Journal of Global Antimicrobial Resistance 24 (2021) 360–362

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Letter to the Editor

Novel SCC*mec* element containing the methicillin resistance gene *mecD* in *Macrococcus bohemicus*

Sir,

The methicillin resistance gene *mecD* was originally found on chromosomal resistance islands (McRI$_{mecD}$) in *Macrococcus caseolyticus* [1]. So far, three related islands (McRI$_{mecD}$-1 to -3) have been reported for *M. caseolyticus* strains isolated from cattle and bovine milk in Switzerland, England and Wales [2]. They are integrated at the 3' end of the 30S ribosomal protein S9 (*rpsI*) gene by the function of the site-specific integrase (*int*) gene located on the left side of the element. In contrast, *mecA*, *mecB* and *mecC* are associated with staphylococcus cassette chromosome *mec* (SCC*mec*) elements that are site-specifically inserted into the chromosomal *orfX* (*rmlH*) locus and carry cassette chromosome recombinase (*ccr*) genes for element mobilisation [3]. The *mecB* gene is also found as part of the transposon Tn*6045* on plasmids. While *mecA* and *mecC* are found in *Staphylococcus* spp., *mecB* and *mecD* were only detected in *Macrococcus* spp. except on one occasion where a *mecB*-containing plasmid was reported in a methicillin-resistant *Staphylococcus aureus* (MRSA) [3]. Here we discovered that the *mecD* gene can also be carried by a novel composite SCC*mec* element (SCC*mec*$_{19Msa422}$) that is completely unrelated to the McRI$_{mecD}$ elements.

Strain 19Msa422 was isolated on BD BBL™ CHROMagar™ MRSA II from a nasal swab of a healthy calf in Switzerland in 2019. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonics) analysis assigned the strain to *Macrococcus* but failed to identify the species (MBT 7854 MSP Library). Resistance to β-lactams was confirmed by susceptibility testing using the broth microdilution technique and Sensititre EUST plate (Thermo Fisher Scientific) following European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (https://www.eucast.org/). Strain 19Msa422 was resistant to cefoxitin [minimum inhibitory concentration (MIC) >16 mg/L] and penicillin (>2 mg/L) but susceptible to the other antibiotics tested, namely clindamycin (≤0.12 mg/L), tetracycline (≤0.5 mg/L), rifampicin (≤0.016 mg/L), streptomycin (≤4 mg/L), fusidic acid (≤0.5 mg/L), chloramphenicol (≤4 mg/L), kanamycin (≤4 mg/L), quinupristin/dalfopristin (1 mg/L), vancomycin (≤1 mg/L), gentamicin (≤1 mg/L), trimethoprim (≤2 mg/L), erythromycin (≤0.25 mg/L), ciprofloxacin (0.5 mg/L), linezolid (≤1 mg/L), mupirocin (1 mg/L) and sulfamethoxazole (≤64 mg/L) using breakpoints defined for *Staphylococcus* spp. that have previously been used to interpret the resistance phenotype of *Macrococcus* spp. [2]. Strain 19Msa422 carried the *mecD* gene, but PCR-based typing for McRI$_{mecD}$ [2], adapted with a universal primer for macrococcal *rpsI* genes (rpsI-MC-F: 5'-TTAGACTTAAACCAACCATTCGA) that replaced the *M. caseolyticus*-specific *rpsI* primer, could not associate the *mecD* gene with any of the three McRI$_{mecD}$. To properly identify and characterise the strain, the genome of 19Msa422 was sequenced using MinION Oxford Nanopore (ONT) and Illumina HiSeq (2 × 150-bp paired-end) technologies. A hybrid assembly was obtained with Unicycler v.0.4.8 software using pre-filtered ONT reads with a minimum length of 10 kb (Cutadapt v.2.5). Genome features were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Strain 19Msa422 contained a circular 2 358 024-bp chromosome and a 4393-bp plasmid (GenBank accession nos. **CP054482** and **CP054483**). Sequence analysis of the 16S rRNA gene revealed that 19Msa422 belonged to either *Macrococcus bohemicus*, *Macrococcus epidermidis* or *Macrococcus goetzii*, a group of highly related species that cannot be discriminated based on 16S rRNA genes (99.94–100% identity) [4]. Based on whole-genome sequencing analysis, strain 19Msa422 was identified as *M. bohemicus* since it shared a digital DNA:DNA hybridisation (dDDH) value (formula d_4) of 84% with the *M. bohemicus* type strain CCM7100^T and only 53% with *M. epidermidis* CCM7099^T and 51% with *M. goetzii* CCM4927^T as determined by the Type (Strain) Genome Server (TYGS) (https://tygs.dsmz.de). It was also most related to *M. bohemicus* by average nucleotide identity (ANI) with OrthoANIu values of 98.14% to CCM7100^T and 93.64 and 93.12% to CCM7099^T and CCM4927^T, respectively (OrthoANIu; www.ezbiocloud.net).

