

# Macrococcus canis contains recombinogenic methicillin resistance elements and the *mecB* plasmid found in *Staphylococcus aureus*

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**Objectives:** To analyse the genetic context of *mecB* in two *Macrococcus canis* strains from dogs, compare the *mecB*-containing elements with those found in other *Macrococcus* and *Staphylococcus* species, and identify possible mobilizable *mecB* subunits.

**Methods:** Whole genomes of the *M. canis* strains Epi0076A and KM0218 were sequenced using next-generation sequencing technologies. Multiple PCRs and restriction analysis confirmed structures of *mecB*-containing elements, circularization and recombination of *mecB* subunits.

**Results:** Both *M. canis* strains contained novel composite pseudo ( $\Psi$ ) staphylococcal cassette chromosome *mec* (SCC*mec*) elements. Integration site sequences for SCC flanked and subdivided composite  $\Psi$ SCC*mec*<sub>Epi0076A</sub> (69569 bp) into  $\Psi$ SCC1<sub>Epi0076A</sub>– $\Psi$ SCC*mec*<sub>Epi0076A</sub>– $\Psi$ SCC2<sub>Epi0076A</sub> and composite  $\Psi$ SCC*mec*<sub>KM0218</sub> (24554 bp) into  $\Psi$ SCC<sub>KM0218</sub>– $\Psi$ SCC*mec*<sub>KM0218</sub>. Putative  $\gamma$ -haemolysin genes (*hlgB* and *hlgC*) were found at the 3' end of both composite elements.  $\Psi$ SCC*mec*<sub>KM0218</sub> contained a complete *mecB* gene complex (*mecI<sub>m</sub>*–*mecR1<sub>m</sub>*–*mecB*–*blaZ<sub>m</sub>*) downstream of a new IS21-family member (ISMaca1).  $\Psi$ SCC*mec*<sub>Epi0076A</sub> carried a *blaZ<sub>m</sub>*-deleted *mecB* gene complex similar to that reported in '*Macrococcus goetzii*' CCM4927<sup>1</sup>. A second *mecB* gene was found on the 81325 bp MDR plasmid pKM0218 in KM0218. This plasmid contained a complete Tn6045-associated *mecB* gene complex distinct from that of  $\Psi$ SCC*mec*<sub>KM0218</sub>. pKM0218 was almost identical to the *mecB*-containing plasmid recently reported in *Staphylococcus aureus* (overall 99.96% nucleotide identity). Mobilization of *mecB* within an unconventional circularizable structure was observed in Epi0076A as well as chromosomal plasmid insertion via recombination of *mecB* operons in KM0218.

**Conclusions:** Our findings provide evidence of both the continuing evolution of *mecB*-containing elements in macrococci and *M. canis* as a potential source of the *mecB*-containing plasmid found in staphylococci.

## Introduction

The species *Macrococcus canis* was recently recognized as a new member of *Macrococcus*, a genus closely related to *Staphylococcus*.<sup>1</sup> The bacterium is found on the skin of healthy dogs but has also been isolated from infection sites, suggesting that it is a novel canine opportunistic pathogen.<sup>1,2</sup> It is the only *Macrococcus* species that produces complete haemolysis on sheep blood agar and contains the putative haemolysin genes *hlgB* and *hlgC*.<sup>3</sup> Similar to staphylococci, *Macrococcus* species can acquire methicillin resistance by expressing an alternative-penicillin binding protein (PBP), called PBP2a. Whereas PBP2a is encoded by either the *mecA* or *mecC* gene in *Staphylococcus* that has acquired a staphylococcal cassette chromosome *mec* (SCC*mec*) element, *Macrococcus* carries distantly related *mec* homologues, either

*mecB* or *mecD*, on more diverse genetic elements.<sup>4,5</sup> Recently, *mecB* was detected for the first time on a plasmid (pSAWWU4229\_1) in a *Staphylococcus aureus* isolate of human origin, suggesting a possible gene transfer between the two genera.<sup>6</sup>

In *Macrococcus caseolyticus*, the *mecB* gene complex (*mecI<sub>m</sub>*–*mecR1<sub>m</sub>*–*mecB*–*blaZ<sub>m</sub>*) is associated with the presence of Tn6045 transposases and forms part of different mobile genetic elements including plasmids and staphylococcal cassette chromosome *mec* (SCC*mec*)-like elements.<sup>4,7</sup> In '*Macrococcus goetzii*', a *mecB* gene complex without a *blaZ<sub>m</sub>* has been identified on a pseudo ( $\Psi$ ) SCC*mec*.<sup>8</sup> In *M. canis*, the only SCC*mec* element described so far contains a Tn6045-independent complete *mecB* complex that has the ability to form an unconventional circularizable structure

(UCS)<sup>9,10</sup> Recently, *mecB* was detected in several *M. canis* isolates from dogs that differed in their genetic background by MLST and antimicrobial resistance gene profiles.<sup>2</sup> Here, two diverse strains were selected for deeper characterization of their *mecB*-containing elements using WGS. One strain was from a healthy carrier and the other was from a dog with otitis externa. The latter contained antimicrobial resistance genes similar to those found on plasmid pSAWWU4229\_1 and was suspected to contain an MDR plasmid.

