GT) was also included since PBP2A depends on at least one native GT enzyme for expression of β-lactam resistance.[22] The second part provides a detailed presentation and analysis of the versatile mobile genetic elements that carry *mecB* and *mecD*. The elements characterized so far in *Macrococcus* were compared with each other and with sequences from the GenBank database. SCC*mec* elements containing *mecA* and *mecC* are not discussed in detail here. These elements have been well illustrated in many other review articles.[14,16,23]

The genera *Macrococcus*, *Mammaliicoccus* and *Staphylococcus*

The genera *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* contain catalase-positive cocci with low DNA G+C content[2,4,24] that cluster into three related clades within the family of *Staphylococcaceae*.[3] Historically, *Macrococcus* and *Mammaliicoccus* species had been included in the genus *Staphylococcus* until assigned to the separate genus *Macrococcus* in 1998[1] and *Mammaliicoccus* in 2020.[3]

At the time the genus *Macrococcus* was introduced, it contained only four species, namely the reclassified *M. caseolyticus* (former *S. caseolyticus*[25]) and the novel species *M. bovicus*, *M. carouselicus* and *M. equipercicus*.[1] These species could be distinguished from *Staphylococcus* species based on significantly lower 16S rRNA gene similarities (93.4%–95.3%) and a smaller genome size (1.5–1.8 Mb estimated based on macrorestriction pattern) with a higher G+C content (38%–45%).[1] Additional members of the genus *Macrococcus* were later identified, including *M. brunensis*, *M. hajekii* and *M. lamae* in 2003,[26] *M. canis* in 2017,[27] *M. goetzii*, *M. bohemicus* and *M. epidermidis* in 2018[28] and *M. armenti* in 2022.[29] Considering WGS data, the criteria for the genus *Macrococcus* defined by Kloos *et al.*[1] need to be updated with regard to genome size and G+C content. A rather small genome of 2.0 to 2.2 Mb is observed for *M. caseolyticus*, *M. bovicus*, *M. brunensis*, *M. carouselicus*, *M. equipercicus*, *M. hajekii* and *M. lamae*.[4,30] Larger genomes of 2.3 to 2.4 Mb are seen for *M. armenti*, *M. canis* and *M. bohemicus*[28,29,31] and of 2.5 to 2.6 Mb for *M. epidermidis* and *M. goetzii*.[28] The genome size of *Staphylococcus* ranges from 2.1 to 3.0 Mb (NCBI Genome database). *S. aureus* has an average genome size of 2.8 Mb,[32] although an exceptionally small genome of 2.1 Mb is found for *S. muscae* (GenBank: CP027848), and small genomes of around 2.3 Mb are also reported for *S. hominis*,[33] *S. auricularis* (GenBank: NZ_LS483491), *S. chromogenes*,[34] *S. rostri* (GenBank: PPRF00000000) and *S. canis*.[35] *Mammaliicoccus* species have genome sizes of 2.4 to 2.8 Mb and variable G+C content of 32% to 36%.[3] The G+C content of 40% to 44% is higher for the *Macrococcus* species, *M. bovicus*, *M. brunensis*, *M. carouselicus*, *M. equipercicus*, *M. hajekii* and *M. lamae*[30] than that of *Mammaliicoccus* and *Staphylococcus* (33%–40%).[2] However, *M. armenti*, *M. caseolyticus* and *M. canis*[4,29,31] as well as *M. epidermidis*, *M. bohemicus* and *M. goetzii*[28] have lower G+C contents of 36%, 37% and 34%, respectively, that fall within the G+C range of *Staphylococcus*.

The reclassification of the five former *Staphylococcus sciuri* group species into the novel genus *Mammaliicoccus* (*M. sciuri*, *M. fleurettii*, *M. lentus*, *M. stepanovicii* and *M. vitulinus*) was recently undertaken based on a phylogenetic analysis of the *Staphylococcaceae* family.[3] Clustering of species using conserved core genes grouped the *Mammaliicoccus* species in a monophyletic clade that was similarly distant from *Staphylococcus* species as the clade of *Macrococcus* species.[3]

In December 2021, the genus *Staphylococcus* contained 61 validly published species in the List of Prokaryotic names with Standing in Nomenclature (LPSN).[36] All recognized species belonging to the genera *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* were clustered into a species tree based on orthologous protein coding genes identified with the OrthoFinder software (Figure 1).[37,38] The result showed closer relationship of *Macrococcus* species with *Mammaliicoccus* than with *Staphylococcus* species. The *Macrococcus* and *Mammaliicoccus* strains used in our analysis shared 1178 orthogroups (OG) with each other and only 724 and 814 with the *Staphylococcus* species, respectively (See Figure 2 for distribution of shared OG genes in the genome of *M. canis*). The evolutionary proximity between *Macrococcus* and *Mammaliicoccus* was already suggested by Kloos *et al.*[1] based on DNA–DNA hybridization studies. Furthermore, *Macrococcus* species shared slightly higher mean amino acid identities (AAI) with *Mammaliicoccus* (64%) than with *Staphylococcus* species (62%) in the study of Madhaiyan *et al.*[3] Moreover, members of the genus *Macrococcus* and *Mammaliicoccus* can be biochemically distinguished from *Staphylococcus* by a positive oxidase test reaction.[39] For *M. caseolyticus*, machineries for electron transport chain similar to

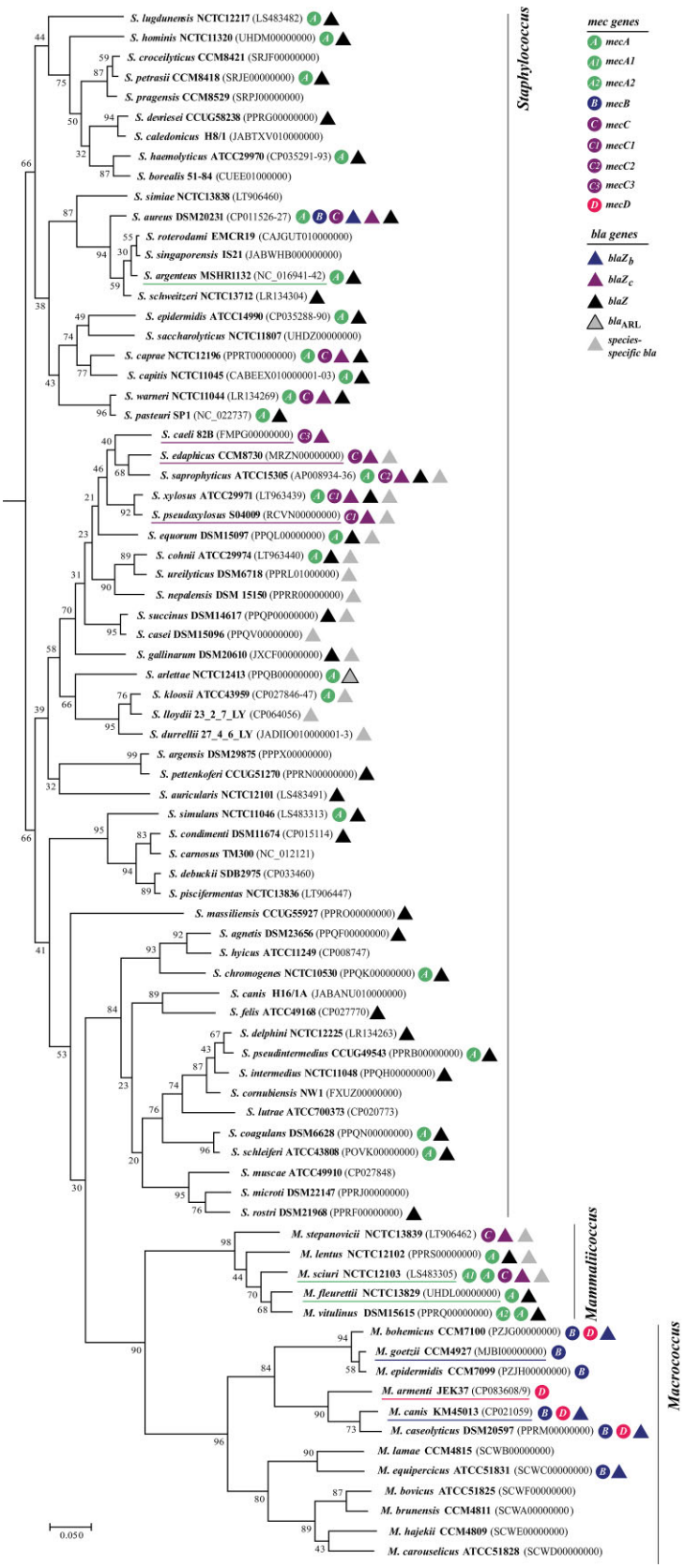


Figure 1.

those present in *Bacillus* species have been reported.[4] Four c-type cytochromes (encoded by *ctaC*, *qcrC*, *cccA* and *cccB*) are present in *Macrococcus* und *Mammaliicoccus* species and are absent in all *Staphylococcus* species. These cytochromes belong to a heterogeneous protein family containing a covalently attached haem group[40] that can be identified by the presence of cytochrome c family profile CYTC in PROSITE database (PROSITE entry PS51007).[41,42] The c-type cytochromes *qcrC*, *ccrA*, *ccrB* as well as genes identified in *B. subtilis* essential for cytochrome c biogenesis such as *ctaG*,[43] *resB*, *resC* and *ccdA*[44] were identified by OrthoFinder in OG only shared between *Macrococcus* and *Mammaliicoccus* species (Table S1, available as Supplementary data at *JAC* Online). The quinol oxidase components encoded by *qoxABCD* are absent in *Macrococcus*, but present in *Mammaliicoccus* as well as in *Staphylococcus* and *B. subtilis*.[4]

Mammaliicoccus were originally considered to contain a group of ancestral bacteria.[3,5] The *Mammaliicoccus* species *M. sciuri* and *M. fleuretti* have attracted special attention because they encode a native PBP2A in the chromosome that is highly similar to the acquired *mecA* gene of SCC*mec* elements (see below).[45–47] *Mammaliicoccus* isolates are in general classified as commensals, but can occasionally cause infections in humans and animals.[5] They were found in a wide range of hosts including domestic and wild animals and the environment and they may represent a reservoir for resistance genes.[5,48]

Macrococcus species are, like *Mammaliicoccus*, also considered as commensals of mammals with low pathogenic potential. However, data about ecological distribution is rather scarce and often biased by selecting for methicillin-resistant isolates. The best documented *Macrococcus* species is *M. caseolyticus*. It was first isolated in 1916 from bovine milk and named for the ability for rapid and complete peptonization of milk (casein-dissolving).[49] Later, *M. caseolyticus* was isolated in many occasions from skin and organs of cattle and from raw milk[1,50–54] as well as during cheese production.[55–57] The proteolytic enzymes from *M. caseolyticus* also play a role in the food industry and are associated with the development of aroma and flavour in fermented products.[58,59] Besides cattle, which seem to represent a natural host for *M. caseolyticus*, isolates were also obtained from other farm animals such as sheep,[60] chickens[4,61,62] and pigs.[63] Other *Macrococcus* species were also isolated from the skin and mucous membranes of cattle, namely *M. bovicus*,[1] *M. goetzii* and *M. canis*,[53] *M. bohemicus*[64] and *M. armenti*.[29] The skin of dogs was found to harbour *M. caseolyticus* and *M. canis*,[65,66] as well as a few other *Macrococcus* species, such as *M. equipercicus* (GenBank: CP073809), *M. epidermidis* (GenBank: CP073819) and *M. bohemicus*.[67] The skin of llamas was reported to be colonized with *M. lamae*, *M. hajekii* and *M. brunensis*,[26] that of horses and ponies with *M. bovicus*, *M. equipercicus* and *M. carouselicus*,[1] and that of pigs with *M. armenti*.[29]

Macrococcus species seem not to be commensal bacteria of the human skin. Only one report described *M. caseolyticus* in the nose of a healthy carrier.[68] However, *M. caseolyticus*, *M. goetzii*, *M. bohemicus*, *M. epidermidis* and *M. canis* were recovered from human clinical specimens.[28,69] Association with infections in animals was reported for *M. canis*,[65] *M. bohemicus*[67] and *M. caseolyticus*.[50,60,62]

Staphylococcus are also adapted to the skin and mucous membranes of mammals, hence their habitats overlap with that of *Macrococcus* and *Mammaliicoccus*. Especially, coagulase-negative *Staphylococcus* have been isolated from farm animals, pets, and fermented food and dairy products.[70] Accurate identification of species in the genera *Staphylococcus* and *Macrococcus* is not always simple. Cut-off values proposed for species definition using whole-genome sequences such as digital DNA–DNA hybridization (dDDH) (<70%),[71] average nucleotide identity (ANI) (<95% to 96%),[72] revealed many new species in these genera, some of them cannot be discriminated using 16S rRNA gene sequences. For example, four new species with near-identical 16S rRNA genes (99.8% to 100%) to that of *S. aureus* were described in the last 6 years.[73–75] These highly related species, *S. aureus*, *S. argenteus*, *S. schweitzeri*, *S. singaporensis* and *S. roterodami*, constitute the members of the clinical relevant *S. aureus* complex (Figure 1). 16S rRNA gene identity of 99.94% to 100% is also not appropriate to assign the closely related *Macrococcus* species *M. bohemicus*, *M. epidermidis* and *M. goetzii* (Figure 1). For identification, other markers such as the *hsp60* gene in *Macrococcus*[28,54] and the *rpoB* gene in *Staphylococcus*[76] have been shown to be more discriminative than the 16S rRNA gene. WGS data reveals new insights into phylogeny of *Macrococcus*, *Mammaliicoccus* and *Staphylococcus*. We used these WGS data to uncover and present putative peptidoglycan assembly components and β-lactam resistance genes in these genera.

Peptidoglycan and penicillin-binding proteins

Peptidoglycan structure and assembly

β-Lactam antibiotics inhibit the synthesis of the peptidoglycan layer of bacterial cell walls by targeting the bacterial assembly enzymes. Recent insights into the cell wall structure of *S. aureus* by atomic force microscopy showed a dense mesh of inner peptidoglycan, followed by a more disordered outer peptidoglycan layer with larger pores.[77] This architecture suggests remodelling of peptidoglycan after synthesis and an interplay between the cell wall synthesis and hydrolysis enzymes.[77] The crosslinks in the cell wall peptidoglycan of *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* species are similar and assigned to the A3α type.[78] Their linear glycan strands composed

Figure 1. Continued
Rooted tree for *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* species based on orthogroups of protein-coding genes. Amino acid sequences of representative strains were derived from GenBank entries indicated in parenthesis. Type strain sequences were used except for *S. pasteuri* and *S. carnosus* where another representative sequence was used. OrthoFinder software (version 2.3.8)[37,38] identified 710 orthogroups with all strains present and used them to build the species tree with the integrated STAGE[199] and STRIDE[200] algorithms. Presence of *mec* and *bla* genes in the species is indicated according to key on the right side. Used representative strains that carry a *mec* gene are underlined. *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* species specified as validly published in the List of Prokaryotic names with Standing in Nomenclature (LPSN) December 2021 were used for analysis (https://lpsn.dsmz.de/). Sequences available in GenBank database in January 2022 were used to screen for the distribution of *mec* and *bla* genes in the different species. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

of β-1,4-linked alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) are crosslinked via glycine-rich interpeptide bridges between D-Ala at position 4 and L-Lys at position 3 of stem peptides attached to MurNAc.[79] The stem-pentapeptide of MurNAc usually consists of L-Ala^1-γ-D-Glu^2-L-Lys^3-D-Ala^4-D-Ala^5 in most Gram-positive bacteria.[79] More variations are seen in the composition and length of the interpeptide bridges. L-Lys^3-Gly$_{2-4}$, L-Ser interpeptide bridges are described for *M. armenti*, *M. caseolyticus*, *M. bovicus*, *M. carouselicus*, *M. equipercicus*, *M. canis* and *M. epidermidis*.[1,27–29] L-Lys^3-Gly$_{1-2}$ was found in *M. bohemicus* and *M. goetzii*.[28] L-Lys^3-Gly$_{4-6}$, and L-Lys^3-Gly$_4$, L-Ser are reported for most *Staphylococcus*[2] while L-Lys^3-L-Ala-Gly$_{3-5}$ was found in *Mammaliicoccus*.[80,81]

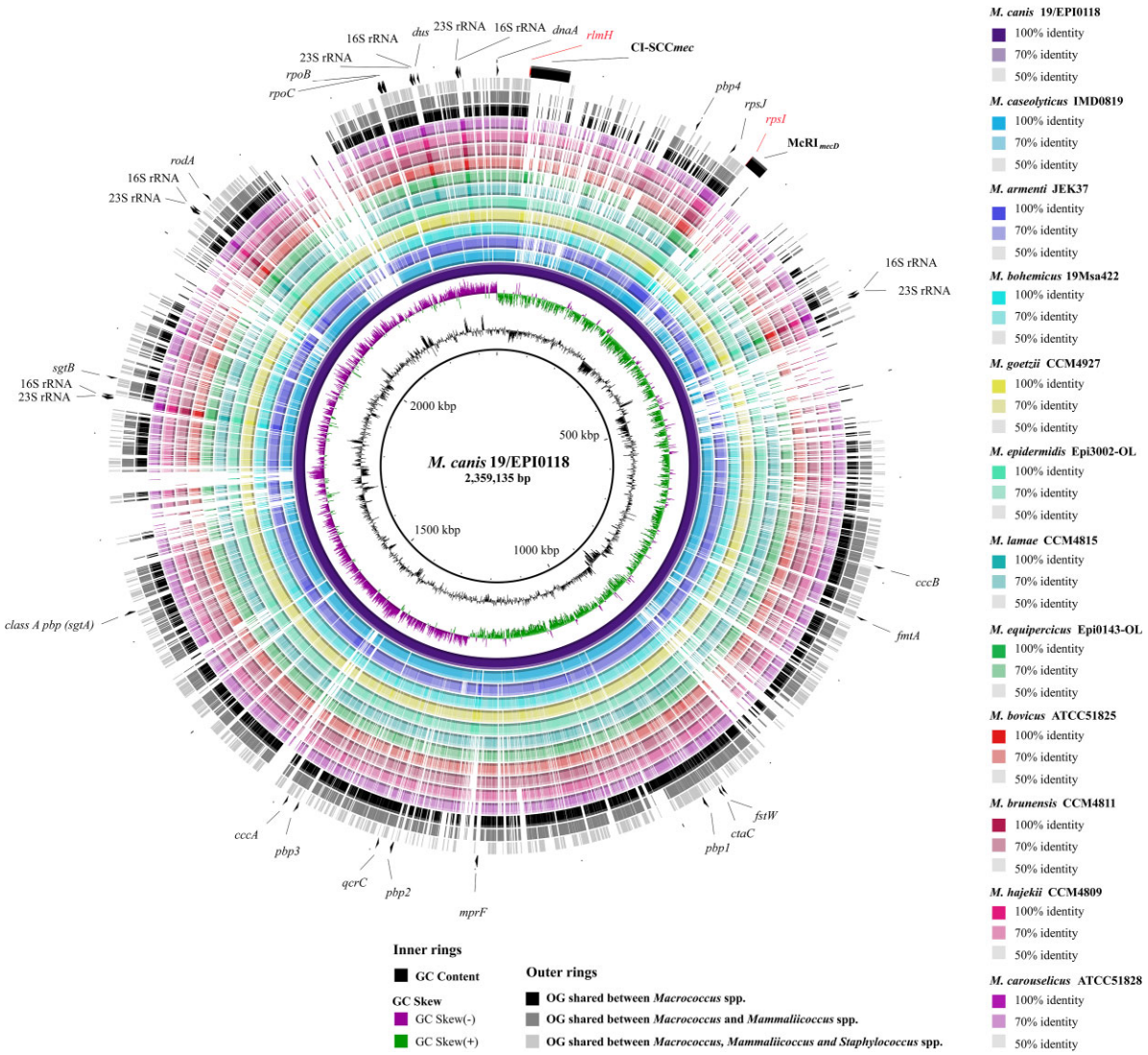


Figure 2. Chromosome comparison of the twelve *Macrococcus* species. *M. canis* 19/EPI0118 (GenBank: CP072837) that contains a composite ΨSCC*mecB* islands and McRI$_{mecD}$-1[187] was used as reference in BLAST Ring Image Generator (BRIG).[201] Query sequences and coverage are indicated in the key at the right. If available, a complete genome sequence was used instead of draft type strain sequence, namely for *M. caseolyticus* (strain IMD0819; GenBank: CP021058.1), *M. bohemicus* (19Msa422; CP054482), *M. epidermidis* (Epi3002-OL; CP073819) and *M. equipercicus* (Epi0143-OL; CP073809). Orthogroups (OG) with genes present in all *Macrococcus*, all *Macrococcus* and *Mammaliicoccus*, and all *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* species were identified with the OrthoFinder software[37,38] and indicated in black, grey and light grey, respectively. Annotations are given for PBP1 (*pbp1*), PBP2 (*pbp2*), PBP3 (*pbp3*), PBP4 (*pbp4*), FmtA (*fmtA*) and the additional class A PBP (*sgtA*); the monofunctional glycosyltransferase *sgtB*; SEDS family proteins (*ftsW* and *rodA*); the 16S-23S ribosomal DNA clusters; the four c-type cytochromes (*ctaC*, *qcrC*, *cccA* and *cccB*); RNA polymerase subunits (*rpoB* and *rpoC*); ribosomal protein (*rpsJ*); and tRNA dihydrouridine synthase (*dus*). The gene for the chromosomal replication initiator protein *dnaA* found at *oriC*, the genes associated with attachment sites for SCC*mec* (*rlmH*) and McRI (*rpsI*) are indicated in red. The location of a lysylphosphatidylglycerol flippase/synthetase (*mprF*) gene is also indicate, which is found upstream of alternative island integration related to McRI. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