The *mecD* gene of 19Msa422 was found downstream of the *orfX* gene in a 79 619-bp composite island subdivided by five integration site sequences (ISS) for SCC into one SCC*mec*$_{19Msa422}$, two ΨSCC and one SCC elements (Fig. 1A). The *mecD* gene was preceded by complete regulatory genes *mecR1*$_d$ and *mecI*$_d$ (subscript 'd' for regulators of *mecD*). The 4731-bp fragment containing the *mec* operon was the only part of the element that showed high (99%) nucleotide (nt) identity to the corresponding fragments of McRI$_{mecD}$ (Fig. 1). However, the *mecD* gene of 19Msa422 contained 13 single nucleotide polymorphisms (SNPs) compared with all *mecD* sequences in the NCBI databases (accessed December 2020), suggesting that it was not directly acquired from McRI$_{mecD}$ of *M. caseolyticus*.

The composite SCC*mec*$_{19Msa422}$ structure was associated with two copies of novel *ccrABm3* genes that have recently been identified in a *mecB*-containing SCC*mec* element of *M. bohemicus* strain H889678/16/1 isolated from a dog in Scotland (Fig. 1A) [5]. The two *ccrAm3* genes of 19Msa422 shared 88% nt identity with each other, 93% and 87% with the *ccrAm3* of H889678/16/1, and 52–53% with the other described *ccrAm* genes (*ccrAm1* and *ccrAm2*) in *Macrococcus* spp. (Fig. 1A). The two *ccrBm3* genes of 19Msa422 displayed 93% nt identity between each other, 87% and 88% with the *ccrBm3* of H889678/16/1, and 60–61% with the reported *ccrBm1* and *ccrBm2* of *Macrococcus* spp. Moreover, SCC*mec*$_{19Msa422}$ showed