## Materials and methods

### Bacterial strains and antimicrobial resistance profiles

*M. canis* strain KM0218, ST12 [*mecB*, *blaZ<sub>m</sub>*, *aac(6')-aph(2'')*, *sat4*, *aph(3')-IIIa*, *erm(B)*, *ant(6)-Ia*, *tet(S)*], originated from a dog with otitis externa, and *M. canis* strain Epi0076A, ST2 [*mecB*, *GrlA* (Ser80Leu)], was isolated from a healthy dog.<sup>2</sup> Antibiotic susceptibility was determined by the measurement of MICs using the microdilution method and Sensititre EUST plates (Thermo Fisher Scientific, TREK Diagnostics Systems, East Grinstead, UK) according to CLSI guidelines M07 and VET01.<sup>11,12</sup> Results were interpreted using the clinical breakpoints of CLSI recommended for *Staphylococcus* spp. for human and dog isolates when available as described.<sup>2</sup> Antibiotic resistance genes and mutations were identified by PCR, sequencing and microarray analysis.<sup>2</sup> *M. canis* strains were routinely cultured under aerobic conditions at 37°C on trypticase soy agar with 5% sheep blood (TSA-S; Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

### DNA extraction, PCR and restriction analysis

DNA was extracted from pure cultures using a peqGOLD bacterial DNA kit (PepLab Biotechnologie GmbH, Jena, Germany). To improve lysis, *M. canis* cells were incubated in solution I of the kit supplemented with 50 µg/mL lysostaphin and 2 mg/mL lysozyme for 20 min at 37°C. PCRs were performed with FIREPol DNA polymerase (Solis BioDyne, Tartu, Estonia) (amplicons <2.5 kb) and GoTaq Long PCR Master Mix (Promega, Madison, WI, USA) for long amplicons (up to 20 kb) according to the manufacturer's instructions. All relevant primers used in this study are listed in Tables S1 and S2 (available as [Supplementary data](#) at JAC Online). Restriction digestion was performed according to conditions suggested by the manufacturers (New England Biolabs, Ipswich, MA, USA and Roche Diagnostics, Rotkreuz, Switzerland) using up to 0.25 volume of PCR product in the reaction. If the manufacturer's instructions were not followed, reaction conditions are indicated in [Supplementary data](#).

### WGS, assembly and annotation

*M. canis* strains KM0218 and Epi0076A were sequenced using Illumina MiSeq technology and Reagent Kit v2 (Illumina, Inc., San Diego, CA, USA). Strain KM0218 was additionally sequenced using the PacBio Sequel system (Pacific Biosciences, Menlo Park, CA, USA). Sequence reads from Illumina were assembled *de novo* with Geneious (version R9.1.5) and generated 67 and 50 contigs for Epi0076A and KM0218, respectively. Contigs were analysed for characteristics of SCCmec elements [*orfX* gene, *mec* and *ccr* gene complexes and the integration site sequences for SCC (ISSs)], transposon Tn6045, and sequences related to the *mecB*-containing plasmid pMCCL2 of *M. caseolyticus*<sup>4</sup> using BLAST (<http://blast.ncbi.nlm.gov/>). Gaps between contigs of interest (composite ΨSCCmec<sub>Epi0076A</sub>: a composite of a 27 kb and a 194 kb contig; plasmid pKM0218: a composite of a 13 kb, a 57 kb and a 16 kb contig) were filled by PCR and Sanger sequencing (ABI Prism 3100 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). The structures of these *mecB*-containing elements (ΨSCCmec<sub>Epi0076A</sub> and pKM0218) were verified by long-range PCRs and restriction analysis (Tables S3 and S4). ORFs were predicted using Prodigal software,<sup>13</sup> and gene annotation was performed manually by BLAST homology. PacBio reads were assembled

*de novo* with canu (v1.6) and provided the complete chromosomal sequence of KM0218. Features present in the *mecB*-containing region (ΨSCCmec<sub>KM0218</sub>) were annotated with prokka (v1.13) and edited manually. The complete chromosome was additionally annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) service.