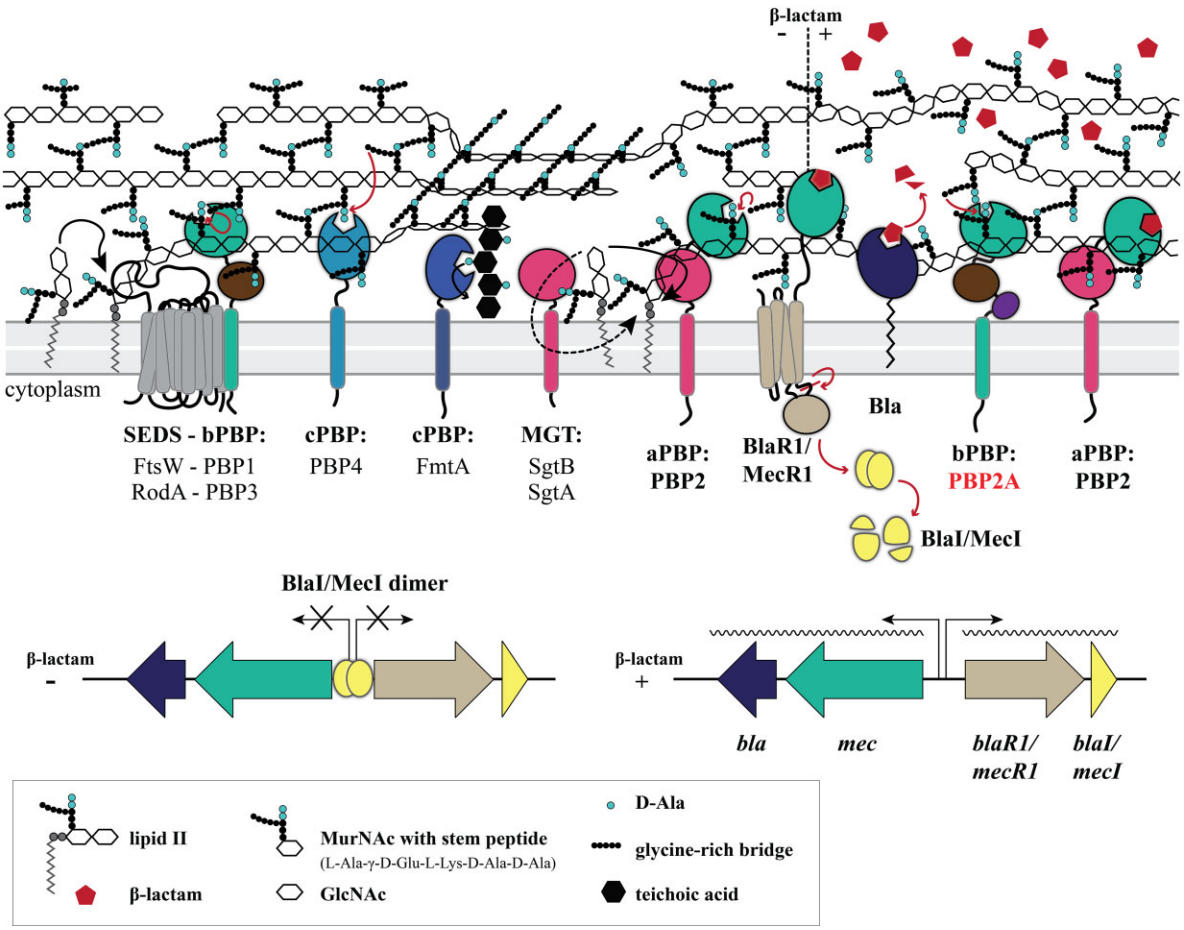


Figure 3. Model for cell wall peptidoglycan assembly in the absence and presence of β-lactam antibiotics. Linear glycan strand polymerization from N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) peptide precursor (lipid II) is conducted by shape, elongation, division and sporulation (SEDS) family proteins and unrelated proteins with glycosyltransferase activity including monofunctional glycosyltransferases (MGT) and class A PBP (aPBP). Crosslinking of glycan strands via glycine-rich bridges is performed by transpeptidases belonging to class A, B, and C PBP (aPBP, bPBP and cPBP). FmtA is a cPBP involved in D-alanylation of teichoic acid. β-Lactam antibiotics bind with high affinity to intrinsic PBP and inhibit their crosslinking activity. Killing of susceptible cells is thought to result from loss of cell wall integrity. Resistance to β-lactams can be obtained by acquisition of *mec* and/or *bla* genes encoding an alternative bPBP (PBP2A) and a β-lactamase (Bla), respectively. PBP2A has a lower affinity for β-lactam binding than intrinsic PBP. Hence, PBP2A can carry out crosslinking of glycan strands in the presence of virtually all classes of β-lactams. Bla can only hydrolyse and inactivate β-lactams belonging to the class of penicillins. *mec* and *bla* genes are organized in an operon with divergently transcribed regulatory genes *blaR1/mecR1* and *blaI/mecI*. These regulators are responsible for inducible expression of *mec* and *bla* genes. BlaR1/MecR1 are transmembrane sensor/transducer proteins that become activated through binding of β-lactam to the extracellular penicillin-binding domain. The signal is transmitted to the cytoplasmic domain leading to the proteolytic auto-activation of its metalloprotease activity and subsequently proteolytic inactivation of BlaI/MecI transcriptional repressors. Cleaved repressors lose their ability to bind the promoter-operator sequence of the β-lactam operon and transcription and translation of *mec* and *bla* genes is induced. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Proteins that have peptidoglycan glycosyltransferase (GT) and transpeptidase (TP) activity carry out final peptidoglycan assembly. GT enzymes polymerize GlcNAc-MurNAc disaccharide peptide precursor (lipid II) into linear glycan strands (Figure 3).[82,83] Crosslinking of the neighbouring glycan strand is catalysed by TP that recognizes the D-Ala^4-D-Ala^5 end of the stem pentapeptide. Due to the structural resemblance of β-lactam and D-Ala-D-Ala, TP enzymes are sensitive to β-lactams and prone to form a stable acyl-enzyme intermediate with them that impairs crosslinking capability.[84] The affinity for β-lactams is also the reason why TP are referred to as penicillin-binding proteins (PBP).

Intrinsic penicillin-binding proteins and glycosyltransferases in S. aureus

S. aureus has four native PBP: one single bifunctional enzyme (PBP2) which has TP and GT activity and belongs to class A PBP (aPBP), as well as two class B enzymes (bPBP: PBP1 and PBP3) and one class C enzyme (cPBP: PBP4) which have TP activity only (Figure 3).[83] For decades, PBP2 has been presumed to be the key GT enzyme for glycan chain polymerization from lipid II precursor.[85] However, RodA and FtsW, two proteins belonging to the shape, elongation, division and sporulation (SEDS) family,

have been shown to have GT activity[86,87] and are suggested to represent the primary peptidoglycan polymerases which build the cell wall peptidoglycan together with bPBPs (Figure 3).[88] Nevertheless, PBP2 as well as PBP1 have been shown to be essential in *S. aureus*.[89–91] PBP1 is crucial for the GT activity of FtsW, suggesting that FtsW-PBP1 forms a functional SEDS-bPBP pair in *S. aureus* that is responsible for the septal peptidoglycan incorporation (Figure 3).[87,92] While FtsW is essential, the second SEDS protein RodA, involved in lateral peptidoglycan synthesis, as well as its cognate bPBP (PBP3), are not required for viability of *S. aureus*.[92,93] PBP4 is a non-essential enzyme which has both TP and carboxypeptidase activities (Figure 3).[93,94] It is able to control the level of crosslinks in peptidoglycan by exchanging D-amino acids and removing the terminal D-Ala residues from stem-pentapeptides.[94] Increased production of PBP4 correlates with the formation of highly cross-linked peptidoglycan and increased β-lactam resistance.[95–98] While overproduction of the wild-type PBP4 protein can already cause this effect,[99] decreased β-lactam susceptibility was also associated with mutations in the TP domain of PBP1, PBP2 and PBP3.[100–102]

In addition to the SEDS proteins FtsW and RodA, *S. aureus* has two monofunctional glycosyltransferases (MGT) that show similarity to the GT domain of class A PBP (Figure 3).[103,104] These enzymes, referred to as SgtB (or MGT) and SgtA in *S. aureus*, are not essential in the presence of functional PBP2.[104] However, in cells with a mutated PBP2 that has lost its GT activity, SgtB but not SgtA becomes essential for viability suggesting that SgtB can act as a redundant TG in cell wall assembly.[104]

Penicillin-binding proteins and glycosyltransferases in non-S. aureus staphylococci, mammaliicocci and macrococci

OrthoFinder analysis revealed orthologues for all the four native PBP of *S. aureus* in the representative *Staphylococcus*, *Mammaliicoccus* and *Macrococcus* strains (shown in Figure 1) except for *S. epidermidis* ATCC 14990 and *S. canis* H16/1A that lack a gene for PBP4 (Table S2). The identified PBP all possess a transpeptidase superfamily domain, NCBI Conserved Domain Database (CDD) entry cl21491,[105] that can be further subdivided into protein families pfam00905 and pfam00768 described in Pfam database[106] (Figure 4). The bifunctional PBP2 proteins hold an additional conserved GT domain in the N-terminal part (Pfam entry pfam00912) (Figure 4).

A search for the TP (cl21491) and GT (pfam00912) domains in the sequences of the representative *Staphylococcus*, *Mammaliicoccus* and *Macrococcus* strains (shown in Figure 1), revealed additional proteins that could be involved in peptidoglycan assembly. Around half of the *Staphylococcus* species analysed here had two [number of species (n) = 27] or three (n = 2) orthologues in the PBP4 group (orthogroup OG0000097) while *Macrococcus* and *Mammaliicoccus* had only one orthologue (Table S2).

Four additional types of cPBP were detected (Figure 4). These putative monofunctional transpeptidases/carboxypeptidases (MTP) are usually annotated as serine hydrolases in the GenBank database and were named in this review MTP1 (OG0003124), MTP2 (OG0003455), FmtA-1 (OG0001592) and FmtA-2 (OG0002209). Proteins of the two FmtA orthogroups are distantly related to each other and include the FmtA protein of *S. aureus* (Protein accession WP_000671243.1) in OG0001592 (Table S2 and Figure 4). Most *Staphylococcus* and *Mammaliicoccus* species as well as *M. armenti*, *M. canis*, *M. caseolyticus*, *M. equipercicus* and *M. hajekii* contained one or two FmtA-like proteins (Table S2). The *fmtA* gene (formerly *fmt*) of *S. aureus* was identified as a factor that affects methicillin resistance[107] and belongs to those genes that become upregulated during cell wall stress conditions.[108] A weak D,D-carboxypeptidase activity was demonstrated *in vitro*.[109] FmtA interacts with teichoic acid and modulates the D-alanylation of teichoic acid (Figure 3).[109,110] Nothing is known about the function of MTP1 and MTP2 proteins. MTP1 was present in all *Mammaliicoccus* and some *Macrococcus* species, while MTP2 was detected in *M. armenti*, *M. fleurettii* and in a few *Staphylococcus* species (Table S2).

Unexpectedly, a second bifunctional aPBP was detected in all *Macrococcus* and *Mammaliicoccus* species. The TG domain of this bifunctional PBP is related to the monofunctional TG protein SgtA that is present in all other *Staphylococcus* species with the exception of *S. auricularis* NCTC 12101 (Figure 4). OrthoFinder groups the SgtA proteins and the additional bifunctional aPBP of *Macrococcus* and *Mammaliicoccus* species into one orthogroup (Table S2, OG0001169) suggesting that the genes descended from a common ancestor. Experimental evidence for a different set of PBP between *S. aureus* and *M. sciuri* exists. Analysis of membrane fractions detected five high molecular mass PBP (aPBP and bPBP) in *M. sciuri* species instead of the four present in MRSA.[111] All species encoded additionally an orthologue of SgtB (MGT) of *S. aureus* (Table S2, OG0000530).

The native *mec* genes of *M. sciuri* and *M. fleurettii* species and those coding for the acquired low-affinity PBP2A were found grouped in a separate orthogroup (Table S2, OG0003334) (Figure 4). The transpeptidase superfamily domain cl21491 was further detected in the β-lactam sensor/transducer protein belonging to MecR1-BlaR1 family (Table S2, OG0002527) and class A β-lactamases (Table S2, OG0002159) (Figures 3 and 4). The latter contains the acquired well-characterized BlaZ penicillinase[7] and distantly related proteins that seems to be native and species-specific.[12]

Three conserved sequence motifs are described for PBP and β-lactamases, motif 1 SxxK, motif 2 [S/Y]x[N/C], and motif 3 [K/H][S/T]G ('x' indicates any amino acid and a slash stands for 'or').[112] Motif 1 contains the active site serine which can form a long-lived covalent acyl-enyzme complex with β-lactam.[112] Most of the cl21491-containing PBP of the analysed *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* species contained motifs 1 to 3 (all proteins belonging to aPBP and bPBP, all PBP4 and MTP1 proteins except MTP1 of *M. armenti*) (Figure 4). However, some proteins of the MTP2 and FmtA orthogroups lack one or two of the motifs (indicated in parentheses within the motif box in Figure 4). Proteins that do not contain motif 1 are not expected to be PBP. Those proteins that lack motif 3 could still be PBP as illustrated by FmtA, which lacks motif 3 but forms covalent intermediates with β-lactams.[113]

SEDS proteins are unrelated to MGT and aPBP. They contain 10–12 transmembrane segments and a large extracytoplasmic loop that is required for GT activity.[86] All species in our analysis contained one RodA orthologue (Table S2, OG0000565) and a least one FtsW orthologue (OG0000111). Two FtsW orthologues were detected in some *Mammaliicoccus* (n = 3) and *Staphylococcus*

($n=12$) species, and a few *Staphylococcus* species even contained three ($n=2$) or four ($n=1$) FtsW proteins (Table S2, OG0000111). In all RodA and FtsW proteins, a conserved domain typical for FtsW, RodA and SpoVE cell cycle proteins (CDD entry cl00511) was identified (Figure 4).

The screen for putative PBP in *Staphylococcus*, *Mammaliicoccus* and *Macrococcus* species indicates that many species may contain additional PBP, such as an additional bifunctional aPBP in *Macrococcus* and *Mammaliicoccus*, a second PBP4 variant in many *Staphylococcus* species or other types of monofunctional TP/carboxypeptidase such as MTP1 or FmtA variants.

Alternative low affinity PBP2A

Classification and structure of PBP2A

PBP2A are encoded by one of the currently four known *mec* genes, *mecA*, *mecB*, *mecC* and *mecD* (the corresponding proteins are named throughout this review PBP2A$_a$, PBP2A$_b$, PBP2A$_c$ PBP2A$_d$ with subscript indicating *mec* gene type, A, B, C, D, respectively). As defined in the guidelines for reporting novel *mecA* gene homologues, the different *mec* gene types share <70% nucleotide (nt) identities among each other.[114] For *mecA* and *mecC* gene types, allotypes (*mecA1*, *mecA2*, *mecC1*, *mecC2* and *mecC3*) have been allocated that share ≥70% and <95% nt identity to the prototypes *mecA* of *S. aureus* N315 and *mecC* of *S. aureus* LGA251, respectively (Figure 5).

PBP2A are membrane-anchored proteins that possess a N-terminal non-penicillin binding domain (nPD), also called the allosteric domain, and a C-terminal TP domain (Figure 4). The N-terminal extension MecA_N (CDD: pfam05223) in the nPD is unique to PBP2A, while the pfam03717 domain is also present in other bPBP. Pfam03717 is misleadingly called PBP dimerization domain, but its function has not been precisely defined. PBP2A$_a$ has a significantly lower affinity for β-lactam binding and enzyme acylation than native PBP.[115,116] PBP2A can therefore maintain TP activity in the presence of β-lactam concentrations inhibitory for native PBP. The crystal structure of apo- and acylated-PBP2A$_a$ suggested that poor acylation efficiency is due to a closed active site that must undergo conformational change for binding of β-lactam.[117] Increased acylation of PBP2A$_a$ by nitrocefin in the presence of synthetic peptidoglycan fragments suggested that interaction with peptidoglycan could cause conformational changes that open the active site and activate the PBP2A$_a$ catalytic function.[118] Later, structural analysis of PBP2A$_a$ confirmed that conformational change in the active site of the TP domain is controlled through binding of allosteric ligands to the allosteric nPD domain.[119] Interestingly, not only natural ligands such as muramic acid and cell wall peptidoglycan can cause opening of active site, but also the anti-MRSA cephalosporin ceftaroline.[119] The good efficacy of ceftaroline against MRSA is thought to be caused through allosteric binding of one ceftaroline molecule to the allosteric site of PBP2A$_a$, which predisposes the TP domain to become inactivated by a second ceftaroline molecule.[119]

Structural details are not known for PBP2A$_b$, PBP2A$_c$, and PBP2A$_d$. Amino acid (aa) comparison suggests a rather conserved TP domain with identities between 59% to 75% among the different PBP2A types and less-conserved nPD domains with identity values between 37% to 56%. The *mecA* gene is more closely related to *mecC* and the *mecB* gene is more closely related to *mecD* in both nt sequence and deduced aa sequence (Figure 5).

