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Brief Report

Methicillin-Resistant *Macrococcus bohemicus*
Encoding a Divergent SCCmecB Element

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Abstract: A methicillin-resistant *Macrococcus* isolate from canine otitis, H889678/16/1, was whole-genome sequenced using HiSeq technology to identify the species, antimicrobial resistance determinants and their genomic context. H889678/16/1 belonged to the newly described species *Macrococcus bohemicus*. It encoded mecB within a novel SCCmec element most similar to that of *Macrococcus canis* KM45013T. This SCCmec_H889678/16/1 element also encoded blaZm and fusC, but no other resistance determinants were found in the H889678/16/1 genome. The ccrA and ccrB recombinase genes within SCCmec_H889678/16/1 were distinct from those previously described in staphylococci and macrococci and therefore designated here as ccrAm3 and ccrBm3. Our study represents, to the best of our knowledge, the first description of mecB being encoded by *M. bohemicus* and of methicillin resistance in this species. Furthermore, the SCCmec described here is highly dissimilar to other such elements and encodes novel ccr genes. Our report demonstrates a wider distribution of mecB among *Macrococcus* species and expands the genomic context in which mecB may be found. The potential for dissemination of mec genes from *Macrococcus* to related but more pathogenic *Staphylococcus* species highlights the need to understand the epidemiology of these genes in macrococci.

Keywords: *Macrococcus*; SCCmec; mec genes mecB; methicillin-resistance; veterinary microbiology; antimicrobial resistance

1. Introduction

The genus *Macrococcus* is closely related to *Staphylococcus* and consists of eleven species typically found as commensals in a range of animal hosts [1]. However, there is a growing appreciation that some macrococci may also act as opportunistic pathogens in different animals. For instance, *M. caseolyticus* has been isolated from mastitis in dairy cattle [2,3], canine dermatitis [4], canine otitis [2], an outbreak high-mortality systemic infection in broiler chickens [5], ovine abscesses [6] and cases of embryo mortality in greater white-fronted geese (*Anser albifrons*) [7]. *Macrococcus canis* has also been isolated from a range of canine infections [4] and a small number of isolates of different *Macrococcus* species have come from human clinical samples, suggesting a potential role, albeit infrequently, in human infections as well [8].

As with staphylococcal species, macrococci can acquire methicillin resistance through mec genes which encode an alternative penicillin-binding protein, PBP2a [9]. While PBP2a is encoded by mecA in *Staphylococcus*, and to a lesser extent by mecC [10], methicillin resistance in *Macrococcus* is encoded by the mec gene alleles mecB and mecD. mecB has been reported from *M. caseolyticus* [5,11–13], *M. canis* [4,14] and *Macrococcus goetzii* [8,15]. To date, mecD has only been reported from *M. caseolyticus* [2–4,12].

mecB has been found to be encoded by various genetic elements in *Macrococcus*; different SCCmec elements [11,13], different plasmids [11,14,16] and different ΨSCC elements [8,14]. Importantly,
there has been a single report of a human isolate of *Staphylococcus aureus* encoding mecB [17] on a plasmid almost identical to a *M. canis* plasmid also encoding mecB [14]. This raises the strong possibility of the exchange of methicillin resistance determinates between these two genera and highlights the need to better understand the epidemiology and genomics of mec genes among *Macrococcus*.

*Macrococcus bohemicus* was first described in 2018 on the basis of a single isolate cultured from a human traumatic knee wound sample collected in 2003 in the Czech Republic [8]. Subsequently, a second *M. bohemicus* isolate coming from bovine milk in the Republic of Ireland and collected in 2017 has been described [18]. Both isolates have been genome sequenced.

To the best of our knowledge, no mec gene or methicillin resistance has been described in *M. bohemicus*, and herein we describe the first example of such, a canine otitis isolate H889678/16/1 encoding mecB within a distant SCCmec element and carrying novel ccrA and ccrB alleles, designated here as ccrAm3 and ccrBm3.

2. Results and Discussion

2.1. Isolation and Whole-Genome Sequencing of Methicillin-Resistant *M. bohemicus* H889678/16/1

H889678/16/1 was isolated from mixed growth cultured from a canine otitis sample collected from a cocker spaniel in Scotland in 2016. Also isolated were an *Enterobacter* sp., yeast (likely *Malassezia pachydermatis*) and *Aerococcus viridans*. H889678/16/1 was identified phenotypically as a presumed *Macrococcus* sp. and considered to be methicillin resistant on the basis of resistance to oxacillin when tested by Vitek2. H889678/16/1 was also resistant to benzylpenicillin and fusidic acid but susceptible to all the other antimicrobials tested. H889678/16/1 was whole-genome sequenced using HiSeq technology to resolve its identity to the species level and to determine the genetic basis for methicillin resistance. The resultant assembled draft genome consisted of 49 contigs totalling 2,497,285 bp in length, with a GC% content of 33.89%. The average genome coverage was 42.9-fold. H889678/16/1 was identified as belonging to *M. bohemicus* using the Type Strain Genome Server [19] and showed a dDDH value of 83.3 against *M. bohemicus* type strain CCM 7100.