### Detection of *mecB*-containing recombination products

Excision of *mecB*-containing elements including circular Tn6045<sub>KM0218</sub>, circular subunits of composite ΨSCCmec<sub>Epi0076A</sub>, and UCS<sub>Epi0076A</sub> was analysed by PCR (Table S5). Sanger sequencing confirmed the positive PCR results obtained for UCS<sub>Epi0076A</sub> and the remaining chromosomal segment (ΔUCS<sub>Epi0076A</sub>) (Figure S1). Chromosomal integration and excision of plasmid pKM0218 was also analysed by PCR (Table S6) and further confirmed by restriction endonuclease digestion (Figure S2).

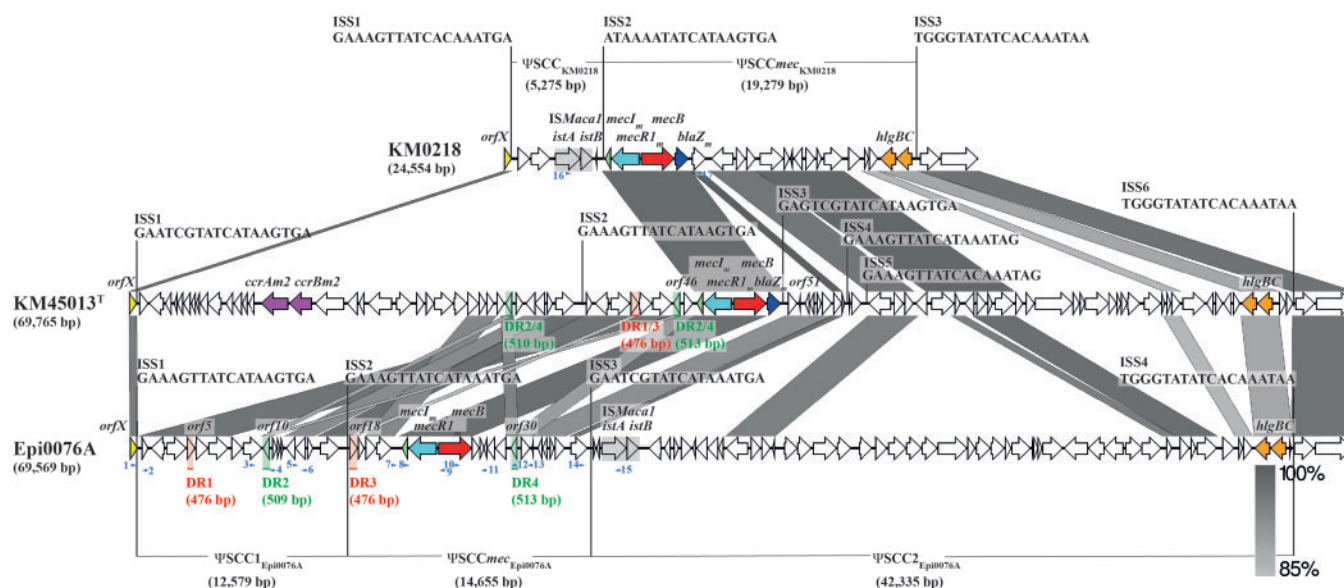
### Nucleotide sequence accession numbers

The nucleotide sequences of the composite ΨSCCmec elements of *M. canis* Epi0076A and *M. canis* KM0218 have been deposited in GenBank under accession numbers MF477835 and MK422159, respectively. The plasmid pKM0218 and the complete chromosomal sequence of KM0218 have been deposited under accession numbers MF477836 and CP035309, respectively.