β-Lactam resistance mediated by PBP2A

Phenotypic detection of a *mec* gene can be problematic independently of the *mec* type. A remarkable diversity of β-lactam resistance levels mediated by *mec* genes have been observed in *Staphylococcus*, *Mammaliicoccus* and *Macrococcus*. As documented for *mecA* in *Staphylococcus*, even within a strain, methicillin resistance is often expressed heterogeneously with small subpopulations presenting high-level resistance while the majority of cells show low-level resistance.[120] Induction of the stringent stress response can alter this heterogeneous resistant phenotype to a homogeneous, high-level β-lactam-resistant phenotype.[121] Nutrient limitations cause the stringent stress response in order to slow-down bacterial growth. In contrast to most classes of proteins that are suppressed under these growth conditions, the production of *mecA* transcript and PBP2A protein is clearly increased.[122] In addition, several chromosomal factors were identified that can influence methicillin resistance.[123] In line with this observation, a resistance phenotype caused by *mecA* and *mecC* genes expressed from recombinant plasmids was found to be dependent on the genetic background of the recipient strain.[124,125]

Differences in the properties for β-lactam binding that may influence the resistance phenotype among the different PBP2A types are likely to exist. For instance, low aa identity (37%) of the nPD domain of PBP2A$_d$ with that of PBP2A$_a$ questions similar allosteric control of the active site of PBP2A$_d$.[19] Deletion of *mecD* in the *M. caseolyticus* strain IMD0819 had a clear effect on the MIC of ceftaroline that was reduced at least 16-fold.[19] Biochemical experiments showed that PBP2A$_c$ has higher affinity for oxacillin than PBP2A$_a$ while both proteins show comparable cefoxitin affinity.[126] *S. aureus* strains containing *mecC* have been reported to frequently show the phenotypic resistance pattern 'cefoxitin resistance/oxacillin susceptibility' when analysed with semi-automated AST systems.[127] Low MICs situated around the clinical breakpoint for oxacillin and cefoxitin were also observed in a fraction of *S. aureus* strains carrying *mecC*.[17] However, cefoxitin has been shown to be more reliable than oxacillin for prediction of *mecC* gene presence in *S. aureus*[127,128] and is recommended by CLSI and EUCAST for methicillin resistance screening in *S. aureus* (https://www.eucast.org/clinical_breakpoints/).[129]

For *Macrococcus*, no clinical breakpoints and screening values are defined for the prediction of methicillin resistance. To evaluate β-lactam resistance, criteria defined for *Staphylococcus* species have been so far used tentatively.[50,52,65] For *M. caseolyticus* carrying *mecD*, reported MICs ranged from 6 to >256 mg/L for cefoxitin and 1 to 16 mg/L for penicillin, values clearly higher than those for *M. caseolyticus* strains without a *mec* gene (penicillin MIC ≤0.25 mg/L, cefoxitin MIC range of 1 to 2 mg/L).[19,50,52] However, McFadyen *et al.*[52] observed that *mecD*-containing *M. caseolyticus* strains were classified as cefoxitin susceptible and oxacillin resistant when tested by the bioMérieux VITEK 2 system, which poses the risk of overlooking these isolates with AST systems using cefoxitin screening only. Moreover, whether oxacillin is more reliable than cefoxitin for *mec* gene prediction in *Macrococcus* still needs to be evaluated. High oxacillin MICs

Figure 4.

were measured for the *mecB*-containing *M. caseolyticus* strain JCSC5402 (64 mg/L)[4] and the *mecD*-containing *M. caseolyticus* IMD0819 (128 mg/L).[19] Presence of the *mecB* gene in *M. canis* strains also correlates with reduced susceptibility to penicillin and cefoxitin, with cefoxitin MIC in the range of 4 to >16 mg/L, but data for oxacillin are lacking.[65]

PBP2A types: difference in crosslinking preference and cooperation with GT enzymes?

PBP2A proteins seem to be more flexible in crosslinking glycan strands than originally thought. Genetic studies had shown that inactivation of *femA* or *femB* abolishes β-lactam resistance in MRSA strains.[130,131] FemA and FemB are peptidyltransferases required for synthesis of the branched pentaglycine chains which are used for crosslinking of neighbouring glycan strands (L-Lys^3-Gly$_5$ in *S. aureus*).[132] The observation that β-lactam resistance was abolished in MRSA strains with inactivated *femA* or *femB* genes suggested that complete pentaglycine chains are needed for PBP2A$_a$ to function in peptidoglycan crosslinking.[132] However, detection of *mecA*-mediated methicillin resistance in *Mammaliicoccus* species with interpeptide crosslinks different from pentaglycine indicated that alterations can be tolerated; e.g. L-Lys^3-L-Ala-Gly$_{3-4}$ in *M. vitulinus*, *M. lentus* and *M. sciuri* species.[80] *In vitro* experiments further confirmed that PBP2A$_a$ can crosslink peptidoglycan bearing penta- and tri-glycine, but not those with monoglycine.[133] Furthermore, *mecB* and *mecD* are functional in *S. aureus*,[4,19] and hence can crosslink glycan strands via both the longer pentaglycine bridges in *S. aureus* and the shorter interpeptide bridges with serine substitutions in *Macrococcus* (L-Lys^3-Gly$_{2-4}$, L-Ser and L-Lys^3-Gly$_{1-2}$).[1,27,28]

All PBP2A proteins depend on a functional GT to express β-lactam resistance. For *mecA*-mediated resistance in *S. aureus*, the GT function of the native PBP2 is needed and is thought to cooperate with the TP function of PBP2A$_a$.[22,122,134] However, in the absence of β-lactam, PBP2A$_a$ can function without PBP2 and compensate for the essential function of PBP2.[90] This result suggests that under non-selective conditions, the TP activity of PBP2A$_a$ and a GT other than PBP2 are involved in peptidoglycan assembly. Moreover, *mecC* can also confer β-lactam resistance in the absence of PBP2,[126] suggesting that PBP2A$_c$ also cooperates under selective conditions with another GT than PBP2. Hence, the cooperation of PBP2A with native GT may depend on the PBP2A type, as well as on the growth conditions. There seems to be a complex interplay of many TP and GT enzymes, which also contributes to the maintenance of integrity of peptidoglycan structure under stress conditions. In the current model, the SEDS-bPBP pairs are suggested to build the main peptidoglycan and the aPBP may have an essential role in repairing gaps in the cell wall.[88] Our analysis also revealed different sets of peptidoglycan assembly proteins in the different species. Cooperation of a PBP2A enzyme may also depend on the different GT available in the host, and in this regard, the role of SgtA as GT and the additional aPBP present only in *Macrococcus* and *Mammaliicoccus* would be interesting to elucidate.

The β-lactam resistance operons

Regulation of β-lactam resistance

The *mecA*, *mecB*, *mecC* and *mecD* genes are usually under control of the regulators MecR1 and MecI. Together with the regulatory genes, they constitute an operon with divergently transcribed *mec* and *mecR1-mecI* genes from intergenic promoter-operator sequence (Figure 3). The structure is similar to β-lactamase operons *blaZ-blaR1$_z$-blaI$_z$* found on transposon Tn*522* and plasmids in many *Staphylococcus* species[11,135] and to the native *bla$_{ARL}$-blaR1$_{arl}$-blaI$_{arl}$* of *S. arlettae*[12] (subscripts next to regulatory genes indicate the accompanying *mec* and *bla* type) (Figure 5). In bacilli, related β-lactamase operons have also been described; the organization of the regulatory genes differs however with *blaP-blaI$_p$-blaR1$_p$* as shown for *Bacillus licheniformis* (Figure 5).[136] The *blaR1/mecR1* genes encode transmembrane sensor/transducer proteins with an extracellular penicillin-binding domain (PBD) and cytoplasmic zinc metalloprotease domain (MPD) (CDD: pfam05569) that contains a classical zinc-binding motif (HExxH) (Figure 4). The *blaI/mecI* genes encode transcriptional repressors with a C-terminal dimerization domain and a N-terminal winged helix-turn-helix (HTH) domain that functions in sequence-specific DNA binding.[137,138] Induction of *blaZ* and *mecA* gene follows exposure to β-lactam through a proteolytic signalling pathway: β-lactam binding to the PBD of BlaR1$_z$ results in cleavage of both BlaR1$_z$, which autoactivates its MPD, and cleavage of BlaI$_z$, which inactivates the repressor (Figure 3).[139] Similar proteolytic inactivation was also observed for the repressor MecI$_a$.[140] However, a further regulator, the antirepressor protein MecR2 was suggested to be required for optimal *mecA* expression from *mecA* operon with complete regulators (Figure 5).[141] Several lines of evidence exist that the *mecA* gene can also be regulated by *blaR1$_z$* and *blaI$_z$* even more

Figure 4. Continued
Structure of proteins containing transpeptidase domain (superfamily cl21491) and peptidoglycan polymerase domains (transglycosylase pfam00912 and FtsW-RodA-SpoVE superfamily cl00511). Proteins are divided into class A to C PBP (aPBP, bPBP, cPBP), monofunctional glycosyltransferases (MGT), β-lactam sensor/transducer and β-lactamases and shape, elongation, division and sporulation (SEDS) family proteins. Proteins used in the analysis were from the representative *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* species listed in Figure 1 and clustered into orthogroups using OrthoFinder software (version 2.3.8) (Table S2).[37,38] For each protein group, name, orthogroup (in parenthesis), size range of protein members and species distribution is given. Protein domains present in all members of an orthogroup are indicated and specified on the right side. NCBI conserved protein domains: cl21491, cl00511, pfam00912, pfam00905, pfam00768, pfam00144, pfam05569, pfam03717, pfam09211 and pfam05223. PROSITE pattern: PS00146 and PS51178. The conserved sequence motifs 1 to 3 for PBP[112] (see text) are indicated if present in all members as 'Motif 1–3' or only present in a subset of members as 'Motif (1,2) 3' (Motif 1 or Motif 1 and Motif 2 was not found in all members of MTP2) and 'Motif 1,2 (3)' (Motif 3 was not found in all members of MTP1 and FmtA). *PS00146 was not recognized in BlaZ$_b$ due to an isoleucine substitution at the last pattern position where a leucine or cysteine is expected. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

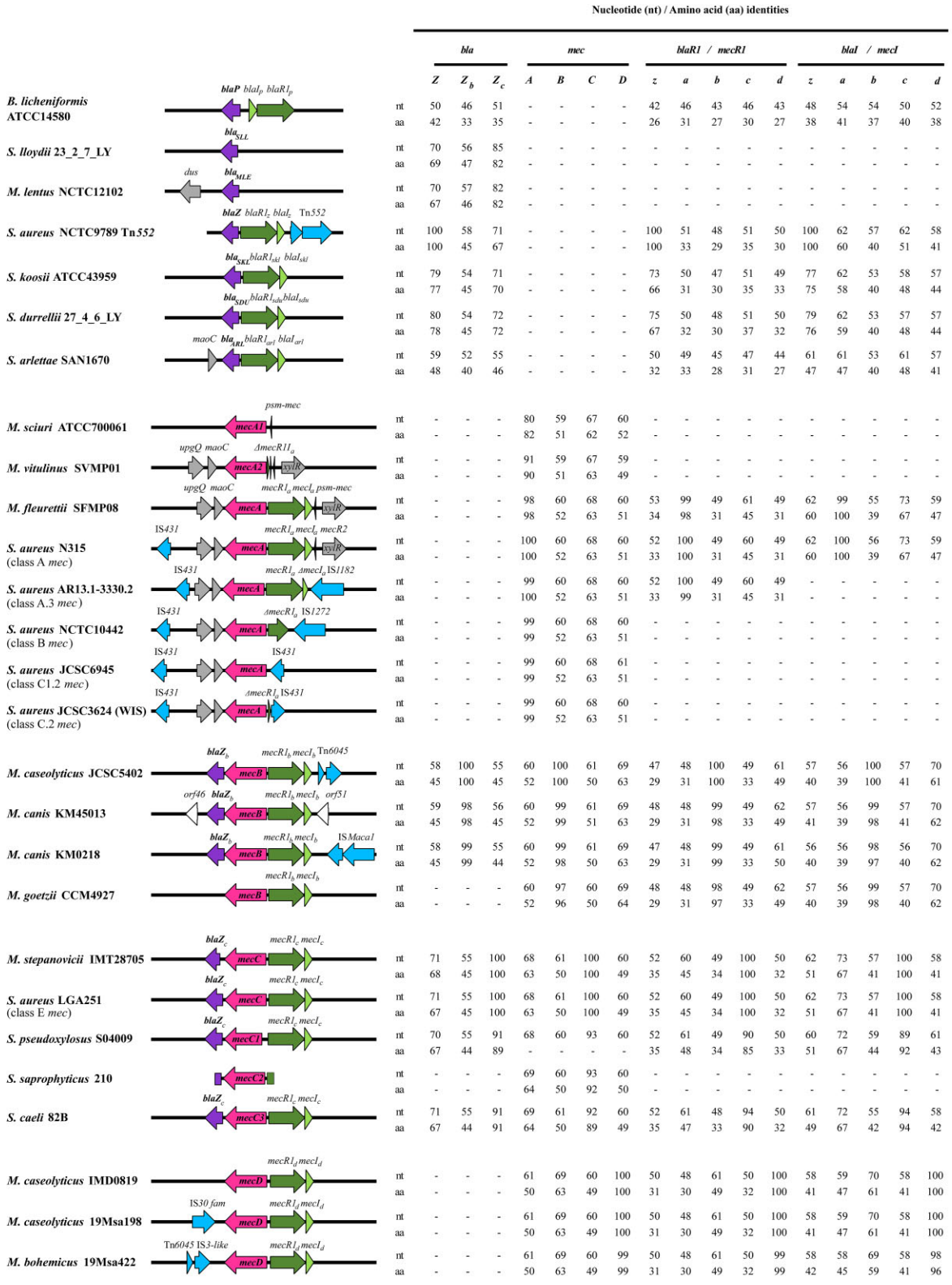


Figure 5. Genetic organization of different *mec* and *bla* operons. The *bla* and/or *mec* genes, the regulators *blaR1*/*mecR1* and *blaI*/*mecI* as well as flanking genes are shown as arrows according to the direction of transcription. Nucleotide (nt) and amino acid (aa) identities between the different genes of the indicated strains were calculated using multiple sequence alignment tool of Clustal Omega.[202] This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

efficiently than by its cognate regulators.[142–145] MecR1$_a$- and BlaR1$_z$-mediated induction functions only via their cognate repressor, but MecI$_a$ and BlaI$_z$ are functionally interchangeable.[144] They bind to both *mecA* and *blaZ* operator DNA and repress transcription.[143,146,147] Comparison of *bla* (*blaZ* and *bla$_{ARL}$*) and *mec* (*mecA*, *mecB*, *mecC* and *mecD*) regulatory genes showed nt identities from 44% to 62% for *blaR1/mecR1* genes and higher identities from 53% to 73% for *blaI/mecI* genes (Figure 5). It is possible that beside *mecA* and *blaZ*, crosstalk between other systems could also take place. The intergenic region between *bla/mec* and *blaR1/mecR1* usually contains two 18 bp palindromic consensus sequences [A/G]NATTACA[A/T]NTGTA[A/G][T/G]NT recognized by MecI$_a$/BlaI$_z$ (Figure S1a, available as Supplementary data at *JAC* Online).[137] Moreover, the sequence of the recognition helix α3 of BlaI$_z$ and MecI$_a$ that interacts specifically with DNA[137,138] is highly conserved in all BlaI and MecI proteins (Figure S1b).

In any case, the expression of *mec* genes is regulated because the PBP2A production is not beneficial for the cell in the absence of β-lactam. Experimental introduction of the *mecA* gene into *S. aureus* showed that either *blaR1$_z$-blaI$_z$* or *mecR1$_a$-mecI$_a$* regulatory genes are needed for stable maintenance of *mecA*.[124,148] Clinical MRSA isolates often contain a *mecA* operon with truncated regulator *mecR1$_a$* and *mecI$_a$* (Figure 5). In such isolates, a correlation with the presence of *blaI$_z$* was shown indicating the need for at least one functional repressor.[149] Furthermore, *S. aureus* transformed with an SCC*mec* containing a truncated *mecA* operon (class B *mec* gene complex; see Figure 5) showed reduced growth rate, and the selected faster-growing variants expressed reduced oxacillin resistance.[150] The reason for the proposed fitness cost for *mecA* expression is not clear. An altered peptidoglycan with reduced crosslinks was observed in MRSA strains when grown under β-lactam selection.[151] The switch to this altered peptidoglycan was already detected with subinhibitory concentrations of methicillin, suggesting this is the result of the PBP2A$_a$ function.[151] These results indicate that PBP2A disturbs the normal peptidoglycan synthesis in the absence of a β-lactam concentration, which is inhibitory for other PBP.

The *mecB* and *mecC* operons contain downstream of the *mec* gene a *blaZ*-like β-lactamase, *blaZ$_b$* and *blaZ$_c$* (subscripts next to the *bla* genes indicate the accompanying *mec* type), respectively (Figure 5).[4,17,18] While the intergenic region between the start codon of *blaZ$_b$* and the stop codon of *mecB* is short (6 to 21 bp, depending on annotation), the intergenic region between *blaZ$_c$* and *mecC* consists of 93 bp, which contains one 18 bp palindrome that could be recognized by MecI/BlaI repressors (Figure S1a). The *blaZ$_b$* gene is less related to the recognized β-lactamases *blaZ* and *bla$_{ARL}$* than *blaZ$_c$*, which shares 71% nt identity with *blaZ* (Figure 5). Both β-lactamases *blaZ$_b$* and *blaZ$_c$* are scarcely characterized. The *blaZ$_b$* gene was suggested to be active in *M. canis* KM45013 since the strain displayed a positive nitrocefin test and contained a complete *mecB* operon but no further *bla* gene.[152] Single and double deletions of *blaZ$_c$* and *mecC* in *mecC*-carrying MRSA indicate that both *blaZ$_c$* and *mecC* contribute to penicillin resistance, although the measured inhibition zone in disc diffusion assays was larger in the single *blaZ$_c$* deletion mutant compared with single *mecC* deletion mutants.[153] The penicillinases *blaZ*, *blaZ$_b$*, *blaZ$_c$* and *bla$_{ARL}$* are found together with the regulatory genes *mecR1/blaR1-mecI/blaI* suggesting that inducible expression of *bla* penicillinases could also be advantageous.