http://dx.doi.org/10.1016/j.jgar.2021.02.001

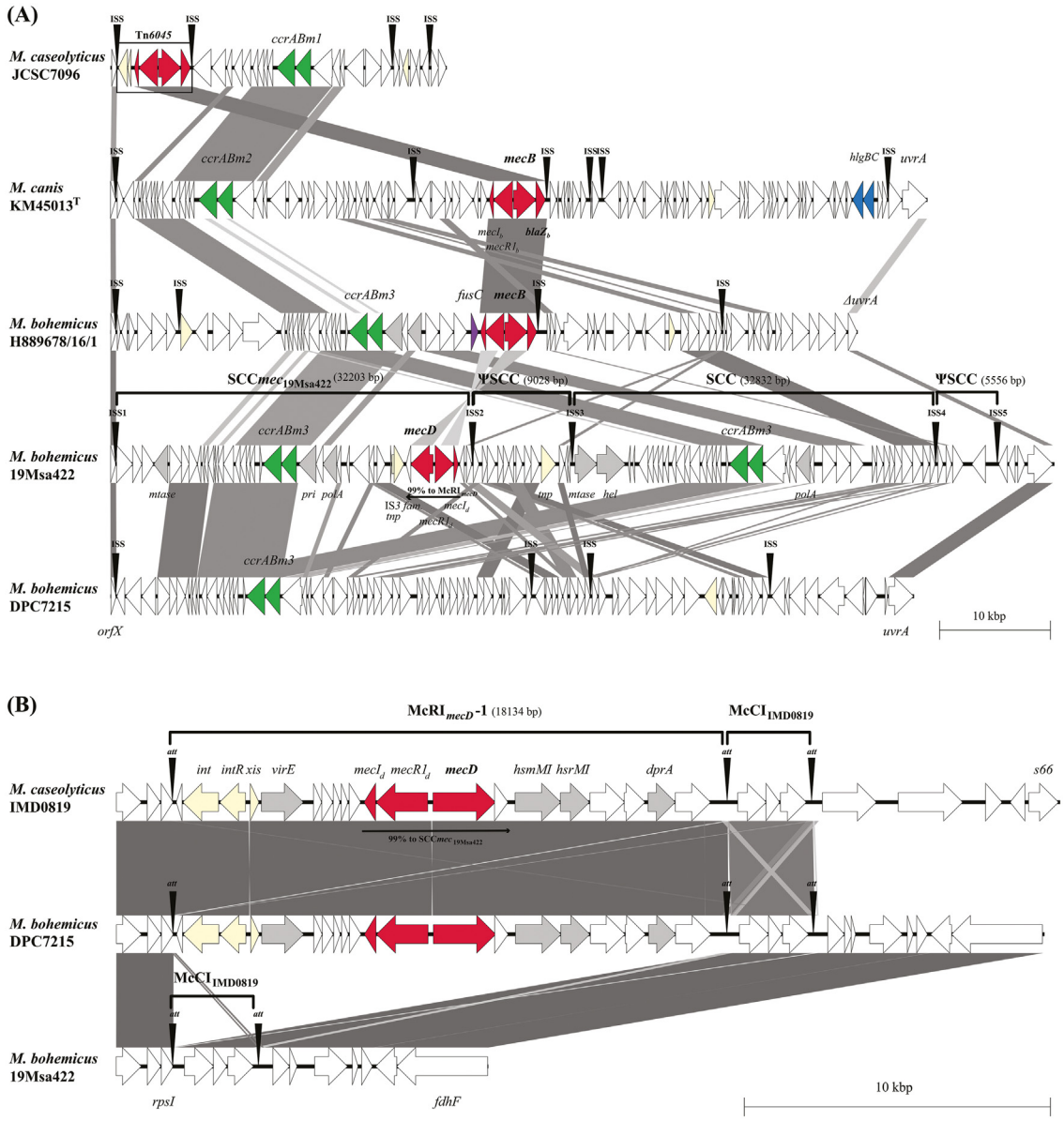

Fig. 1. The *mecD*-containing genetic elements SCC*mec* and McRI$_{mecD}$ in *Macrococcus bohemicus*. (A) Comparison of the composite SCC*mec* element of *M. bohemicus* 19Msa422 (GenBank accession no. **CP054482**, positions 31 904 to 117 158) with the SCC*mec*(-like) of *Macrococcus caseolyticus* JCSC7096 (**AB498756**), *Macrococcus canis* KM45013^{T} (**CP021059**, positions 31 942 to 105 740), *M. bohemicus* H889678/16/1 (**JACEGF010000003**, positions 81 170 to 148 668) and SCC of *M. bohemicus* DPC7215 (**SELR01000003**, positions 46 000 to 118 600). All elements are chromosomally integrated downstream of the *orfX* (*rmlH*) gene. Integration site sequences (ISS) for SCC that subdivide the elements into SCC*mec*, SCC and ΨSCC structures are indicated and have the following sequences for *M. bohemicus* 19Msa422: ISS1, 5'-GAAAGTTATCACAAATGA; ISS2, 5'-GAAAGTTATCATAAATGA; ISS3, 5'-GAAAGTTATCACAAATGA; ISS4, 5'-GAAAGTTATCATAAGTAA; and ISS5, 5'-GAGTCGTATCATAAATGA. Open-reading frames (ORFs) are represented by arrows and colour-coded as follows: red, *mec* operon genes; green, *ccr* genes; yellow, other recombinase and associated genes; blue, putative haemolysin genes (*hlgBC*); purple, fusidic acid resistance gene; grey, gene with functional annotation for primase (*pri*), helicase (*hel*), polymerase (*polA*), methyltransferase (*mtase*), restriction–modification systems (*hsmMI–hsrMI*), putative virulence gene (*virE*) and DNA recombination-mediator protein (*dprA*). Specific genes found in the flanking region of the elements are: *orfX* (*rmlH*), 23S rRNA (pseudouridine^{1915}-N^{3})-methyltransferase *rlmH*; *uvrA*, UvrABC system protein A gene; *rpsI*, 30S ribosomal protein S9 gene; *s66*, peptidase S66 gene; and *fdhF*, formate dehydrogenase subunit alpha gene. (B) Comparison of the McRI$_{mecD}$ of *M. bohemicus* DPC7215 (GenBank accession no. **SELR01000007**, positions 112 048 to 142 395) with the McRI$_{mecD}$-1 of *M. caseolyticus* IMD0819 (**CP021058**, positions 258 729 to 289 587) and the corresponding *rpsI*-downstream region of *M. bohemicus* 19Msa422 (**CP054482**, positions 339 300 to 351 464). The attachment sites (*att*) that delimitate McRI$_{mecD}$ and other small chromosomal island (McCI$_{IMD0819}$) found at the *rpsI* loci are indicated. ORFs are labelled as described above. Figures were generated using Easyfig software. Grey connections indicate regions with between 67% and 100% nucleotide sequence identity in (A) and between 80% and 100% in (B).