## Results and discussion

### *M. canis* strain Epi0076A carries *mecB* on a composite ΨSCCmec element

*M. canis* strain Epi0076A was resistant to ciprofloxacin (MIC >4 mg/L), penicillin (MIC >2 mg/L), cefoxitin (MIC >16 mg/L) and erythromycin (MIC >8 mg/L). Quinolone and β-lactam resistance could be attributed to a mutation in the gyrase subunit *GrlA* (Ser80Leu) and the *mecB* gene, respectively. The mechanism of macrolide resistance remains unknown. The *mecB*-carrying element of Epi0076A was found downstream of the *orfX* (*rmlH*) gene in a 69569 bp region that contained four characteristic imperfect repeats containing the ISS (ISS1, ISS2, ISS3 and ISS4) (Figure 1). A *ccr* gene complex was not found either in this region or elsewhere in the genome. Following the criteria of the International Working Group on Staphylococcal Cassette Chromosome Elements (IWG-SCC),<sup>14</sup> the ISS-flanked segments of 12579, 14655 and 42335 bp were named ΨSCC1<sub>Epi0076A</sub>, ΨSCCmec<sub>Epi0076A</sub> and ΨSCC2<sub>Epi0076A</sub> and constituted a composite ΨSCCmec<sub>Epi0076A</sub> element. The ΨSCCmec<sub>Epi0076A</sub> segment contained 20 ORFs and a *mec* gene complex that lacked the *blaZ<sub>m</sub>* gene (*mecI<sub>m</sub>*-*mecR1<sub>m</sub>*-*mecB*) and was not associated with Tn6045. Such an incomplete *mecB* gene complex has thus far only been detected in the '*M. goetzii*' strain CCM4927<sup>T</sup> isolated from human clinical samples.<sup>8</sup> The *mecB* operon of Epi0076A also shared the highest nucleotide identity of 99% with that of '*M. goetzii*' CCM4927<sup>T</sup>. The remaining parts of ΨSCCmec<sub>Epi0076A</sub> and ΨSCC1<sub>Epi0076A</sub> showed the highest sequence similarity to SCCmec of *M. canis* KM45013<sup>T</sup> (Figure 1).<sup>9</sup> However, homologous sequences were arranged differently in Epi0076A, and additional small inserts coding for hypothetical proteins (ORFs 8, 9, 11–13, 19 and 25–29) were present between them. In KM45013<sup>T</sup>, the *mecB* gene complex was flanked by extensive DRs that included *orf46* or *orf51* and were involved in UCS formation (Figure 1).<sup>9</sup> Epi0076A contained only a single copy of such a homologous ORF (*orf31*) downstream of the *mecB* gene complex.



**Figure 1.** Genetic structure of the composite  $\Psi\text{SCCmec}_{\text{KM0218}}$ ,  $\text{SCCmec}_{\text{KM45013}}$  and  $\Psi\text{SCCmec}_{\text{Epi0076A}}$  elements. Comparison was performed with the sequences of *M. canis* strain KM0218 (GenBank accession number MK422159), KM45013<sup>T</sup> (CP021059.1 region: 31942...105740) and Epi0076A (MF477835) using the EasyFig program.<sup>23</sup> Grey areas indicate regions with nucleotide sequence identities between 85% and 100%. The composite elements were inserted in the 3' region of the *orfX* gene (yellow) and subdivided by ISSs. White arrows represent ORFs and are indicated by colour as follows: *mecI<sub>m</sub>* gene, light green; *mecR1<sub>m</sub>* gene, sky blue; *mecB* gene, red; *blaZ<sub>m</sub>* gene, blue; *ccr* genes, purple; and bicomponent  $\gamma$ -haemolysin genes (*hlgB* and *hlgC*), orange. The *ISMaca1* region, comprising *istA* and *istB*, is shaded in grey, and the extended DR regions are shaded in red (DR1 and DR3) and green (DR2 and DR4). Small blue arrowheads represent the primers used for PCR analysis (see Table S1 in the Supplementary data).

Extensive DR sequences were also present in  $\Psi\text{SCC1}_{\text{Epi0076A}}-\Psi\text{SCCmec}_{\text{Epi0076A}}$  within *orf5* (DR1) and *orf18* (DR3) (476 bp overlap with 85% identity) as well as within regions containing *orf10* (DR2) and *orf30* (DR4) (509/513 bp overlap with 88% identity) (Figure 1). Interestingly, *orf10* (DR2), *orf18* (DR3) and *orf30* (DR4) were located next to the insertion sites, suggesting that recombination events could have taken place in Epi0076A and thus diversified the *mecB* element. Furthermore, we observed circular excision of the *mecB*-containing fragment between the extended DRs comprising *orf10* (DR2) and *orf30* (DR4) by PCR (Table S5). UCS formation and its excision from the chromosome were confirmed by sequencing. Recombination and random strand exchange occurred at least within a 270 bp core of the DR sequences (Figure S1). Although we present evidence for the unconventional mobilization of the *mecB* gene in Epi0076A, the location of  $\Psi\text{SCC1}_{\text{Epi0076A}}-\Psi\text{SCCmec}_{\text{Epi0076A}}$  downstream of *orfX* and the flanking ISS suggest a *Ccr*-mediated acquisition of the element followed by the loss of the *ccr* genes. The structure of *SCCmec*(-like) elements in *Macrococcus*<sup>7,9</sup> with *ccr* gene complexes on a separate SCC next to  $\Psi\text{SCCmec}$ , as found in *M. canis* KM45013<sup>T</sup> (Figure 1), would allow both integration of  $\Psi\text{SCCmec}$  only or integration of the composite island with subsequent loss of SCC and *ccr* genes. As expected, in the absence of *ccr* genes, circularization and excision of  $\Psi\text{SCC1}_{\text{Epi0076A}}-\Psi\text{SCCmec}_{\text{Epi0076A}}$  was not detected by PCR (Table S5).