Distribution of β-lactam resistance operons

Limited sequence data available in the past and similar organization of regulatory genes and operator upstream of *mecA* and *blaZ* led to the speculation that the *mecA* gene complex could be formed through recombination of the *blaR1-blaI-blaZ* operon with a gene encoding a low-affinity PBP.[154,155] However, with increasing data from WGS, more *mec* and *bla* operons were identified in the genomes of *Staphylococcus*, *Macrococcus* and *Mammaliicoccus* indicating that these structures appear to be more abundant. These data also suggest that these structures may have derived from a primordial β-lactam resistance operon that contained both *bla* and *mec* genes under control of regulatory genes *mecR1* and *mecI*. The unfavourable effect of *mecA* in the absence of β-lactam (see Regulation of β-lactam resistance) could favour an evolutionary process that selects for loss or inactivation of these operons.

The origin, reservoir and transfer of *mec* and *bla* genes among different strains, species and genera is an open field of research. The *mecA* allotypes are intrinsically present in *M. fleurettii* (*mecA*) and *M. sciuri* (*mecA1*) (see Native *mecA* genes). The *mecA* gene is also the predominantly acquired *mec* gene type found in different species of *Staphylococcus* and *Mammaliicoccus*, whereas *mecC* is less widespread, *mecB* was only found in one occasion in a *S. aureus* strain,[20] and *mecD* has not been detected in the *Staphylococcus* and *Mammaliicoccus* (Figure 1). The *mecB* and *mecD* genes seem to be most prevalent in the *Macrococcus* species with low G+C content, such as *M. armenti*, *M. caseolyticus*, *M. canis*, *M. epidermidis*, *M. bohemicus* and *M. goetzii* (Figure 1). The *mecA* and *mecC* genes were not detected in *Macrococcus* species. The *blaZ* gene is widespread in different *Staphylococcus* and *Mammaliicoccus* species due to spread of *blaZ*-carrying mobile elements (Figure 1). Its origin is unknown; however, several other putative *bla* genes are intrinsically present in the chromosome of phylogenetic-related *Staphylococcus* species and may represent remnants of an inactivated β-lactam resistance operon (see Native *bla* genes) (Figure 1). The *blaZ$_c$* gene was so far always present in the *mecC* operon, while some *mecB* operons with deleted *blaZ$_b$* have been detected in *M. geotzii*, *M. canis*, *M. epidermidis* (Figure 5).[21,28]

Native mecA genes

The *mecA* carried on SCC*mec* elements is considered to originate from *M. fleurettii*.[47] In this species, *mecA* and the regulatory genes *mecR1$_a$* and *mecI$_a$* are not associated with an SCC*mec* element and located approximately 200 kb downstream of the *rlmH* gene (also known as *orfX*), the attachment site for SCC*mec*.[156] The *mecA* gene of *M. fleurettii* is found as a native part of the chromosome located between a mevalonate (*mvaACS*) and xylose operon (*xylRABE*) followed by *nadBCA* gene cluster (Figure S2a).[47] Besides *mecA*, *mecR1$_a$* and *mecI$_a$*, flanking genes present in *M. fleuretti* are also found in the *mecA* region of SCC*mec* elements, including genes *upgQ*, *maoC*, the phenol soluble modulin *psm-mec* and the xylose repressor *xylR*, later named *mecR2* in MRSA (Figure 5).[141] In SCC*mec* elements of *Staphylococcus*, the *mecA* and *mecC* genes and their

surrounding regions are classified into five different *mec* gene complexes (class A to E reviewed in Lakhundi & Zhang 2018[14]). Figure 5 shows only a selection of them to illustrate variations in completeness of *mec* regulatory genes and flanking IS. IS elements might be involved in both, mobilization of *mec* genes and driving diversity of *mec* gene complexes in SCC*mec* elements.

Like *M. fleurettii*, *M. sciuri* also contains a native *mecA* gene, allotype *mecA1* that shares around 80% nt identity with *mecA*.[45,46] In contrast to *mecA* in its native host *M. fleurettii* and *mecA*-carrying SCC*mec* in *Staphylococcus* species, *mecA1* does not usually confer resistance to β-lactams in *M. sciuri*.[45,47] This observation was explained by a silent *mecA1* gene that is not or only expressed in low amount in susceptible *M. sciuri* strains.[157,158] Activation of *mecA1* through mutation or insertion of IS*256* in the promoter sequence can lead to increased transcription and translation and mediates β-lactam resistance in *M. sciuri* and *S. aureus*.[157–159] The third *mecA* allotype, *mecA2*,[160] is found with a frequency of around 30% to 50% in *M. vitulinus* strains that are usually susceptible to β-lactam.[47,156] The *mecA2* allotype is also not associated with SCC*mec* elements and located between the *mvaACS* and *xylRABE and/or nadACB* locus like *mecA* of *M. fleurettii* and *mecA1* of *M. sciuri* (Figure S2a).[47] Surrounding genes including, *upgQ*, *maoC*, *psm-mec* and *xylR* are also present next to *mecA2* (Figure 5). However, remnants of the *mec* regulators are present in *M. vitulinus* while they are completely missing upstream of *mecA1* in *M. sciuri*.[47,156]

Native bla genes

Among the representative *Staphylococcus*, *Mammaliicoccus* and *Macrococcus* strains analysed in this review, a native (species-specific) class A β-lactamase (*bla*) gene related to *blaZ*, $blaZ_b$, $blaZ_c$, and bla_{ARL} was detected in 14 species of *Staphylococcus* and 3 species of *Mammaliicoccus* (Figure 1 and Table S2), 9 of them already reported in the study by Andreis *et al*.[12] The staphylococcal *bla* genes were all present in the novobiocin-resistant *Saprophyticus* group[70] (species from *S. caeli* to *S. durellii*) to which *S. arlettae* also belongs to (Figure 1). *S. caeli* was the only species that lacks a *bla* gene in the novobiocin-resistant *Saprophyticus* group. Regulatory genes *blaR1* and *blaI* upstream of the *bla* gene were only seen in *S. arlettae*, *S. kloosii* and *S. durrellii* (Figure 5). Most of the native *bla* genes seem not to confer penicillin resistance. An exception is bla_{ARL} of *S. arlettae*, which was shown to function as penicillinase by cloning experiments in *S. aureus* as well as by low level penicillin resistance of the *S. arlettae* isolates.[12] It is not known if the bla_{SKL}-$blaR1_{skl}$-$blaI_{skl}$ of *S. kloosii* and bla_{SDU}-$blaR1_{sdu}$-$blaI_{sdu}$ of *S. durrellii* also mediate penicillin resistance through β-lactamase production. The $blaR1_{skl}$ gene was annotated as a pseudogene in strain ATCC 43959 due to a frameshift at the 3′ end (GenBank: CP027846). However, the frameshift caused only a 10 aa truncation at the C terminus of $BlaR1_{skl}$ and the PBD and MPD domains were still complete. The bla_{ARL} operon as well as the bla_{SKL} and bla_{SDU} operons were located in the *oriC* environs, a highly plastic region around the origin of replication (−0.3 Mb to +0.5 Mb in *Staphylococcus*) that contains only a few conserved genes, especially to the right of *oriC*[161] (see also Figure 2 for distribution of shared orthologues). The bla_{ARL} operon of *S. arlettae* NCTC 12413 was located around 140 kb upstream of *rlmH* on the left side of *oriC*, next to a *maoC* gene and near the *xylRABE* and *nadACB* gene clusters that have been observed to be in the vicinity of native *mecA* allotypes in *M. fleurettii*, *M. sciuri* and *M. vitulinus* (Figure S2a). The bla_{SKL} gene of *S. kloosii* ATCC 43595 and the bla_{SDU} gene of *S. durrellii* 27_4_6_LY were highly similar (94% nt identity) and both genes were carried by a similar chromosomal region around 200 kb downstream of *rlmH* (Figure S2a). This region was not related to the bla_{ARL}-containing region of *S. arlettae* and the native *mecA* regions in *Mammaliicoccus* species. Interestingly, *S. lloydii* 23_2_7_LY that is highly related to *S. kloosii* and *S. durrellii* (Figure 1) did not contain a *bla* gene in the corresponding region (Figure S2a). Instead, *S. lloydii* 23_2_7_LY contained a *bla* gene (bla_{SLL}) around 100 kb downstream of the *rlmH* gene that was more related to bla_{MLE} of *M. lentus* (83% nt identity) and $blaZ_c$ of the *mecC* gene complex (85% nt identity) than to bla_{SKL} and bla_{SDU} (71%) (Figure 5 and Figure S2b).

All the other native *bla* genes in the novobiocin-resistant *Saprophyticus* group were located on the left side of *oriC* in a related region around 15 to 30 kb downstream of the conserved genes *tuf*, *fusA*, *rpsG*, *rpsL*, *rpoC* and *rpoB* (Figure S2c, see Figure 2 for location of *rpoBC*). The relatedness of these *bla* genes corresponds to the degree of relatedness of the species that carry them. For instance, the *bla* genes of the closely related *S. succinus* and *S. casei* species shared 93% nt identities, other *bla* genes in this group shared from 63% to 91% nt identities among each other and around 60% with bla_{ARL}, bla_{SKL}, bla_{SDU} and bla_{SLL}. The bla_{ARL} gene was also distantly related to bla_{SKL} and bla_{SDU} as well as to bla_{SLL} and shared around 57% nt identities with them.

The native *bla* genes of *Mammaliicoccus* were found in *M. lentus*, *M. sciuri* and *M. stepanovicii* and not in *M. fleurettii* and *M. vitulinus* (Figure 1). These *bla* genes were not associated with SCC elements and were located 20 to 70 kb downstream of *rlmH* in a highly diverse region in the analysed strains *M. lentus* NCTC 12102, *M. sciuri* NCTC 12103 and *M. stepanovicii* NCTC 13839 (Figure S2b). A tRNA dihydrouridine synthase gene (*dus*) was the only conserved gene found in their vicinity. The *dus* gene belongs to the orthologues shared between *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* and is located either on the right or the left side of the *oriC* (see Figure 2 for location of *dus* in *M. canis*). The native *bla* genes in *M. lentus*, *M. sciuri* and *M. stepanovicii* shared 68% to 75% nt identities with each other, 69% to 85% with bla_{SLL} of *S. lloydii*, 67% to 71% to bla_{SKL} and bla_{SDU}, and 56% to 61% identities to all the other *bla* genes in the novobiocin-resistant *Saprophyticus* group. The bla_{MLE} of *M. lentus* and bla_{SLL} of *S. lloydii* showed high nt identity with $blaZ_c$ (82% and 85%) (Figure 5). The sequence upstream of bla_{MLE} contains an 18 bp palindrome that could be recognized by $MecI/BlaI_z$ and which was absent upstream of bla_{SLL} (Figure S1a). Furthermore, the *bla* operons of *S. kloosii* and *S. durrellii* showed relatively high similarity with the *blaZ* operon of Tn*552* with identities of 79% to 80%, 73% to 75% and 77% to 79% for *bla*, *blaR1* and *blaI* genes, respectively (Figure 5). These gene identities are clearly higher than those resulting from comparison of the different *mec* gene complexes with each other. Values ≥70% are only observed for the repressor genes $mecI_a$ and $mecI_c$ (73%) and $mecI_b$ and $mecI_d$ (70%).

Native *bla* genes are abundant in certain phylogenetic clusters of *Staphylococcus* species. They seem to be homologues of acquired *bla* genes and may represent a relict of the β-lactam

operon lost in most *Staphylococcus*. Accordingly, these native *bla* genes also evolved with the species that carry them. An exception is bla_{SLL} of *S. lloydii*, which shows higher similarity to bla_{MLE} and $blaZ_c$ than to all other *bla* genes in the novobiocin-resistant *Saprophyticus* group. Judging from their relatedness, bla_{MLE}, bla_{SLL} and $blaZ_c$ of the *mecC* gene complex may descended from a common ancestor.

The $blaZ_b$ gene of the *mecB* gene complex shows less than 60% nt identity with all *bla* genes in *Staphylococcus* and *Mammaliicoccus*. Similar to *mecB* and *mecD*, $blaZ_b$ was only recognized as an acquired gene in *Macrococcus* associated with mobile genetic elements in a subpopulation of species. The origin of *mecB* and *mecD* is unknown: they seem to belong to the accessory genes in *Macrococcus* that can be mobilized through different genetic elements including SCC*mec* (*mecB* and *mecD*), transposon Tn*6045* (*mecB*), plasmid (*mecB*) and resistance island $McRI_{mecD}$ (*mecD*) (see below).

Acquired *mec* genes on mobile genetic elements

This second part of the review summarizes the mobile genetic elements involved in spreading of *mec* genes. Distinct *mecA*- and *mecC*-carrying SCC*mec* elements from *Staphylococcus* and *Mammaliicoccus* are not described here since these structures have already been extensively presented in previous reviews. The present review is therefore limited to the general characteristics of SCC*mec* elements that have been mainly discovered in *S. aureus*, and focuses on the versatile elements carrying *mecB* and *mecD* in *Macrococcus*. These elements are described in detail and compared with the structures present in *Staphylococcus* and *Mammaliicoccus*.

mecA and mecC on SCCmec elements in Staphylococcus and Mammaliicoccus

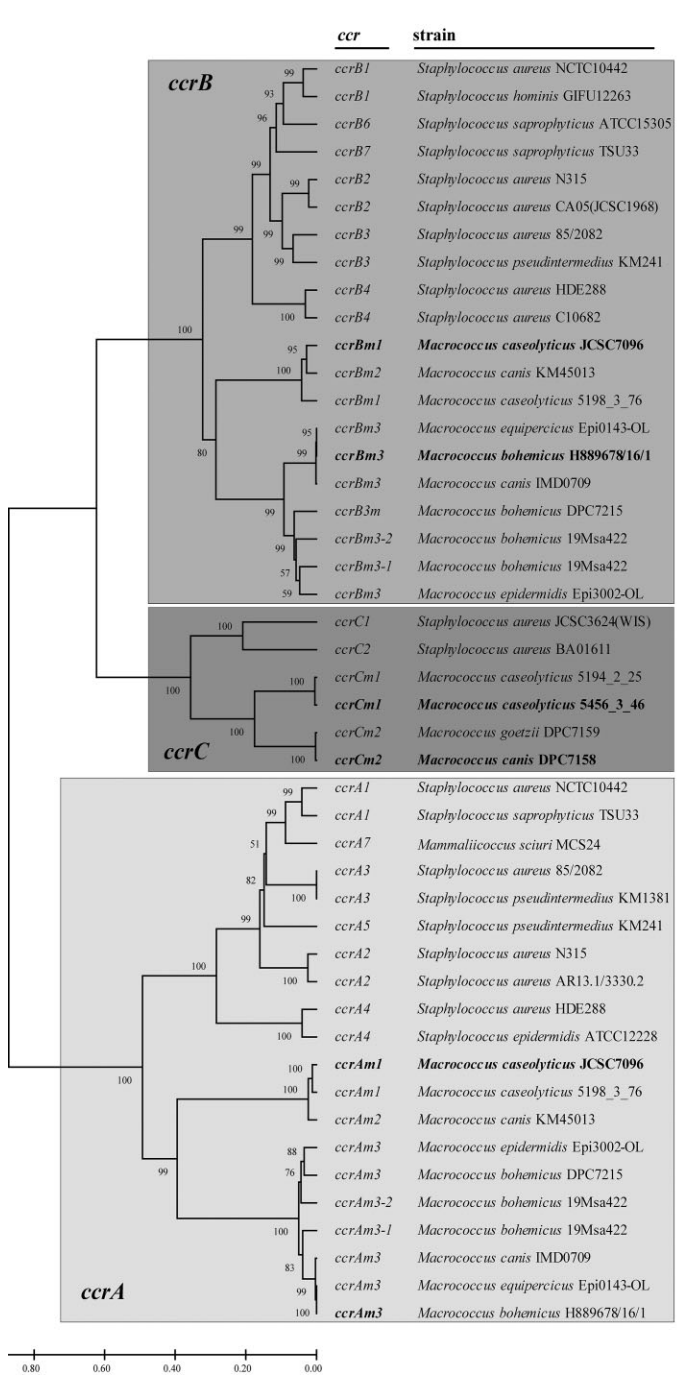
SCC*mec* was first found to be the mobile genetic element that carries *mecA* in MRSA N315.[162,163] Later, SCC*mec* elements with *mecC* were identified.[17,18] To date, 13 different *mecA*-carrying SCC*mec* types and one *mecC*-carrying SCC*mec* type have been recognized in *Staphylococcus* according to the classification scheme that is based on the combination of the *mec* gene complex and unique recombinase genes named cassette chromosome recombinases (*ccr*) responsible for element mobility.[14,15,164] All SCC*mec* elements have a common integration site at the 3′ end of the *rlmH* (*orfX*) gene (Figure 2), which is a conserved orthologue in the *oriC* environ and encodes a ribosome methyltransferase.[165] SCC*mec* are flanked by characteristic direct repeats (DR), also called attachment (*att*) sites, that contain a sequence similar to the last 18 bp of the *rlmH* gene referred to as the integration site sequence (ISS) for SCC.[166] Typically, SCC*mec* carry either the recombinase genes *ccrA* and *ccrB* or a *ccrC* gene. These three *ccr* gene types are phylogenetically distinct, with nt identities between them of less than 50%.[164] Within these *ccr* types, gene variants are classified into allotypes with nt identities between 50% to 85% (currently *ccrA1* to *ccrA7*, *ccrB1* to *ccrB7*, *ccrC1* and *ccrC2* in *Staphylococcus*) (Figure 6). All *ccr* genes encode large serine recombinases that catalyse site-specific DNA recombination between *att* sites. Reaction between an *att* site on a circular SCC*mec* (*attS*) and the *att* site at the 3′ end of *rlmH* gene (*attB*) leads to chromosomal SCC*mec* integration. Recombination between the two *att* sites at each end of integrated SCC*mec* (*attL* and *attR*) results in reconstituted *attB* and extrachromosomal circular SCC*mec*, the molecule thought to be required for horizontal transfer of SCC*mec*. The *ccrA* and *ccrB* genes are found together in a two-gene operon while *ccrC* is found alone. Accordingly, CcrC alone can catalyse both the integration and excision recombination reaction[166] while functional activity of both CcrA and CcrB is in principle required in recombination reactions.[163,167,168] However, CcrA and CcrB are not equivalently involved in recombining *att* sites. CcrB is more involved in target DNA binding and can, at least with certain *att* site sequences, conduct the excision reaction without CcrA protein.[167–169] The *ccrAB* system seems to favour SCC*mec* integration over excision.[168] This could be one of the reasons for the low spontaneous SCC*mec* excision observed in *Staphylococcus*, for which an excision rate of approximately 2×10^{-6} was measured by quantitative PCR in MRSA N315.[170] Another reason is that *ccrAB* gene expression seems to be absent in the majority of cells as assessed by measuring promoter activity with green-fluorescent protein translation fusion.[171] These characteristics ensure relatively stable carriage of SCC*mec* elements and therefore only rare conversion from methicillin resistance to methicillin susceptibility. However, the organization of sequence downstream of *rlmH* in *att*-flanked segments suggests a considerable impact of *ccr* activity on diversifying this locus. SCC structures, *att*-flanked elements with *ccr* but without *mec* genes, are common basic mobile elements for exchange of genes between staphylococcal species.[172–174] Moreover, several *att*-flanked elements with *ccr* genes (SCC/SCC*mec*) and without *ccr* genes (ΨSCC/ΨSCC*mec*) can be found in arrays as structures called composite islands (CI). Considerable flexibility in recombining different *att* site pairs of CI has been observed experimentally in PCR assays detecting circular DNA excisions in *Staphylococcus* and *Mammaliicoccus*.[175–177]