overall little relatedness to the *mecB*-containing SCC*mec*(-like) elements described so far in *Macrococcus* spp. (Fig. 1A). Similarity was only observed for the region containing the *ccrABm3* genes, which overlaps with the corresponding region of SCC*mec* of *M. bohemicus* strain H889678/16/1 (5.9-kb segment with 85% nt identity) (Fig. 1A). A SCC not associated with a *mec* gene that contains the *ccrABm3* genes was detected in the NCBI GenBank database in *M. bohemicus* strain DPC7215 isolated from bovine milk in Ireland (GenBank accession no. **SELR01000003**). The elements of 19Msa422 and DPC7215 contained a similar 11.2-kb segment (94% identity) with *ccrABm3* (Fig. 1A). Interestingly, DPC7215 also contained the *mecD* gene but as part of an *rpsI*-integrated resistance island (GenBank accession no. **SELR01000007**) highly similar (3 SNPs in 18 134 bp) to McRI$_{mecD}$-1 of *M. caseolyticus*

IMD0819 (Fig. 1B). At the *rpsI* locus, 19Msa422 carried only the chromosomal island McCI$_{IMD0819}$ that is flanked by the characteristic attachment (*att*) sites and can be mobilised by the *int* of McRI$_{mecD}$ (Fig. 1B) [1].

This study demonstrated that the *mecD* gene is not solely acquirable by *M. caseolyticus* and can be associated with different mobile genetic elements in *M. bohemicus*. While *M. bohemicus* DPC7215 carried *mecD* on an McRI$_{mecD}$-1 highly similar to the one found in *M. caseolyticus*, strain 19Msa422 carried *mecD* on a SCC*mec*, a mobile cassette so far not reported to carry *mecD*. Moreover, the SCC*mec*$_{19Msa422}$ contained novel *ccrABm3* genes that were recently found on both a *mecB*-containing SCC*mec* and on a SCC in *M. bohemicus* strains. These findings illustrate that *mecD* can be mobilised via a novel type of SCC that appears to be prevalent in *M. bohemicus* and contribute to the dissemination of methicillin resistance genes.

Funding

This study was financed by internal funds of the Institute of Veterinary Bacteriology, University of Bern (Bern, Switzerland).

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgments

The authors thank Susanne Rickli, Brigitte Ljungcrantz and Alexandra Collaud for technical assistance.

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Received 24 June 2020

Available online 8 February 2021