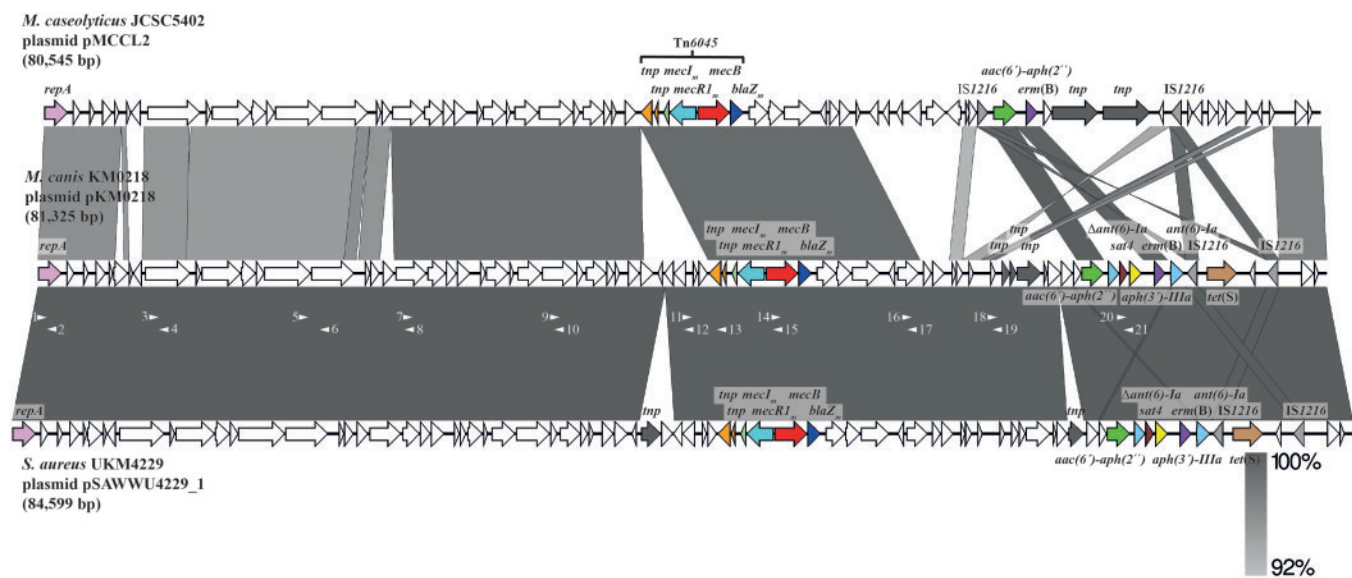
The third segment,  $\Psi\text{SCC2}_{\text{Epi0076A}}$ , contained 50 putative ORFs, including the IS21-family element *ISMaca1* (see below) on the left side, bicomponent  $\gamma$ -haemolysin genes (*hlgB* and *hlgC*) on the right side and multiple hypothetical sugar metabolism gene clusters that are not present in the other *M. canis* genomes but are present in '*M. goetzii*' CCM4927<sup>T</sup> sequences (Figure 1).

### *M. canis* strain KM0218 contains *mecB* on a composite $\Psi\text{SCCmec}$ element and on a plasmid

*M. canis* strain KM0218 contained two *mecB* operons, one on the MDR plasmid pKM0218 and another on a chromosomal  $\Psi\text{SCCmec}_{\text{KM0218}}$  element. PacBio long-read sequencing technology was used to confirm the location of the two *mecB* operons in the genome. PCR and Sanger sequencing further confirmed the presence of two distinct classical *mecB* gene complexes (*mecI<sub>m</sub>*-*mecR1<sub>m</sub>*-*mecB*-*blaZ<sub>m</sub>*) that shared overall 99% nucleotide identities (57 SNPs in a 5073 bp overlap). Careful analysis of the Sanger chromatograms revealed double peaks at alternative bases within the plasmidic and chromosomal *mecR1<sub>m</sub>*-*mecB* region, suggesting that recombination between the homologous sequences had occurred. Evidence for plasmid integration and excision was further demonstrated by restriction analysis of long-range PCR fragments amplified with primers specific to unique regions upstream and downstream of the *mecB* operons (Table S6). Expected products for the closed circular plasmid pKM0218 as well as for the chromosomal  $\Psi\text{SCCmec}_{\text{KM0218}}$  element without and with plasmid pKM0218 integration were obtained (Figure S2).