Several review articles have presented the variety and distribution of SCC*mec* elements in *Staphylococcus*. For example, a detailed description of the SCC*mec* types and clonal lineages of MRSA present in hospital, community and livestock is given by Lakhundi & Zhang.[14] Other MRSA reviews focused on genetic features,[161,178] the molecular basis of β-lactam resistance[8] or clinical aspects of MRSA.[179,180] General reviews also exist about SCC*mec* structures in non-*S. aureus* staphylococci,[16] as well about *mecC*-carrying SCC*mec*[23,181] and about possible evolutionary processes leading to SCC*mec* formation in *Mammaliicoccus* species.[182]

mecB and mecD associated with different mobile elements in Macrococcus

mecB on plasmid

The *mecB* gene (formerly $mecA_m$) was first identified on plasmid pMCCL2 in *M. caseolyticus* strain JCSC5402 isolated from a chicken in Japan.[4] Shortly after, another two *M. caseolyticus* strains from chicken were found to carry *mecB* either on a plasmid in an isolate from Thailand (strain JCSC7528) or on a SCC*mec*-like element in an isolate from China (JCSC7096).[61] In these strains, the *mecB* operon was accompanied by an IS



ccr	strain	GenBank	Nucleotide indentities (%)					
			ccrAm1	*ccrAm3*	*ccrBm1*	*ccrBm3*	*ccrCm1*	*ccrCm2*
ccrB1	*Staphylococcus aureus* NCTC10442	AB033763	46	46	57	62	47	47
ccrB1	*Staphylococcus hominis* GIFU12263	AB063171	45	47	55	60	44	45
ccrB6	*Staphylococcus saprophyticus* ATCC15305	AP008934	46	47	54	59	45	45
ccrB7	*Staphylococcus saprophyticus* TSU33	AB353724	43	46	55	58	43	44
ccrB2	*Staphylococcus aureus* N315	D86934	45	46	55	59	43	45
ccrB2	*Staphylococcus aureus* CA05(JCSC1968)	AB063172.2	46	46	56	59	44	45
ccrB3	*Staphylococcus aureus* 85/2082	AB037671	44	46	56	58	44	44
ccrB3	*Staphylococcus pseudintermedius* KM241	AM904731	44	45	56	59	45	45
ccrB4	*Staphylococcus aureus* HDE288	AF411935	44	45	56	60	46	45
ccrB4	*Staphylococcus aureus* C10682	FJ390057	43	46	56	58	45	44
ccrBm1	***Macrococcus caseolyticus* JCSC7096**	**AB498756**	42	43	100	61	42	42
ccrBm2	*Macrococcus canis* KM45013	CP021059	42	43	96	61	41	41
ccrBm1	*Macrococcus caseolyticus* 5198_3_76	PIWO01000006	42	43	94	61	41	41
ccrBm3	*Macrococcus equipercicus* Epi0143-OL	CP073809	45	46	61	100	42	45
ccrBm3	***Macrococcus bohemicus* H889678/16/1**	**JACEGF01000000**	45	46	61	100	42	45
ccrBm3	*Macrococcus canis* IMD0709	CP073808	45	46	61	99	42	45
ccrB3m	*Macrococcus bohemicus* DPC7215	SELR01000003	45	47	61	86	43	43
ccrBm3-2	*Macrococcus bohemicus* 19Msa422	CP054482	44	46	61	88	43	45
ccrBm3-1	*Macrococcus bohemicus* 19Msa422	CP054482	45	46	61	87	43	44
ccrBm3	*Macrococcus epidermidis* Epi3002-OL	CP073819	45	47	60	87	44	44
ccrC1	*Staphylococcus aureus* JCSC3624(WIS)	AB121219	43	44	46	47	56	57
ccrC2	*Staphylococcus aureus* BA01611	KR187111	44	43	47	48	54	55
ccrCm1	*Macrococcus caseolyticus* 5194_2_25	PIWL01000018	43	42	43	44	93	71
ccrCm1	***Macrococcus caseolyticus* 5456_3_46**	**PIWR01000025**	43	42	44	43	100	70
ccrCm2	*Macrococcus goetzii* DPC7159	SDGN01000005	43	43	44	46	71	99
ccrCm2	***Macrococcus canis* DPC7158**	**SDQI01000003**	43	43	44	46	70	100
ccrA1	*Staphylococcus aureus* NCTC10442	AB033763	49	50	43	45	38	42
ccrA1	*Staphylococcus saprophyticus* TSU33	AB353724	49	49	44	44	39	42
ccrA7	*Mammaliicoccus sciuri* MCS24	AB587080	49	49	43	47	38	41
ccrA3	*Staphylococcus aureus* 85/2082	AB037671	50	50	44	44	41	43
ccrA3	*Staphylococcus pseudintermedius* KM1381	AM904732	50	50	44	44	41	43
ccrA5	*Staphylococcus pseudintermedius* KM241	AM904731	49	50	43	45	39	43
ccrA2	*Staphylococcus aureus* N315	D86934	49	50	43	46	40	42
ccrA2	*Staphylococcus aureus* AR13.1/3330.2	AJ810120	49	50	43	46	40	42
ccrA4	*Staphylococcus aureus* HDE288	AF411935	49	48	44	43	41	42
ccrA4	*Staphylococcus epidermidis* ATCC12228	NC_004461	49	48	44	44	41	42
ccrAm1	***Macrococcus caseolyticus* JCSC7096**	**AB498756**	100	53	42	43	40	42
ccrAm1	*Macrococcus caseolyticus* 5198_3_76	PIWO01000006	98	53	43	42	40	41
ccrAm2	*Macrococcus canis* KM45013	CP021059	94	52	42	43	40	41
ccrAm3	*Macrococcus epidermidis* Epi3002-OL	CP073819	52	92	47	42	38	40
ccrAm3	*Macrococcus bohemicus* DPC7215	SELR01000003	53	89	46	43	38	41
ccrAm3-2	*Macrococcus bohemicus* 19Msa422	CP054482	53	87	47	43	39	41
ccrAm3-1	*Macrococcus bohemicus* 19Msa422	CP054482	53	93	48	41	38	40
ccrAm3	*Macrococcus canis* IMD0709	CP073808	53	99	47	42	38	41
ccrAm3	*Macrococcus equipercicus* Epi0143-OL	CP073809	53	100	47	42	38	40
ccrAm3	*Macrococcus bohemicus* H889678/16/1	JACEGF01000000	53	100	47	42	38	40

Figure 6. Phylogenetic tree of cassette chromosome recombinase (*ccr*) genes present in *Staphylococcus*, *Mammaliicoccus* and *Macrococcus*. Reference strains used and their GenBank accession numbers are indicated. The tree was constructed in MEGA7 using the UPGMA method and 1000 replicates in the bootstrap test.[203] The percentage of nucleotide identities shared between the macrococcal *ccr* allotypes, *ccrAm1*, *ccrAm3*, *ccrBm1*, *ccrBm3*, *ccrCm1* and *ccrCm2*, and all other *ccrs* were calculated using multiple sequence alignment tool of Clustal Omega.[202]

forming a *mec* transposon designated Tn*6045* (Figure 7a).[61] The IS of Tn*6045* belongs to the IS*3* family and carries two consecutive genes, the first encoding a DNA-binding protein (CDD: pfam01527), the second an integrase with a catalytic DD35E triad motif (PROSITE: PS50994). Fusion of such consecutive reading frames by −1 programmed translational frameshifting forms the full-length transposase.[183] Coexcision of the IS genes and the *mec* operon as a circular DNA molecule suggested that *mecB* can be mobilized with the Tn*6045* element.[61]

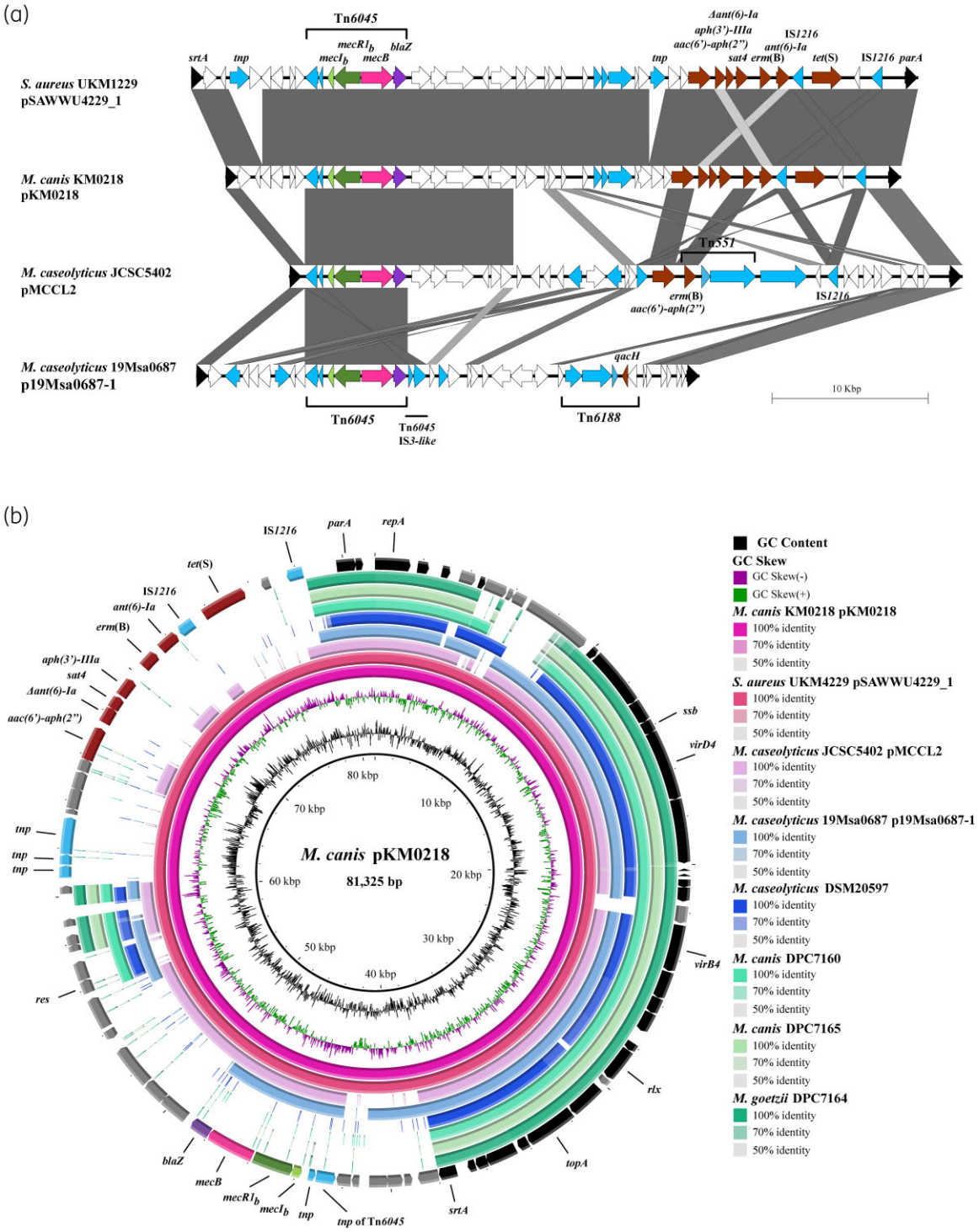


Figure 7. Resistance region and backbone of pMCCL2-like plasmids. (a) Comparison of *mecB*-containing resistance region between the *srtA* and *parA* genes of pMCCL2-like plasmids. Genetic structures of *S. aureus* UKM4229 (GenBank: LT799381), *M. canis* KM0218 (MF477836), *M. caseolyticus* JCSC5402 (AP009486) and *M. caseolyticus* 19Msa0687 (CP079966) were visualized using EasyFig software.[204] (b) Alignment of pMCCL2-like plasmid with reference pKM0218 of *M. canis* KM0218 (GenBank: MF477836) using BLAST Ring Image Generator (BRIG).[201] Query sequences used for comparison were from *S. aureus* UKM4119 (GenBank: LT799381), *M. caseolyticus* JCSC5402 (AP009486), *M. caseolyticus* 19Msa0687 (CP079966), *M. caseolyticus* DSM20597 (GenBank: PPRM01000034, PPRM01000014 and PPRM01000071), *M. canis* DPC7160 and DPC7165 and *M. goetzii* DPC7164 (Bioproject: PRJNA515496). The outermost ring contains the annotation for pKM0218, resistance genes are shown as brick red arrows, recombinase and IS genes are shown in blue and plasmid backbone genes present in all sequences are given in black. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Plasmid-encoded *mecB* was further detected in *M. caseolyticus* strains from bovine milk in the UK[52] (strains 5194_2_25, 5783_EF107, 5812_BC73, 5814_BC75 and 5816_BC109 in NCBI BioProject PRJNA420921), in *M. caseolyticus* isolated from beef in Switzerland[54] (strains 19Msa0687, 19Msa0499 and 19Msa1047 in BioProject PRJNA744395), in MRSA from a human carrier in Germany[20] and in an *M. canis* strain isolated from a dog in Switzerland.[21] In all these strains, *mecB* was part of Tn*6045* on plasmids related to pMCCL2 of *M. caseolyticus* JCSC5402. The pMCCL2-like plasmids seem to be abundant in *Macrococcus* and were also detected in GenBank sequences of several *mecB*-negative strains including *M. caseolyticus* type strain DSM20597 (GenBank: PPRM00000000) and *M. caseolyticus*, *M. canis* and *M. goetzii* isolates from bovine source in Ireland (*M. caseolyticus* DPC6291, *M. canis* DPC7160, DPC7162, DPC7163, DPC7165, DPC7168 and DPC7169, and *M. goetzii* DPC7164 and DPC7166 in Bioproject PRJNA515496). Alignment of the *mecB*-containing plasmid pKM0218 of *M. canis* KM0218 from a dog with other pMCCL2-like plasmids showed an approximately 40 kb shared backbone region (Figure 7b). This conserved region encoded a characteristic Rep protein with a PriCT_1-domain (CDD: pfam08708) for initiation of theta mode plasmid replication[184] and a plasmid partition protein A (ParA) possibly involved in segregational stability. Genes related to conjugative transfer functions were also detected in the conserved backbone, namely a DNA relaxase (Rlx) with a MobL domain (CDD: pfam18555), a VirD4 coupling protein (CDD: COG3505), a conjugative transfer ATPase VirB4 (CDD: TIGR00929), a single-strand binding protein (SSB) (PROSITE: PS50935) and a DNA topoisomerase IA (TopA) (CDD: COG0550) (Figure 7b). The second part of the pMCCL2-like plasmids spanning the region from a gene annotated as class A sortase (*srtA*) up to the *parA* gene was highly diverse in most of the plasmids. The Tn*6045*-associated *mecB* operon as well as other antibiotic resistance and recombinase genes were found in this region (Figure 7a).

The plasmids pKM0218 of *M. canis* KM0218 and pSAWWU4229_1 of *S. aureus* UKM4229 contained a highly similar backbone and resistance region with an identical cluster of IS elements and resistance genes for aminoglycosides [*aph(3′)-IIIa*, *ant(6)-Ia* and *aac(6′)-Ie – aph(2″)-Ia*], macrolides [*erm*(B)], streptothricin (*sat*4) and tetracyclines [*tet*(S)].[21] The two plasmids differed mainly in two additional inserts of transposases in the resistance region of pSAWWU4229_1 (Figure 7a). A common precursor and a probable recent transfer of such a pKM0218-like plasmid from *Macrococcus* to *S. aureus* is therefore very likely.[21] Of note, pSAWWU4229_1 is the only detected pMCCL2-like plasmid in staphylococcal sequences in the GenBank database (accessed January 2022), suggesting a rare inter-genus transfer of a plasmid which does not yet seem to be established in the genus *Staphylococcus*. Plasmid pMCCL2 of *M. caseolyticus* JCSC5402 also carries in addition to *mecB*, the resistance genes *aac(6′)-Ie – aph(2″)-Ia* and *erm*(B) as well as IS*1216*, but the structural organization of these genes differs from that of pKM0218 and pSAWWU4229_1 (Figure 7a). The plasmid p19Msa0687-1 from *M. caseolyticus* shown in Figure 7a and plasmids of the other *M. caseolyticus* strains from beef in Switzerland were similar with each other and contain no further antibiotic resistance genes apart from *mecB*.[54] They had all an additional copy of a Tn*6045*-like IS*3* inserted downstream of Tn*6045* (Figure 7a). The same IS arrangement around the *mecB* gene was also found in plasmids of *M. caseolyticus* strains from UK milk (NCBI BioProject: PRJNA420921). The plasmid p19Msa0687-1 contains in the resistance region several transposase genes and the transposon Tn*6188* (Figure 7a). Tn*6188* was identified in *Listeria monocytogenes* and carries the *qacH* gene involved in tolerance to benzalkonium chloride.[185] All the *mecB*-negative pMCCL2-like plasmids were not associated with any antibiotic resistance genes, but accumulation of IS elements and unique genes was also observed in the variable region between the *srtA* and *parA* gene.

mecB on SCCmec

Macrococcus species also use SCC elements as a vehicle for gene exchange and accumulation of accessory sequences at the *rlmH* locus (Figure 2). SCC*mec* of *Macrococcus* are not included in the classification scheme for *Staphylococcus*, which is primarily used to type *S. aureus* elements and curated by the IWG-SCC committee.[164] To distinguish between elements that contain *mecB* and *mecD* in *Macrococcus*, we suggest here to indicate the *mec* type and call the element SCC*mecB* and SCC*mecD*, respectively. A suffix with the strain name can additionally be included to refer to specific elements, for example SCC$mecB_{KM45013}$ or SCC$mecD_{19Msa422}$.