The *mecB* gene on plasmid pKM0218 was associated with Tn6045 transposases. This *mec* transposon exhibited >98% nucleotide identity to that of pMCL2 and the *SCCmec*-like element of *M. caseolyticus* strain JCSC5402 and strain JCSC7096, respectively.<sup>4,7</sup> Tn6045 is spontaneously excised in JCSC7096,<sup>7</sup> but PCR assays could not detect this phenomenon in KM0218 even though flanking DRs (DR<sub>tnL</sub> and DR<sub>tnR</sub>, AACC(T/G)CACTCTTAACCGATACGCTG(T/A)GG) and a gene for an intact transposase were present (Table S5). The *mecB* gene on the





**Figure 2** Linear genetic structure of the MDR plasmids pMCCL2 of *M. caseolyticus* strain JCSC5402 (GenBank accession number AP009486), pKM0218 of *M. canis* KM0218 (MF477836) and pSAWWU4229\_1 of *S. aureus* UKM4229 (LT799381). White arrows represent ORFs and other genes are indicated by colour as follows: *repA*, pink; transposases associated with Tn6045, orange; *mecI<sub>m</sub>*, light green; *mecB*, sky blue; *blaZ<sub>m</sub>*, blue; *aac(6')-aph(2'')*, green; *ant(6)-Ia*, light steel blue; *sat4*, brick red; *aph(3')-IIIa*, yellow; and *erm(B)*, purple. IS1216 and other transposases are shown in light grey and dark grey, respectively. Small arrowheads represent the primers used for PCR analysis (see Table S2 in the Supplementary data). This genetic map was created using the EasyFig program.<sup>23</sup>

chromosomal  $\Psi$ SCC*mec*<sub>KM0218</sub> element was found downstream of a new IS21-family element, named ISMaca1 (<https://www-is.bio.toul.fr>),<sup>15</sup> which consisted of the DDE transposase *istA* and the IS21 helper gene *istB* and flanking 61 bp inverted repeats (Figure 1). IS21 elements have been reported to play a role in the mobility of the multiresistance gene *cfr* in *Staphylococcus* and *Micrococcus*.<sup>16,17</sup> ISMaca1 was only distantly related to other IS21 members, including the one associated with *cfr* (<45% overall nucleotide identity). ISMaca1 was found not only in *Micrococcus* species but also in *Staphylococcus pseudintermedius* (GenBank accession numbers MWUZ0100009.1 and CP035741.1), another bacterium typically found on the skin of dogs.

The 81325 bp plasmid pKM0218 contained 73 ORFs, including genes for plasmid replication (*repA*), restriction modification, DNA transposition and antimicrobial resistance (Figure 2). In addition to the *mecB* gene, pKM0218 contained a resistance gene cluster including *aac(6')-aph(2'')* (gentamicin-kanamycin-tobramycin resistance),  $\Delta$ *ant(6)-Ia*, *sat4* (streptothricin resistance), *aph(3')-IIIa* (kanamycin resistance), *erm(B)* (macrolide-lincosamide-streptogramin B resistance), a second complete *ant(6)-Ia* (streptomycin resistance) and *tet(S)* (tetracycline resistance). The resistance genes correlated with the increased MICs of  $\beta$ -lactam antibiotics [penicillin (MIC >2 mg/L) and cefoxitin (MIC 16 mg/L)], gentamicin (MIC 4 mg/L), kanamycin (MIC >64 mg/L), erythromycin (MIC >8 mg/L), clindamycin (MIC >4 mg/L), streptomycin (MIC >32 mg/L) and tetracycline (MIC >16 mg/L) measured for strain KM0218. The Tn5405-associated *ant(6)-Ia-sat4-aph(3')-IIIa* resistance cluster is frequently present next to *erm(B)* in Gram-positive genera.<sup>18–20</sup> The IS1282 of the Tn5405 element was, however, not present in pKM0218, but two copies of an IS1216 element were found downstream of *erm(B)* and *ant(6')-Ia*, which form a composite transposon

containing *tet(S)* (Figure 2). IS1216 has also been previously found in association with resistance genes such as *aac(6')-aph(2'')* and *erm(B)* in staphylococcal plasmids.<sup>21,22</sup> Apart from a 4 kb region upstream of Tn6045 and the 23 kb fragment containing the resistance gene cluster, pKM0218 showed >96% overall nucleotide identity with pMCCL2 (Figure 2). However, pKM0218 was almost identical to the *mecB*-containing plasmid pSAWWU4229\_1 recently reported in *S. aureus*.<sup>6</sup> pKM0218 and pSAWWU4229\_1 carried an identical resistance gene cluster and differed mainly in two additional inserts in pSAWWU4229\_1, both associated with transposases (Figure 2). The remaining sequences of the plasmids (81323/81325 bp) varied only in two indels and 32 point mutations. Thirty of the point mutations were located within the *mecR1<sub>m</sub>-mecB* fragment, indicating again that the *mecB* operon of pKM0218 is mutated by recombination with the chromosomal *mecB* operon. The data presented here indicate that *mecB*-containing plasmids originate from a common backbone and have the potential to evolve and acquire additional resistance genes. The data also suggest that a pKM0218-like plasmid was the potential precursor of pSAWWU4229\_1 and provide further evidence for plasmid exchange between *Micrococcus* and *Staphylococcus*.

The chromosomal  $\Psi$ SCC*mec*<sub>KM0218</sub> consisted of a 19279 bp pseudo-element that carried 20 ORFs and showed the highest similarity to sequences of the SCC*mec* region of strain KM45013<sup>T</sup> (Figure 1). The *orfX* downstream region of KM0218 was less complex than that of KM45013<sup>T</sup>. KM0218 contained only two ISS-flanked segments ( $\Psi$ SCC<sub>KM0218</sub> and  $\Psi$ SCC*mec*<sub>KM0218</sub>) spanning a 24554 bp region. In contrast, KM45013<sup>T</sup> displayed five ISS-flanked segments in a 69765 bp region. Overall, the *orfX* downstream region of the three analysed *M. canis* strains showed different SCC/ $\Psi$ SCC segments arranged in tandem (Figure 1). All three

chromosomal *mecB* elements of *M. canis* contained  $\gamma$ -haemolysin genes (*hlgB* and *hlgC*) at the 3' end of the tandem structures. The *hlgB* and *hlgC* genes were situated on a  $\Psi$ SCC in KM45013<sup>T</sup> and Epi0076A and on the  $\Psi$ SCCmec<sub>KM0218</sub> in KM0218. The location of the *hlg* genes suggests that the haemolytic phenotype of *M. canis* is not stably encoded on the core chromosome and may explain the occurrence of non-haemolytic *M. canis* strains.<sup>1</sup>

Altogether, the *mecB* gene has thus far been identified in three different genetic contexts in *M. canis*, namely, on an SCCmec in KM45013<sup>T</sup>,<sup>9</sup> on a  $\Psi$ SCCmec element in KM0218 and Epi0076A (this study) and on a large MDR plasmid in KM0218 (this study). Transposases and especially active recombination machinery might drive the diversification and plasticity of *mecB*-containing structures. Recombination between the chromosomal and plasmidic *mecB*-*mecR*<sub>1m</sub> sequences was observed in KM0218. Different UCSs containing *mecB* were formed in KM45013<sup>T</sup> and Epi0076A that might be involved in spreading the *mecB* gene. The presence of highly similar *mecB*-containing plasmids in *S. aureus* and *M. canis* strongly suggests that the transfer of plasmids occurs between these species and illustrates the importance of *Macrococcus* from dogs as a reservoir of transferable antimicrobial resistance.

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## Transparency declarations

None to declare.

## Supplementary data

Tables S1 to S6 and Figures S1 and S2 are available as [Supplementary data](#) at JAC Online.