(i) ***SCCmecB elements with ccrABm1/2***. An SCC*mec*-like structure was first observed in *M. caseolyticus* JCSC7096 from chicken in China, which contained the *mec* transposon Tn*6045* downstream of *rlmH*, followed by an SCC element (SCC_{7096}) with *ccrAB* gene variants (*ccrAm* and *ccrBm*) (Figure 8).[61] The 6.7 kb Tn*6045* of JCSC7096 was flanked by ISS of *att* sites and formed a 6.8 kb ΨSCC$mecB_{7096}$ as well as a SCC*mecB*-like structure together with the 18.1 kb SCC_{7096}. PCR assays showed that *mecB* was excised from the chromosome with Tn*6045* only and as a SCC*mecB*-like element through recombination of ISS1 and ISS4′/ISS5 sites. The *ccrAm1* and *ccrBm1* genes of SCC_{7096} differ from all *ccrAB* allotypes present in *Staphylococcus*, *ccrAm1* sharing approximately 50% nt identity with the staphylococcal *ccrA* genes and *ccrBm1* sharing between 54% to 57% identity with staphylococcal *ccrB* genes (Figure 6). A BLAST search in the NCBI database revealed that the *ccrABm1* genes are also present in *M. caseolyticus* strains from milk in UK, which lack the *mecB* gene (strains 5193_3_76, 5450_CC63A, 5458_5_53, 5788_EF188 and 5196_2_38 in Bioproject PRJNA420921). These *ccrABm1* genes shared 98% and 94% nt identities with the *ccrAm1* and *ccrBm1* of JCSC9076, respectively. As shown for the *M. caseolyticus* strain 5193_3_76 from UK, the *ccrABm1* genes were found in an SCC CI structure that displays similarity to the *ccr*-containing region of SCC_{7096} in a 10 kb fragment (94% nt identity) and to a short region upstream of ISS5 of JCSC7096 (1.8 kb fragment, 99%) (Figure 8). A *mecB*-containing SCC*mecB* CI with *ccrAB* genes similar to *ccrABm1* was detected in the *M. canis* type strain KM45013 isolated from a dog in Switzerland (Figure 8).[152,186] The *ccr* genes, called *ccrABm2*, were also carried in separate SCC in KM45013 that shared in a 7.5 kb region 92% nt identity with SCC_{7096}. However, the *mecB* operon of KM45013 was not associated with Tn*6045* sequence and was located in a 12 kb ΨSCC*mecB* flanked by ISS2 and ISS3. Functional activity of *ccrABm2* was suggested since circular

structures of SCC, ΨSCC*mecB* and SCC-ΨSCC*mecB* end-joined by an ISS were observed by PCR.[152] An IS*3* family transposase similar to that of Tn*6045* was also found independently of the *mecB* operon in *M. caseolyticus* strain 5193_3_76 from the UK (89% nt identity to IS*3* of Tn*6045* in JCSC7096) (Figure 8) and in *M. caseolyticus* CCM7927 from Czech Republic (99% nt identity) (GenBank: MJBJ02000005.1). Such IS*3* family transposases were also detected in *S. pseudintermedius* strain 53_60 (GenBank: CP035741) and NA45 (GenBank: CP016072.1) from the US which shared 97% identity with Tn*6045* of JCSC7096.

(ii) ***ΨSCCmecB elements***. ΨSCC*mecB* in CI without *ccr* genes were detected in *M. goetzii* type strain CM4927 from human in Czech Republic and in *M. canis* KM0218 and Epi0076A from dogs in Switzerland, and *M. canis* 19/EPI0118 from veterinary clinic environment in Switzerland (Figure 8).[21,28,187] The CI structure described above with SCC containing functional *ccrABm* genes separated by an ISS from *mecB* in ΨSCC*mecB* could facilitate the loss of SCC and formation of ΨSCC*mecB* CI without *ccr*. In *M. goetzii* CCM4927 and *M. canis* Epi0076A and 19/EPI0118, the $blaZ_b$ gene was additionally missing (Figure 8). The *M. canis* strains KM0218, Epi0076A and 19/EPI0118 showed similarity to the SCC*mecB* CI of *M. canis* KM45013 in several discontinuous fragments, suggesting continuous diversification of the elements by insertion and deletion.[21] Formation of unconventional circularizable structures (UCS), a mechanism involved in mobilization of resistance genes,[188] containing the *mecB* gene was detected in both KM45013 and Epi0076A.[21,152] They were caused by recombination of extended imperfect DR (DR of 0.8 kb in KM45013 and unrelated DR of 0.5 kb in Epi0076A) in the flanking sequences of the *mecB* operons (Figure 8). Furthermore, *M. canis* KM0218 contained two distinct *mecB* operons with overall 99% nt identity, one situated on the multidrug resistance plasmid pKM0218 (Figure 6) and the other on the ΨSCC*mecB* CI (Figure 8). The two homologous *mecB* operon sequences in KM0218 were also used for recombination. Restriction analysis of long-range PCR demonstrated that pKM0218 was not only present as closed circular plasmid but was also found integrated into chromosomal ΨSCC*mecB* CI in KM0218 cells.[21] These observations suggest a recombination-mediated process independent of *ccr* as a further cause for the diversification of *rlmH* region in *Macrococcus*. The *M. canis* strain 19/EPI0118 carried in addition to *mecB* on ΨSCC*mecB* also *mecD* on a $McRI_{mecD}$ indicating that even two different *mec* genes can be present in a strain (Figure 2).[187]

The chromosomal *mecB* operon of *M. canis* KM0218 was located downstream of IS*Maca1*, a member of the IS*21* family, which encodes a DDE transposase (*istA*) and a IS*21* helper gene (*istB*).[21] IS*Maca1* is also present in other *M. canis* species, e.g. Epi0076A contained a IS*Maca1* with 92% identity to that of KM0218 (Figure 8). Similar IS are not frequently seen in the staphylococcal sequences in GenBank database. However, the genome of *S. pseudintermedius* strain 53_60 (GenBank: CP035741) mentioned above contains in addition to the IS*3*-family transposase similar to Tn*6045*, also an IS*Maca1* with 96% identity to that of KM0218. *M. canis* and *S. pseudintermedius* are both colonizers of the canine skin; hence contact of the two species and exchange of genes may take place.

(iii) ***SCCmecB elements with ccrABm3***. New *ccrAB* allotypes (*ccrABm3*) were detected in a *mecB*-containing SCC*mecB* element of *M. bohemicus* H889678/16/1 from a canine otitis sample in Scotland[67] (Figure 8) and a *mecD*-containing SCC*mecD* of *M. bohemicus* 19Msa422 from a calf in Switzerland[64] (see section *mecD* on SCC*mec*). The *ccrABm3* are only distantly related to recognized *ccr* allotypes with *ccrAm3* showing the highest nt identity with *ccrAm1/2* (53%) and *ccrBm3* with *ccrBm1/2* (61%) (Figure 6). Strain H889678/16/1 contained a complete $blaZ_b$-*mecB*-$merR1_b$-$mecI_b$ operon and *ccrABm3* genes without a separating ISS in one SCC*mecB*. Additionally, the fusidic acid resistance gene *fusC* was carried downstream of $blaZ_b$. The *fusC* gene was also present in ΨSCC*mecB* of 19/EPI0118 (99% nt identity) and was previously reported on SCC elements in MRSA and MSSA (98% nt identity).[174,189] Apart from *mec* and *fusc,* these *fusC*-containing SCC*mec* elements show no further similarities.

A GenBank search revealed that *ccrABm3* alloypes are present in different *Macrococcus* species. They were found associated with *mecB* in *M. canis* IMD0709 (GenBank: CP073808), *M. equipercicus* Epi0143-OL (CP073809) and *M. epidermidis* Epi3002-OL (CP073819), and also in an SCC CI without a *mec* gene in *M. bohemicus* DPC7215 (SELR01000003) (Figures 6 and 8).

Where present, *mecB* was found in different gene complexes, namely as part of Tn*6045* in *M. canis* IMD0709, without Tn*6045* transposase and with $blaZ_b$ in *M. bohemicus* H889678/16/1 and Epi0143-OL and without Tn*6045* transposase and without $blaZ_b$ gene in *M. epidermidis* Epi3002-OL. Strikingly, the same 32.4 kb ISS-flanked SCC*mecB* of *M. bohemicus* H889678/16/1 was also present in *M. equipercicus* Epi0143-OL (99.9% nt identity, five SNPs in total) (Figure 8). *M. bohemicus* and *M. equipercicus* are phylogenetic located on different *Macrococcus* branches (Figure 1). The presence of the same element in the two different species suggests that horizontal gene transfers occurred between *M. bohemicus* and *M. equipercicus*. The SCC*mecB* of *M. canis* IMD0709 was also related to the SCC*mecB* of *M. bohemicus* H889678/16/1 sharing 96% nt identity in a 12.2 kb fragment containing the *ccrABm3* (Figure 8). A ΨSCC harbouring the same copper resistance genes (*zntA*, *copY*, *copZ* and *cueO*) was present in *M. caseolyticus*, *M. goetzii*, *M. canis* and *M. bohemicus* species. A ΨSCC with an operon containing putative phytoene desaturase genes (*crtI*) seems to be prevalent in the closely related *M. goetzii*, *M. bohemicus* and *M. epidermidis* species and was also found in a *M. canis* strain (Figure 8).

(iv) ***SCCmecB elements with ccrC***. Two new *ccrC* allotypes were identified during this study in GenBank sequences of *Macrococcus*: *ccrCm1* in *M. caseolyticus* strains 5456_3_46 and 5419_2_25 from UK (Bioproject PRJNA420921), and *ccrCm2* in *M. goetzii* DPC7159 (GenBank: SDGN01000005) and *M. canis* DPC7158 (SDQI01000003) from bovine samples in Ireland (Figure 8). The *ccrCm1* and *ccrCm2* genes shared less than 72% nt identity with each other and less than 58% with the staphylococcal *ccrC1* and *ccrC2* genes (Figure 6). In *M. caseolyticus* 5456_3_46 draft genome, *ccrCm1* was found on the same contig (no. 25) as *mecB* (GenBank: PIWR01000025) representing a new SCC*mecB* type so-far not characterized (Figure 8). One base deviation in the well-conserved centre of the 18 bp consensus ISS[166] was observed in all ISS1 sequences (at the 3′ end of *rlmH*) in strains containing the *ccrCm* allotypes (alternative ISS indicated with ISS′ in Figure 8). This variation suggests that *ccrCm*-containing elements

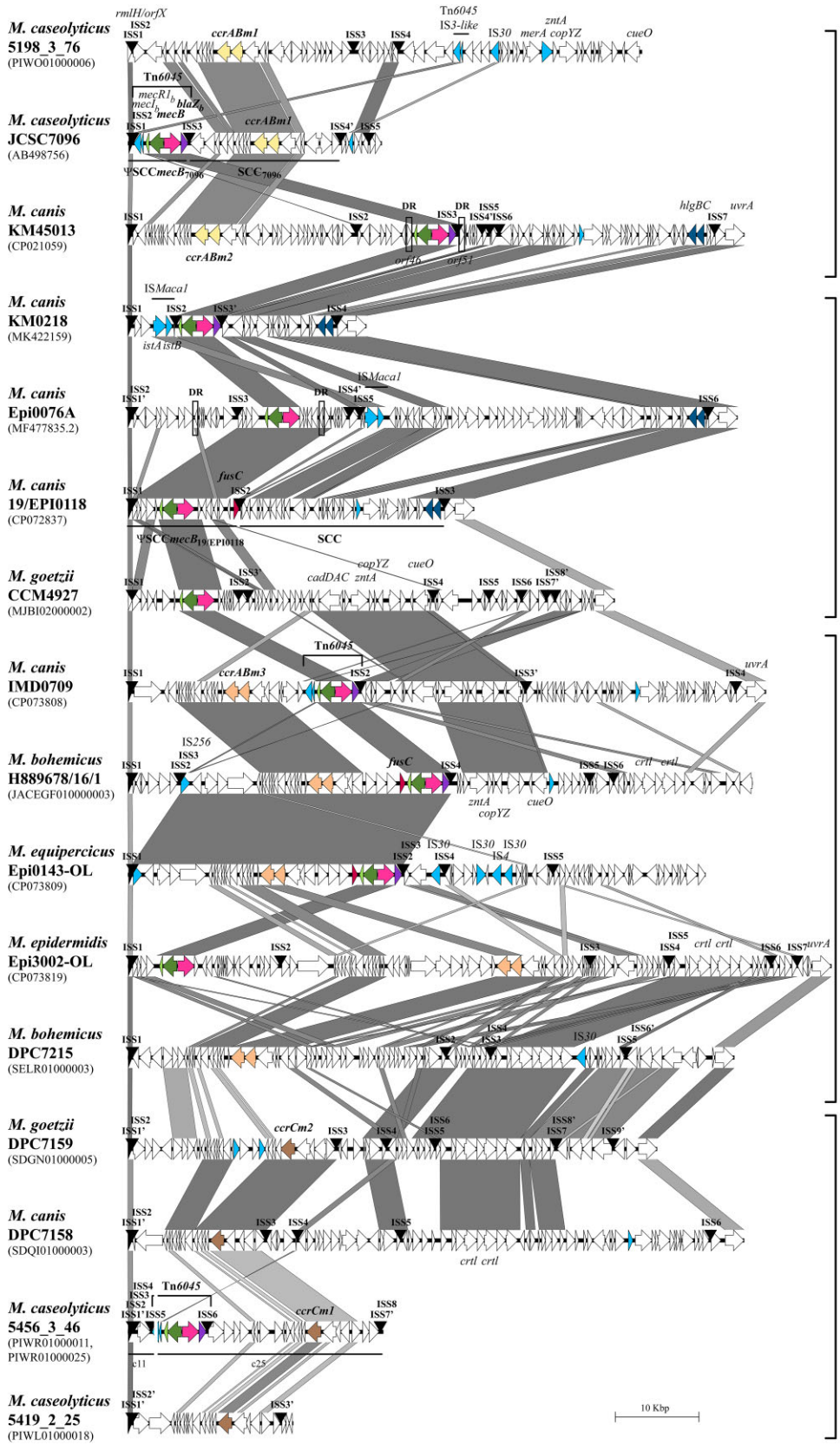


Figure 8.

are associated with alternative *attS* sequences. The deviation consists of a T to C substitution at position 9 of ISS, in the central core that was shown to be essential for CcrB binding by electrophoretic mobility shift assay.[190] An alternative ISS1′ was additionally observed at the 3′ end of *rlmH* in *M. canis* Epi0076A containing a ΨSCC*mecB* CI and in several other *Macrococcus* strains within CI structures (Figure 8).

Overall, *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* harbour orthologous SCC structures that contain specific *mec* genes as well also genus-specific *ccr* genes. The macrococcal *ccrAm*, *ccrBm* and *ccrCm* genes cluster in separate branches as compared with corresponding staphylococcal allotypes (Figure 6). Considering that relatively few SCC*mecB* structures have been sequenced so far in *Macrococcus*, the diversity of the elements is remarkable. The *mecB* gene is found in different genetic contexts containing the *mecB-mecR1*$_b$-*mecI*$_b$ core present in all elements with high nt identity (97%–100% for strains shown in Figure 8) in combination with three different *ccr* genes, *ccrABm1/2*, *ccrABm3* and *ccrCm1* and diverse joining regions.

mecD on McRI$_{mecD}$

Genetic structure and mobility of McRI$_{mecD}$

The *mecD* gene was first detected in 2017 in Swiss *M. caseolyticus* strains from bovine mastitis milk (strains IMD0819 and IMD0473) and from an ear infection of a dog (strain KM0211).[19] The *mecD* gene was located on chromosomal islands, which were unrelated to the other *mec*-containing elements such as SCC*mecB*, pMCCL2-like plasmids and transposon Tn*6045*. They were found site-specifically integrated into the 3′ end of the 30S ribosomal protein S9 (*rpsI*) gene and referred to as *Macroccocus* resistance island *mecD* (McRI$_{mecD}$) (formerly *Macrococcus caseolyticus* resistance island *mecD*) (Figure 9). The *rpsI* gene is conserved among Gram-positive bacteria and is present in *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* at the end of a 19 kb orthologous gene cluster of several ribosomal protein genes (*rpsJ* to *rpsI* in Figure 2). In *Macrococcus* species, this cluster is located approximately +0.3 Mb to the right of the *oriC*. In *Staphylococcus* species, it can be found on either side of *oriC*, at positions around +0.6 to +0.8 Mb or −0.5 to −0.8 Mb.

McRI$_{mecD}$ elements have a size of approximately 16 to 21 kb and display extended imperfect DRs (120–163 bp) in the island-chromosome junction with a 61 bp core attachment site (*att*) similar to the sequence at the 3′ end of the *rpsI* gene (Figure 10a). In *M. caseolyticus* IMD0819, a third imperfect DR (DR3) was found 2.8 kb downstream of McRI$_{mecD}$ that showed an even longer overlap with DR2 in a 405/404 bp fragment and contained a copy of the core *att* site embedded in the middle (Figure 10a). The macrococcal chromosomal island (McCI) formed between DR2 and DR3 was named McCI$_{IMD0819}$ in strain IMD0819 (Figure 9).[19] The 5′ end of McRI$_{mecD}$ encodes a unique integrase (*int*) of the tyrosine recombinase family. The Int protein contains the catalytic residues of tyrosine recombinases in the C-terminal part and an N-terminal SAM-like domain (CDD: pfam14659) present in phage integrases.[19] The *int* gene is preceded by two divergently oriented genes, *intR* and *xis* (Figure 9) encoding putative DNA-binding proteins with an HTH-motif similar to that found in lambda repressor-like proteins and excisionase/Xis family proteins, respectively.[191] The gene organization *int-intR-xis* resembles the *int-stl*(*-str*)*-xis* structure found in pathogenicity islands of *Staphylococcus* species. However, McRI$_{mecD}$ does not contain features characteristic of phage-related chromosomal islands like *pri*, *rep*, *pif* and *terS* genes that are involved in island replication and interaction with helper phage.[192] Furthermore, the Int protein of McRI$_{mecD}$ is only distantly related to tyrosine recombinases of characterized pathogenicity islands. It shares the highest amino acid identity (of around 40%) to Int of *S. aureus* pathogenicity islands SaPIbov1 and SaPIbov2.[19] The latter are also site-specifically integrated elements at the chromosomal GMP synthetase (*guaA*) locus.[193,194]

Spontaneous excision of McRI$_{mecD}$ has been observed in *M. caseolyticus* IMD0819 in PCR assays.[19] Circular recombination products for McRI$_{mecD}$, McRI$_{mecD}$-McCI$_{IMD0819}$ and McCI$_{IMD0819}$ were detected, as well as the corresponding chromosomal part remaining after loss of the elements. Analysis of the joining sequences revealed that recombination occurred within the core *att* site of the DRs. The site-specific reaction was mediated by the *int* gene of *M. caseolyticus* IMD0819 (*int0819*).[191] In experiments with circular model elements containing different 5′ end fragments of McRI$_{mecD}$, the *int0819* gene and the *att* sequence were shown to be sufficient for element integration into the 3′ end of different *rpsI* genes, namely that of *S. aureus*, *S. pseudintermedius* and with lower integration efficiency into that of *Bacillus thuringiensis* (Figure 10a). Variation in the nucleotide sequence of the core *att* site on the circular model and the different *rpsI* genes suggests that strand cleavage for exchange occurs at the 5′ end of the core *att* site before bases at sequence position 1 to 3. Inclusion of *intR* and *xis* alone or together in the circular McRI$_{mecD}$ model revealed that *intR* acts as negative regulator of *int0819* and *xis*, while *xis* stimulates both integration and excision recombination reactions.[191] Xis proteins of phages are known as accessory factors, also called recombination directionality factors (RDF), that control recombination reactions by stimulating

Figure 8. Continued
Genetic structures of *mecB*-containing SCC*mec* and SCC in *Macrococcus* species. Composite islands downstream of the *rlmH/orfX* gene subdivided by attachment site containing the integration site sequence (ISS) for SCC are shown for indicated strains with Genbank accession number in parentheses. The sequences are group according to the presence of cassette chromosome recombinase (*ccr*) genes, *ccrABm1/2*, *no ccr*, *ccrABm3* and *ccrCm* and compared using EasyFig software.[204] Genes are indicated as arrows and colour coded for *mecB* (pink), *mecI*$_b$ (light green), *mecR1*$_b$ (dark green), *blaZ*$_b$ (purple), fusidic acid resistance (red), putative haemolysins (dark blue), *ccrs* (yellow, beige and brown) and other recombinases/transposases (light blue). Genes putatively involved in mercury (*merA*), cadmium (*cadD*, *cadA* and *cadC*) and copper resistance (*zntA*, *copY*, *copZ* and *cueO*) are labelled as well as putative phytoene desaturase genes (*crtI*). The 18 bp consensus ISS suggested by Ito *et al.*,[166] 5′-GA[A/G]GC[A/T/G]TATCA[C/T]AA[A/G]T[A/G][A/G] was adapted for deviations in the 5′-region (ISS) and a central T to C substitution at position 9 (ISS′) using the following extended consensus sequence 5′-**N**[A/**T**/**G**][A/G][G/**A**/**T**]NNTA[T/**C**]CA[C/T]AA[A/G]T[A/G][A/G] (changes indicated in bold). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

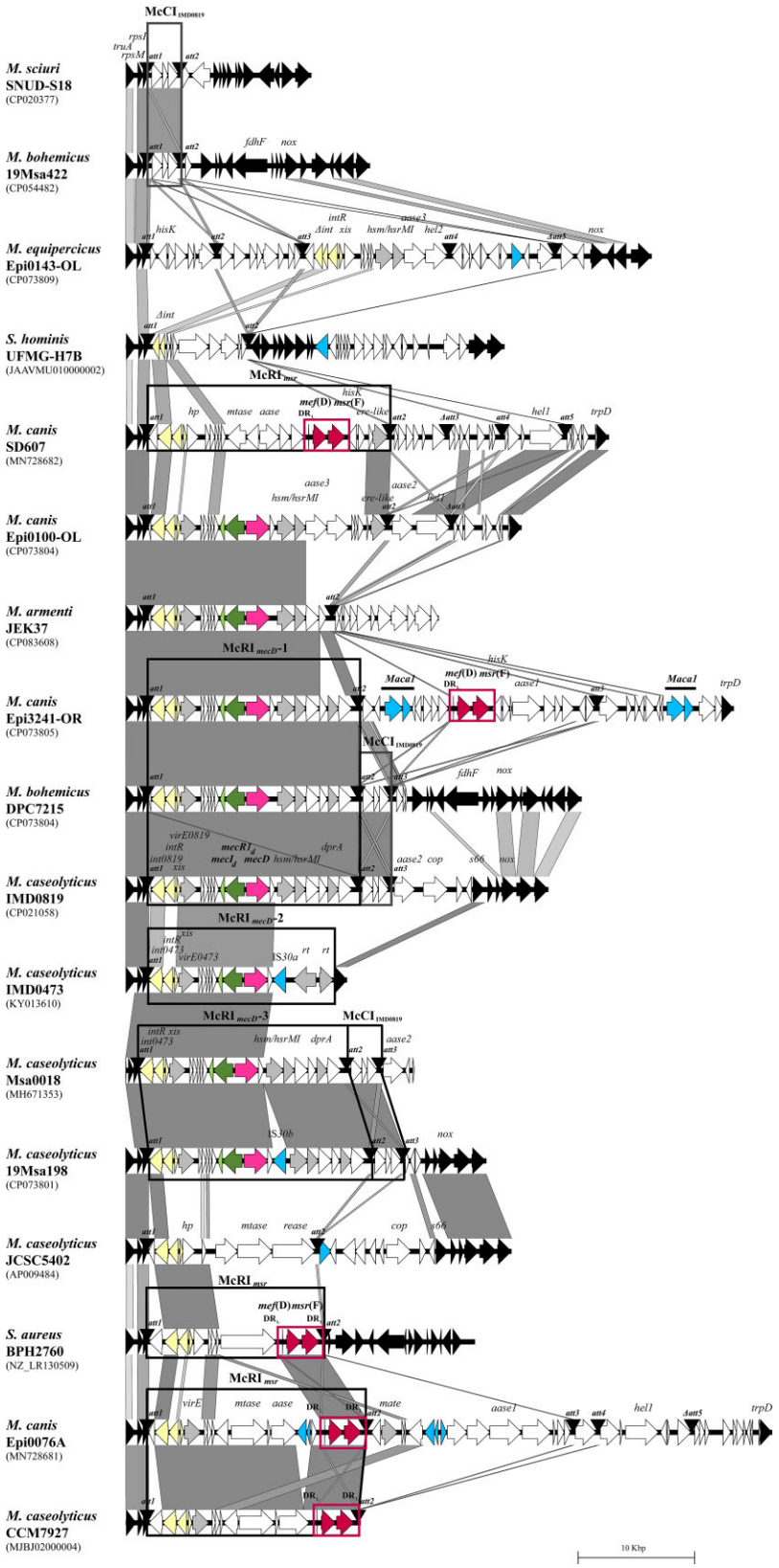


Figure 9.

phage excision while interfering with phage integration.[195] In the experiments with the McRI$_{mecD}$ model, the *xis* gene did not function as a classical RDF. However, *xis* of McRI$_{mecD}$ also directed the *att* site selection of *int0819* in the experiment with *S. pseudintermedius* strain FMV15/08 that contained two chromosomal core *att* sites, *attC1* at the 3′ end of the *rpsI* gene and *attC2*, 2.5 kb downstream of *attC1* (Figure 10a). The *attC1* site was exclusively used for model integration in the absence of *xis* while model integration was observed at both *attC* sites in the presence of *xis*.[191] The *attC2* contains upstream of the core *att* a sequence similar to that of the extended DR2 of McRI$_{mecD}$ which likely also provides a binding site for Xis proteins.

McRI$_{mecD}$ elements in M. caseolyticus

Three McRI$_{mecD}$ islands have been described in *M. caseolyticus* with reference type McRI$_{mecD}$-1 in IMD0819, McRI$_{mecD}$-2 in IMD0473[19] and McRI$_{mecD}$-3 in Msa0018.[50] These islands have in common a highly similar 6.6 kb middle part (99.97% nt identity) containing *mecI$_d$*, *mecR1$_d$* and *mecD* (Figure 9). All contain the *int-intR-xis* cluster and the *virE* gene encoding a protein with a putative virulence-associated protein E-like domain (CDD: pfam05272) at the 5′ end. The 4.2 kb 5′ segment is 99.98% identical in McRI$_{mecD}$-2 and McRI$_{mecD}$-3 but shares only 68% overall nt identity with the corresponding segment of McRI$_{mecD}$-1. The integrase *int0473* present in McRI$_{mecD}$-2 of IMD0473 and McRI$_{mecD}$-3 of Msa0018 exhibits 76% nt identity with *int0819* of McRI$_{mecD}$-1 (Figure 10b). However, the 7.2 kb 3′ segment, which includes genes for restriction modification system (*hsmMI-hsrMI*) and a putative recombination mediator (*dprA*), is identical in McRI$_{mecD}$-1 and McRI$_{mecD}$-3. In McRI$_{mecD}$-2, an *att* site is missing at the right end (Figure 9). Therefore, the island end was tentatively defined at the border of a possible core-genome segment starting with the *s66* gene in IMD0473.[19] The IS*30* and the unique reverse-transcriptase (*rt*) genes downstream of *mecD* were assigned to McRI$_{mecD}$-2. However, comparison of McRI$_{mecD}$-2 with McRI$_{mecD}$-3 suggests that McRI$_{mecD}$-2 could represent a 3′ deletion variant of McRI$_{mecD}$-3.[50] In agreement with the missing *att* site at the right end, circular excision of McRI$_{mecD}$-2 was not observed. The *int0473* gene is suggested to be functional since excision of the *att*-flanked McRI$_{mecD}$-3 in Msa0018, which also contains *int0473* was observed by PCR (Figure 9).

Analysis of McRI$_{mecD}$ types in *M. caseolyticus* strains from bovine source revealed that *mecD* is mainly carried on McRI$_{mecD}$-1 and McRI$_{mecD}$-3 elements.[50,54] This was observed in Swiss isolates which were characterized by multiplex PCR for molecular typing of McRI$_{mecD}$ as well as in strains from UK (NCBI BioProject: PRJNA420921) which were analysed *in silico* by read mapping against the three reference McRI$_{mecD}$.[50] Moreover, the mapping assemblies suggested highly conserved McRI$_{mecD}$ in UK and Switzerland with only few SNPs. Phylogenetic analyses using multilocus sequence typing showed that McRI$_{mecD}$-1 and McRI$_{mecD}$-3 were present in genetically diverse strains in both geographical regions. This finding indicates that McRI$_{mecD}$-1 and McRI$_{mecD}$-3 are mobile and may spread by horizontal gene transfer. In contrast, McRI$_{mecD}$-2 was only found in isolates from Switzerland, all of which belonged to the same sequence type.[50]

Furthermore, a McRI$_{mecD}$-3 with an IS*30* family element (IS*30b*) downstream of *mecD* was detected in *M. caseolyticus* 19Msa198 from a calf in Switzerland (GenBank: CP073801). Of note, the IS*30b* of 19Msa198 was integrated at the same position into McRI$_{mecD}$-3 as the IS*30* (IS*30a*) in McRI$_{mecD}$-2 of IMD0473 (Figure 9). The two IS*30a* and IS*30b* showed however partial nt identity only and coded for proteins that shared 52% amino acid identity. An McRI$_{mecD}$-1, that displayed seven SNPs compared with the reference strain IMD0819, was also present in an environmental *M. caseolyticus* strain from the US (strain DE0369, GenBank: NZ_VDZI01000004.1) suggesting that McRI$_{mecD}$ element can be found in geographically distant *M. caseolyticus* populations.

McRI$_{mecD}$ in Macrococcus species other than M. caseolyticus

An McRI$_{mecD}$-1 element was recently reported in *M. canis* 19/EPI0118 isolated from the clinical environment of an animal clinic in Switzerland.[187] A GenBank database search revealed that McRI$_{mecD}$-1 was also present in *M. canis* Epi3241-OR isolated from a dog in Switzerland (GenBank: CP073805) and in *M. bohemicus* DPC7215 from bovine milk in Ireland (GenBank: SELR01000007) (Figure 9). *M. armenti* type strain JEK37 (GenBank: CP083608) from a slaughter pig in Switzerland contained a 15.9 kb McRI$_{mecD}$-1 variant with deleted *dprA* gene (Figure 9). Deletion of *dprA* was also seen in a 20.7 kb McRI$_{mecD}$-1 variant in *M. canis* strain Epi0100-OL from a dog in Switzerland (GenBank: CP073804) (Figure 9). The 20.7 kb McRI$_{mecD}$-1 variant of Epi0100-OL contained additional genes for a putative helicase (*hel2*), an AAA family ATPase (*aase3*) and esterase (*ere-like*) in the 3′ end.

Chromosomal islands related to McRI$_{mecD}$

The *rpsI*-downstream region in *Macrococcus* is a locus where accessory genes are often found in *att*-flanked segments (Figure 9). In several different *Macrococcus* species, an *int* gene related to *int0819* and *int0473* is present adjacent to the *rpsI* gene (Figure 10b) and probably involved in accumulation of these accessory islands. Among them are islands, which carry *int-intR-xis* at the 5′ end such as McRI$_{mecD}$ and small chromosomal islands such as McCI$_{IMD0819}$ without an obvious mobilization

Figure 9. Continued
Genetic structures of chromosomal islands integrated downstream of the 30S ribosomal protein (*rpsI*) gene in *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* species. The *mecD*-containing resistance islands (McRI$_{mecD}$) are shown in the middle and compared with islands containing an integrase (*int*) similar to *int0819* of McRI$_{mecD}$-1 (upper part) and *int0473* of McRI$_{mecD}$-2/McRI$_{mecD}$-3 (lower part). The core attachment (*att*) sites that flank the islands are indicated. The figure was generated using EasyFig software[204] and indicated strains with GenBank accession number in parentheses. Gene are shown as arrows and colour coded for *mecD* (pink), *mecI$_b$* (light green), *mecR1$_b$* (dark green), macrolide resistance (red), *int* and accompanying *intR* and *xis* (yellow) and other recombinases/transposases (light blue). Genes usually present in the corresponding species are given in black. Abbreviations used for labelled gene not explained in the main text: *truA*, tRNA pseudouridine synthase A; *rplM*, 50S ribosomal protein L13; *fdhF*, formate dehydrogenase subunit alpha; *nox*, NADH oxidase; *hisK*, histidinol phosphate phosphatase domain-containing protein; *mate*, putative MATE family efflux transporter; *cop*, copper-translocating P-type ATPase; *s66*, peptidase s66; *trpD*, anthranilate phosphoribosyltransferase. DR$_L$ indicate a direct repeat that flank the *mef*(D)-*msr*(F) subunit. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

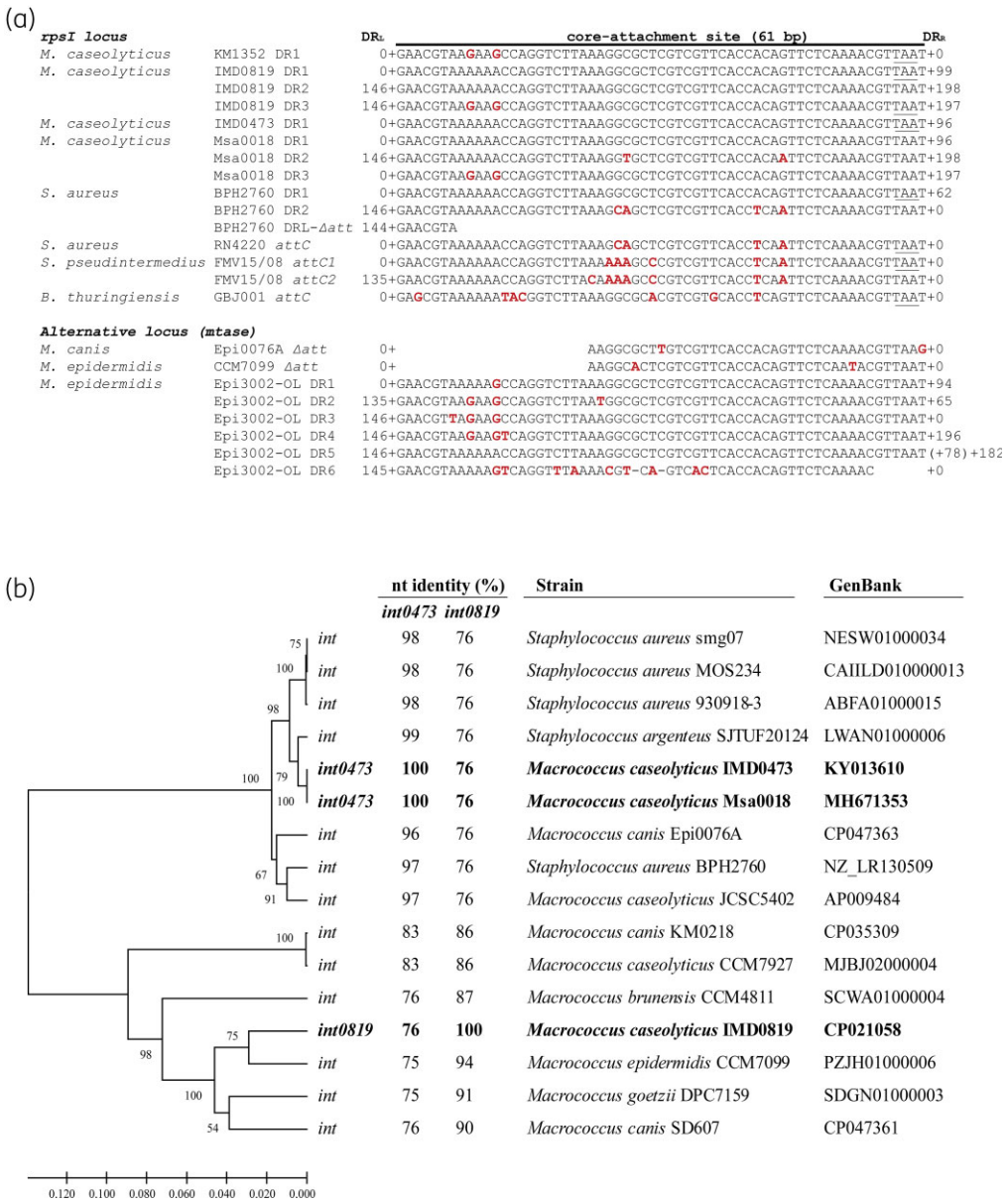


Figure 10. Core attachment site recognized by integrases (*int*) of McRI$_{mecD}$ and phylogenetic tree of *int* present downstream of the 30S ribosomal protein S9 (*rpsI*) gene in *Macrococcus* and *Staphylococcus* species. (a) The sequence of the 61 bp core attachment (*att*) sites found at the *rpsI* locus (upper part) and an alternative locus downstream of a putative methyltransferase (*mtase*) gene in some *Macrococcus* species (lower part). Positions that hold variant bases compared with those of the core *att* site flanking McRI$_{mecD}$-1 in strains IMD0819 are highlighted in red. Numbers at the right and left of the sequence indicate additional bases belonging to extended direct repeats that are found upstream (DR$_L$) and downstream (DR$_R$) of the core *att* sites. *att* sites present at the 3′ end of the *rpsI* gene are indicated with underlined *rpsI* stop codon. DR5 of *M. epidermidis* Epi3002-OL is followed by 78 bases not related to DR$_R$ (+78) and 182 bases related to DR$_R$. (b) Relatedness of integrases genes to *int0473* of McRI$_{mecD}$-2/McRI$_{mecD}$-3 and *int0819* of McRI$_{mecD}$-1. Phylogenetic tree was generated for nucleotide sequences using the UPGMA method in MEGA7.[203] The percentage of nucleotide (nt) identity shared with *int0473* and *int0819* was determined using the multiple sequence alignment tool of Clustal Omega.[202] Strains and GenBank accession numbers are indicated. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

function. Moreover, site-specific acquisition of accessory DNA at the *rpsI* locus is not limited to *Macrococcus* species; islands related to McRI$_{mecD}$ can also be present in *Staphylococcus*. Site-specific integrases similar to *int0819*/*int0473* were found downstream of *rpsI* in few *S. aureus* and *S. argenteus* strains (Figure 10b). The high identities seen among the *int* genes of *Staphylococcus* and *Macrococcus* suggest that elements using the *int0819*/*int0473-intR-xis* recombination system may be exchanged between the two genera. For instance, resistance islands related to McRI$_{mecD}$ that were integrated at the *rpsI* site and carry the macrolide resistance *mef*(D) and *msr*(F) (McRI$_{msr}$) have been reported in *M. canis*, *M. caseolyticus* and *S. aureus* (Figure 9).[196] A

highly similar 3.9 kb subunit carrying *mef*(D) and *msr*(F) (99% nt identity) was seen in *M. canis* Epi0076A and *S. aureus* BPH2670 isolated from dog in Switzerland and human blood in Australia, respectively. This subunit was flanked by DR sequence often found upstream of the core *att* site (DR_L) followed by a complete *att* site on the right side and by the first seven bases of the *att* (5′-GAACGTA) only on the left side (Figures 9 and 10a). A circular form of the *mef*(D)-*msr*(F) subunit was detected in Epi0076A cells suggesting that this segment could be possibly mobilized by *int* of $McRI_{msr}$.[196] Apart from the *mef*(D), *msr*(F) and *int* genes, the $McRI_{msr}$ in *S. aureus* BPH2760 and *M. canis* Epi0076A were different. The 5′ end of $McRI_{msr}$ of BPH2760 was most related to an island present in *M. caseolyticus* JCSC5402 (Figure 9) and to sequences of the *int*-containing *S. aureus* strains 930918-3, sgm07 and MOS234 (Figure 10b); none of them contained *mecD* or *mef*(D)-*msr*(F) resistance genes. Islands highly related overall to the entire $McRI_{msr}$ of Epi0076A were found in a human *M. caseolyticus* strain (CCM7927)[28] (Figure 9) and an *M. caseolyticus* strains from a donkey (DaniaSudan) (GenBank: NZ_RBVL01000008). *M. canis* SD607 contained the *mef*(D)-*msr*(F) gene on a different $McRI_{msr}$ whose integrase was more related to *int0819* than *int0473* (Figures 9 and 10b).[196] The *mef*(D)-*msr*(F) genes were also present in an *att*-flanked 20.5 kb island without an *int* downstream of the $McRI_{mecD}$-1 variant in *M. canis* Epi3241-OR (Figure 9). McRI as well as other *att*-flanked islands integrated at the *rpsI* locus frequently contain genes that may function in restriction modification, such as DNA methylases (*mtase*) and restriction endonucleases (*rease*), or genes related to helicases (*hel*) and AAA family ATPases (*aase*). In some cases, similar genes were found in different genetic contexts. For example, *M. canis* strain Epi0100-OL contains within the $McRI_{mecD}$ structure, a helicase (labelled *hel2*) and AAA family ATPase (*aase3*) genes similar to genes present in an integrase-defective islands found in *M. equipercicus* Epi0143-OL (Figure 9). Epi0100-OL also contains a helicase (*hel1*) similar to that present in *att*-flanked islands of SD607 and Epi0076A as well an *aase* (*aase2*) also present in *M. caseolyticus* IMD0819 and Msa0018. 5′-deleted *int* variants resulting from frameshift mutation and nonsense mutations were observed in *M. equipercicus* Epi0143-OL and *S. hominis* UFMG-H7B (Figure 9).