## References

- Gobeli Brawand S, Cotting K, Gómez-Sanz E *et al.* *Macrococcus canis* sp. nov., a skin bacterium associated with infections in dogs. *Int J Syst Evol Microbiol* 2017; **67**: 621–6.
- Cotting K, Strauss C, Rodríguez-Campos S *et al.* *Macrococcus canis* and *M. caseolyticus* in dogs: occurrence, genetic diversity and antibiotic resistance. *Vet Dermatol* 2017; **28**: 559–e133.
- Gobeli Brawand S, Rychener L, Schwendener S *et al.* Complete genome sequence of the type strain of *Macrococcus canis*. *Genome Announc* 2018; **6**: e01507–17.
- Baba T, Kuwahara-Arai K, Uchiyama I *et al.* Complete genome sequence of *Macrococcus caseolyticus* strain JCSC5402, [corrected] reflecting the ancestral genome of the human-pathogenic staphylococci. *J Bacteriol* 2009; **191**: 1180–90.
- Schwendener S, Cotting K, Perreten V. Novel methicillin resistance gene *mecD* in clinical *Macrococcus caseolyticus* strains from bovine and canine sources. *Sci Rep* 2017; **7**: 43797.
- Becker K, van Alen S, Idelevich EA *et al.* Plasmid-encoded transferable *mecB*-mediated methicillin resistance in *Staphylococcus aureus*. *Emerg Infect Dis* 2018; **24**: 242–8.
- Tsubakishita S, Kuwahara-Arai K, Baba T *et al.* Staphylococcal cassette chromosome *mec*-like element in *Macrococcus caseolyticus*. *Antimicrob Agents Chemother* 2010; **54**: 1469–75.
- Mašláňová I, Wertheimer Z, Sedláček I *et al.* Description and comparative genomics of *Macrococcus caseolyticus* subsp. *hominis* subsp. nov., *Macrococcus goetzii* sp. nov., *Macrococcus epidermidis* sp. nov., and *Macrococcus bohemicus* sp. nov., novel macrococci from human clinical material with virulence potential and suspected uptake of foreign DNA by natural transformation. *Front Microbiol* 2018; **9**: 1178.
- Gómez-Sanz E, Schwendener S, Thomann A *et al.* First staphylococcal cassette chromosome *mec* containing a *mecB*-carrying gene complex independent of transposon Tn6045 in a *Macrococcus caseolyticus* isolate from a canine infection. *Antimicrob Agents Chemother* 2015; **59**: 4577–83.
- Gomez-Sanz E, Schwendener S, Thomann A. Correction for Gomez-Sanz *et al.* “First staphylococcal cassette chromosome *mec* containing a *mecB*-carrying gene complex independent of transposon Tn6045 in a *Macrococcus caseolyticus* isolate from a canine infection”. *Antimicrob Agents Chemother* 2018; **62**: e01916–18.
- Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Eleventh Edition: Approved Standard M07-11*. CLSI, Wayne, PA, USA, 2018.
- Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals—Fifth Edition. Approved Standard VET01*. CLSI, Wayne, PA, USA, 2018.
- Hyatt D, Chen GL, LoCascio PF *et al.* Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 2010; **11**: 119.
- International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome *mec* (SCCmec): guidelines for reporting novel SCCmec elements. *Antimicrob Agents Chemother* 2009; **53**: 4961–7.
- Siguier P, Perochon J, Lestrade L *et al.* ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 2006; **34**: D32–6.
- Wang Y, Wang Y, Schwarz S *et al.* Detection of the staphylococcal multiresistance gene *cfr* in *Macrococcus caseolyticus* and *Jeotgalicoccus pinipedialis*. *J Antimicrob Chemother* 2012; **67**: 1824–7.
- Kehrenberg C, Aarestrup FM, Schwarz S. IS21-558 insertion sequences are involved in the mobility of the multiresistance gene *cfr*. *Antimicrob Agents Chemother* 2007; **51**: 483–7.
- Boerlin P, Burnens AP, Frey J *et al.* Molecular epidemiology and genetic linkage of macrolide and aminoglycoside resistance in *Staphylococcus intermedius* of canine origin. *Vet Microbiol* 2001; **79**: 155–69.
- Derbise A, Dyke KG, El Solh N. Characterization of a *Staphylococcus aureus* transposon, Tn5405, located within Tn5404 and carrying the aminoglycoside resistance genes, *aphA-3* and *aadE*. *Plasmid* 1996; **35**: 174–88.

- 20** Werner G, Hildebrandt B, Witte W. Linkage of *erm*(B) and *aadE-sat4-aphA-3* in multiple-resistant *Enterococcus faecium* isolates of different ecological origins. *Microb Drug Resist* 2003; **9**: 9–16.
- 21** Li B, Wendlandt S, Yao J *et al.* Detection and new genetic environment of the pleuromutilin-lincosamide-streptogramin A resistance gene *lsa*(E) in methicillin-resistant *Staphylococcus aureus* of swine origin. *J Antimicrob Chemother* 2013; **68**: 1251–5.
- 22** Liu BH, Lei CW, Zhang AY *et al.* Colocation of the multiresistance gene *cfr* and the fosfomycin resistance gene *fosD* on a novel plasmid in *Staphylococcus arlettae* from a chicken farm. *Antimicrob Agents Chemother* 2017; **61**: e01388–17.
- 23** Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. *Bioinformatics* 2011; **27**: 1009–10.