att-flanked islands integrated at the *rpsI* locus in strains that do not contain a site-specific *int* gene were also observed in *S. pseudintermedius* and *M. caseolyticus*.[50,191] Additionally, $McCI_{IMD0819}$ that shared around 95% nt identity with that of *M. caseolyticus* IMD0819 was found directly downstream of the *rpsI* gene in *M. bohemicus* 19Msa422 from a calf in Switzerland,[64] *M. sciuri* (SNUC 174, SNUC 1323 and SNUC 1326) and *M. fleurettii* (SNUC 182 and SNUC 248) strains from bovine samples in Canada (NCBI BioProject: PRJNA342349)[197] as well as in the *M. sciuri* strain SNUDS-18 from a duck in South Korea[198] (Figure 9). Furthermore, sequences with 99% nt identity to $McCI_{IMD0819}$ of IMD0819 were also present in the *S. argenteus* strain SJTUF20124 from China (LWAN01000103.1 and LWAN01000159.1) that carried an *int0473*-like integrase at the *rpsI* locus (Figure 10b).

Possible alternative chromosomal integration site for McRI

Additional *att*-flanked structures similar to those present at the *rpsI* locus can also be found in *M. epidermidis*, *M. goetzii* and *M. bohemicus* species at a different chromosomal locus downstream of a putative methyltransferase (*mtase*) gene which was only found in some *Macrococcus* species (Figure 11). This locus contains upstream of the *mtase* gene a lysylphosphatidylglycerol flippase/synthetase gene (*mprF*) usually intrinsically present in the chromosome of *Macrococcus* species (see Figure 2 for chromosomal location of *mprF*). The *M. canis* strain 19/EPI0118 contained no *mtase* downstream of *mprF*, while the *M. canis* Epi0076A contained a 3′ fragment of the *mtase* gene at this locus and a 5′-deleted core *att* site (*Δatt*) similarly to *M. epidermidis* type strain CCM7099 (Figures 10a and 11). In contrast, *M. epidermidis* strain Epi3002-OL displayed five complete and one partial core *att* sites, most of them were embedded in sequences also present in extended DR that flank elements integrated at the *rpsI* locus (Figures 11 and 10a). The *att*-flanked segments downstream of the *mtase* gene contained accessory genes encoding mainly hypothetical proteins with unknown function. No *int-intR-xis* containing element was detected. A fragment that carried a putative aminoglycoside 6-adenylyltransferase (*ant*) gene at this alternative locus is found in strains Epi3002-OL (Figure 11) as well as in *M. goetzii* type strains CCM4927 from Czech Republic (GenBank: MJBI02000001) and *M. goetzii* strains from Ireland (strains DPC7159, DPC7164 and DPC7166 in BioProject PRJNA515496). Compared with the *att*-flanked elements at the *rpsI* locus, similarities were only observed in limited fragments adjacent to the *att*-sites (Figure 11).

The *mecD* gene on $McRI_{mecD}$ elements has so far only been detected in *Macrococcus* species. The presence of highly similar *rpsI*-specific *int* genes and McRI with macrolide resistance genes *mef*(D)-*msr*(F) in *Staphylococcus* species indicates that McRI-related elements could be exchanged between *Macrococcus* and *Staphylococcus*. This indicates that $McRI_{mecD}$ may also have the potential to transfer from *Macrococcus* to *Staphylococcus* species. The recombination system *int-intR-xis* of $McRI_{mecD}$ has been shown to be functional in *Staphylococcus* species and showed flexibility to recombine different *att* targets at the *rpsI* site.[191] Whether the system is also active in recombining *att* sites at the alternative locus present in *M. goetzii*, *M. epidermidis* and *M. bohemicus* species can at the moment only be assumed due to similar ends of the subunits found on both loci, but has not been demonstrated.

mecD on SCCmec

The *mecD* gene was detected on an SCC*mec* structure for the first time in 2021 in *M. bohemicus* strain 19Msa422 ($SCCmecD_{19Msa422}$) isolated from the nose of a healthy calf in Switzerland.[64] This observation was unexpected since until then, *mecD* was always associated with fairly related $McRI_{mecD}$ elements in several *M. caseolyticus* strains, some *M. canis* strains and even in an *M. bohemicus* strain (Figure 9). The $SCCmecD_{19Msa422}$ CI of *M. bohemicus* 19Msa422 was integrated at the *rlmH* site and did not show any relatedness to $McRI_{mecD}$ besides the 4.7 kb segment containing the *mecD* operon (Figure 12). In addition, the *mecD* gene of 19Msa422 displayed 13 SNPs compared with the *mecD* sequences of $McRI_{mecD}$, which were so far all identical. This may suggest that the *mecD* gene of 19Msa422 was not directly acquired from an $McRI_{mecD}$.[64] Interestingly, there was an IS*3* element integrated downstream of the *mecD* gene in

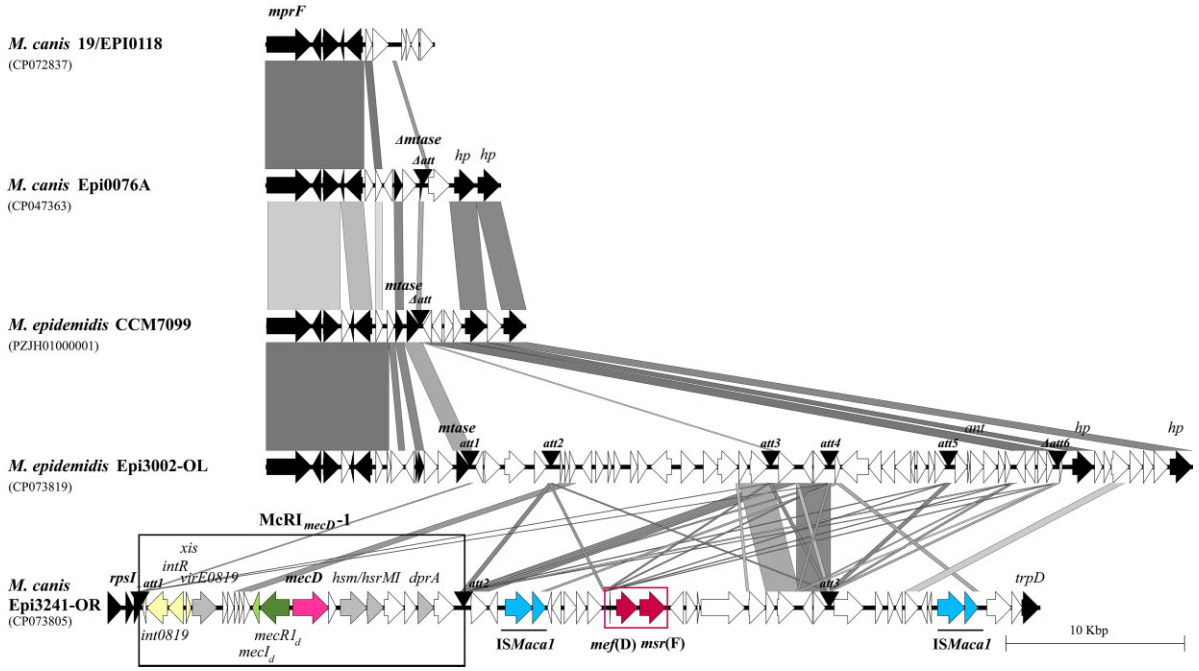


Figure 11. Chromosomal islands integrated downstream of a putative methyltransferase (*mtase*) gene in some *Macrococcus* species. A more conserved lysylphosphatidylglycerol flippase/synthetase (*mprF*) gene is found in the upstream region. The islands are flanked by extended direct repeats (DR) with a core attachment (*att*) site similar to those flanking *mecD*-containing resistance islands (McRI$_{mecD}$) integrated at the 30S ribosomal protein S9 (*rpsI*) gene. The sequences of the *mprF*-downstream region of *M. canis* strains 19/EPI0118 and Epi0076A and *M. epidermidis* strains CCM7099 and Epi3002-OL were compared with the *rpsI*-downstream region of *M. canis* Epi4321-OR using EasyFig software.[204] GenBank accession number are given in parentheses. Gene are shown as arrows and labelled as explained in the legend of Figure 9. Further abbreviations for genes: *hp*, conserved hypothetical proteins; *ant*, putative aminoglycoside 6-adenylyltransferase. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

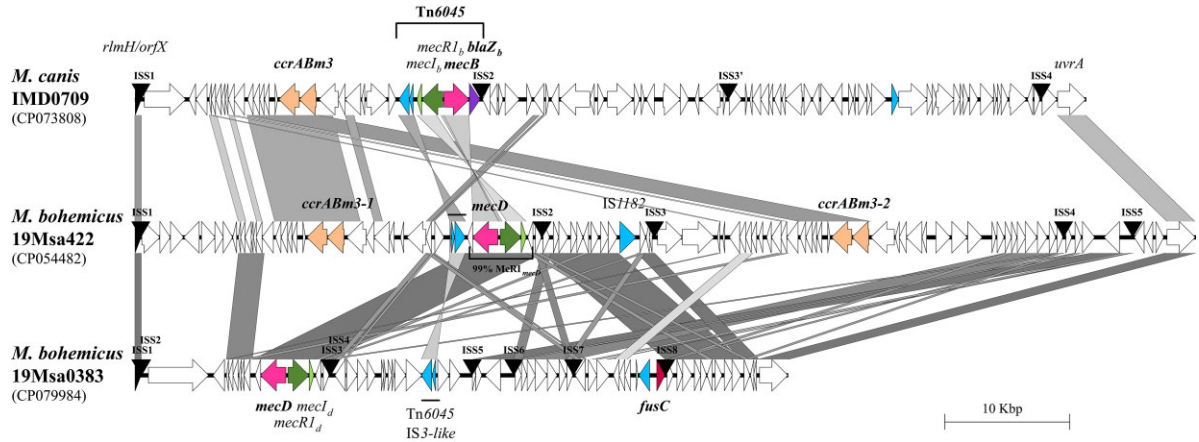


Figure 12. Comparison of the *mecD*-containing SCC*mecD* elements in *Macrococcus bohemicus* 19Msa422 and 19Msa0383 with the SCC*mecB* element of *M. canis* IMD0709. The figure was generated with EasyFig software[204] and sequences downstream of the *rlmH/orfX* gene of the indicated strains (Genbank accession number in parentheses). Gene are shown as arrows and labelled as explained in the legend of Figures 8 and 9. Attachment (*att*) site containing the adapted integration site sequence (ISS) for SCC are indicated (see Figure 8 legend for consensus sequence). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

19Msa422 that shared 84% nt identity with the IS*3* sequence of Tn*6045*, the transposon associated with *mecB* (Figure 12). Whether the *mecD* operon can be mobilized with the IS*3* element similarly to *mecB* in Tn*6045* is not known. Of note, the IS*3* in 19Msa422 was again integrated downstream of the *mecD* gene similarly to IS*30* elements in McRI$_{mecD}$ (Figure 9).

The SCC*mecD*$_{19Msa422}$ CI contained two alleles of *ccrABm3* genes that have been previously found on SCC*mecB* structures

in *M. bohemicus*, *M. canis*, *M. equipercicus* and *M. epidermidis* (Figure 8). The second *ccrAm3* (*ccrAm3-2*) and the two *ccrBm3* genes of 19Msa422 were closely related to *ccrABm3* of *M. bohemicus* DPC7215 and *M. epidermidis* Epi3002-OL (88 to 91% nt identity), while the first *ccrAm3-1* of 19Msa422 was closely related to *ccrAm3* of *M. canis* IMD0709 and *M. bohemicus* H889678/16/1 (93%) (Figure 6). The region containing the *ccrABm3-1* genes in 19Msa422 was also the part of the SCC*mecD* CI that showed high relatedness to SCC*mecB* of *M. canis* IMD0709 (90% over a 5.7 kb segment) (Figure 12). Two other *M. bohemicus* strains that harbour *mecD* on SCC*mecD* elements were recently isolated from pigs in Switzerland. Strain 19Msa0936 had an element nearly identical to SCC*mecD*$_{19Msa422}$ and strain 19Msa0383 carried *mecD* on a ΨSCC*mecD* CI, which was segmented by several ISS and carried the *fusC* gene at the 3′ end of the island (Figure 12).[54] A third *M. bohemicus* strain isolated from a pig in the study of Keller *et al.*[54] (strain 19Msa1083) carried a McRI$_{mecD}$-1 indicating that two different *mecD*-containing elements circulate in porcine *M. bohemicus* strains in Switzerland.

The presence of *mecD* on McRI$_{mecD}$ and SCC*mecD* in different members of the same species illustrates multiplicity of resistance gene mobilization systems in *Macrococcus*. SCC*mec* with *ccrABm3* seem to be prevalent in *Macrococcus* species and contribute to the dissemination of both *mecB* and *mecD* genes.

Conclusions

Clustering based on orthologous genes showed the closest relationship of *Macrococcus* species to *Mammaliicoccus* species. This relatedness had already been reported before based on DNA–DNA hybridization and a similar biochemical phenotype such as a positive oxidase test reaction.[1,2] Our WGS-based analysis showed that species of *Macrococcus* and *Mammaliicoccus* also differ from other *Staphylococcus* in their native collection of PBP. In addition to PBP2, they contain a second bifunctional aPBP that seems to be related to the monofunctional GT SgtA present in all other *Staphylococcus* species. Whether this aPBP is involved in peptidoglycan assembly and able to cooperate with PBP2A proteins is not known and would be of interest to assess. Some cooperation is not unlikely considering that the *mecA* gene encoding PBP2A$_a$ originated from the *Mammaliicoccus* species *M. fleurettii*.[47] The origin of *mecC* is still not known, but nt identity values >80% of the *blaZ*$_c$ gene of the *mecC* operon with that of *bla*$_{MLE}$ gene of *M. lentus* and *bla*$_{SLL}$ of *S. lloydii* indicate a possible common source of these genes. Our analysis also showed that putative native *bla* genes are abundant in certain phylogenetic clusters of *Staphylococcus* species such as the novobiocin-resistant *Saprophyticus* group. They may represent relicts of a β-lactam operon in the *oriC* environs that were lost in most *Staphylococcus* species.

The origin of the *mecB* and *mecD* genes is unknown. They are both found as accessory genes in *Macrococcus* on diverse mobile genetic elements, including transposons, McRI and SCC*mec*. The SCC*mec* elements seemed to have evolved in parallel in *Macrococcus* and *Staphylococcus*. In *Macrococcus*, SCC*mec* do not only carry the *mecB* and *mecD* genes instead of *mecA* and *mecC*, but also *ccrAm*, *ccrBm* and *ccrCm* genes that cluster into separate genus-specific branches and share nt identities of less than 63% with corresponding staphylococcal *ccr* allotypes. The *ccrABm1/2*, *ccrABm3* and *ccrCm1* genes were also found in structures with and without the *mecB* gene in *Macrococcus*. This indicates that methicillin resistance operons are prone to integrate into SCC to form new SCC*mec*. Formation of a new SCC*mec* was observed recently with a *mecD* operon that became associated with *ccrABm3* genes.[64] If IS*3* family transposases found next to the *mecB* and *mecD* operons play a role in this process is unclear. The association of *mec* gene with IS*3* family transposases is strikingly, but the mobility through these transposases should be experimentally confirmed. Tn*6045* can also be found in the resistance region of pMCCL2-like plasmids that are prevalent in *Macrococcus*. An inter-genus transfer of the *mecB* gene to *S. aureus* was observed through such a plasmid.[20]

The *mecD* gene has so far not been detected in *Staphylococcus*. However, the type of island that usually carries *mecD* in *Macrococcus*, the McRI, was also detected in *Staphylococcus* species associated with macrolide resistance genes.[196] In *Staphylococcus*, these islands are also inserted downstream of the *rpsI* gene and contain integrases that share up to 99% nt identity to those of *Macrococcus*. This high sequence identity indicates that *mecD* could also become part of the accessory genome in *Staphylococcus*.

The current distribution of acquired *bla* and *mec* genes in the three genera shows a wide spread of *blaZ* and *mecA* in *Staphylococcus* and *Mammaliicoccus* and the absence of these genes in *Macrococcus*. *Macrococcus* species carry instead *mecB* and *mecD* genes on mobile elements that have the potential to be acquired by *Staphylococcus*. Our knowledge about the distribution of the different *bla* and *mec* gene types will continuously grow with the availability of more data from WGS. Bacteria from the genera *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* have an extraordinary ability to extend their accessory genome to survive in the presence of antibiotics and they seem to share a common reservoir for resistance genes. Nevertheless, how they access and acquire genes from this reservoir is still poorly understood. Biomolecular studies will be necessary to elucidate the molecular mechanisms that control and permit the acquisition of antimicrobial resistance gene in these groups of Gram-positive bacteria.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2, and Figures S1 and S2 are available as Supplementary data at *JAC* Online.

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