2.2. *M. bohemicus* H889678/16/1 Encodes mecB within a Novel SCCmec Element

ResFinder analysis of the H889678/16/1 genome showed that it encoded mecB and fusC. Further analysis showed that mecB was within a mec gene complex with blaZm, but no other antimicrobial resistance determinates were apparent in the genome. All three genes, mecB, blaZm and fusC, were located on a single contig 265 kbp in size (JACEGF0000000003) and encoded within a SCCmec element in the orfX/rlmH region. The insertion of the element into the orfX/rlmH region has to date been a reliable indication of a chromosomal location for SCCmec elements. Additional evidence for this being the case in H889678/16/1 are the large size of the SCCmec-containing contig, the absence of any plasmid features in this contig as detected by PlasmidFinder and the presence of numerous housekeeping genes on this contig, including those likely to be essential for viability, such as gyrA and gyrB. This SCCmec element, designated as SCCmecH889678/16/1, was most similar to, but distinct from, the mecB-encoding SCCmec of *M. canis* KM45013 [Figure 1] [13]. SCCmecH889678/16/1 is also distinct from the SCCmec element found in the *M. bohemicus* type strain CCM7100 that lacks a mec gene complex and ccr genes (Figure 1) [8]. The only other described *M. bohemicus* isolate, DPC 7215 [18], also lacks any mec gene and possesses an orfX/rlmH region distinct from that of H889678/16/1 (data not shown). SCCmecH889678/16/1 is 57,612 bp in size, as defined by the length from the two outermost direct repeats inclusive (Figure 1). The mec gene complex of mecL, mecR1, mecB and blaZm in SCCmecH889678/16/1 was highly conserved with those of SCCmecKM45013, with each gene pair sharing 98–99% nucleotide identity. The other large region conserved between these two elements is a series of eight genes located near the ccr genes which encode the DNA repair protein RadC, a helix-turn-helix domain protein and six hypothetical proteins. This region of similarity extends into portions of the two flanking genes and, in the apparent absence of adjacent mobile genetic elements, may indicate their acquisition by
homologous recombination. SCCmec<sub>H889678/16/1</sub> also contained blocks of similarity with the SCC element found in the <i>M. bohemicus</i> CCM7100<sup>T</sup>, indicating a mosaic structure likely arising through multiple horizontal genetic transfer events.

2.3. SCCmec<sub>H889678/16/1</sub> Encodes Novel Recombinase Genes <i>ccrAm3</i> and <i>ccrBm3</i>

A notable feature of the SCCmec<sub>H889678/16/1</sub> is the relative lack of similarity shown by the <i>ccr</i> genes with those of SCCmec<sub>KM45013</sub> (Figure 1), with the <i>ccr</i> genes being responsible for the excision and integration of SCCmec elements in and out of the genome. Indeed, SCCmec<sub>H889678/16/1</sub> <i>ccr</i> genes share limited nucleotide identity with known <i>Macrococcus</i> and <i>Staphylococcus</i> <i>ccr</i> genes, 43.5–63.0% in the case of <i>ccrB</i> and 41.5–54.4% in the case of <i>ccrA</i>. Following precedent [11,13], we propose the designation of the SCCmec<sub>H889678/16/1</sub> <i>ccr</i> genes as <i>ccrAm3</i> and <i>ccrBm3</i>. A phylogenetic analysis of <i>ccr</i> genes highlights the distinctness of <i>ccrAm3</i> and <i>ccrBm3</i> (Figure 2). Whilst <i>ccrAm3</i> and <i>ccrBm3</i> belong to their respective <i>Macrococcus</i> <i>ccr</i> gene branches, they are distant to these counterparts, with <i>ccrAm3</i> and <i>ccrBm3</i> closest to the ancestral forms of the macrococcal <i>ccr</i> genes.

In conclusion, we report the first example, to the best of our knowledge, of methicillin resistance and <i>mecB</i> in the newly described species <i>M. bohemicus</i>. This is only the third description of a <i>M. bohemicus</i> isolate and appears to be the first isolation from a dog. The <i>mecB</i> gene in this isolate is encoded within a distinct SCC<sub>mec</sub> element containing novel <i>ccr</i> alleles. This expands our knowledge on the distribution and genomic context of methicillin resistance determinates among <i>Macrococcus</i> which themselves are opportunistic pathogens, but which may also act as a genetic reservoir for the more pathogenic and related <i>Staphylococcus</i>.
Figure 1. Schematic comparison of SCCmec and ΨSCC elements in *M. canis* KM45013^T* (top), *M. bohemicus* H889678/16/1 (centre) and *M. bohemicus* CCM7100^T* (below). The sequences used are as follows: *M. canis* KM45013^T* CP021059.1 region: 31942 … 105740; *M. bohemicus* H889678/16/1 JACEGF00000003.1 region: 81206 … 141735; *M. bohemicus* CCM7100^T* PZJG01000007.1 region: 14792 … 52744. Regions of homology are represented by bands connecting the genomes sequences, with the percentage identity key shown on the key. Red denotes normal sequence alignment (N); blue denotes inverted sequence alignment (I). Selected features are coloured and labelled. Direct repeats are indicated by blue triangles. Colouring of genes denotes the following: *orfX/rmlH*, black; *mec* gene complex, blue; *ccr* genes, yellow; mobile elements, gold; genes putatively involved in heavy metal resistance, brown.
Antimicrobial sensitivity testing was performed using Vitek2 (bioMérieux, Basingstoke, UK) following the manufacturer’s instructions. Using the Vitek2 AST-P634 card, the antimicrobials tested were as follows: cefoxitin (screen), benzylpenicillin, oxacillin, gentamicin, ciprofloxacin, inducible clindamycin resistance, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, tetracycline, nitrofurantoin, fusidic acid, mupirocin, chloramphenicol, rifampicin and trimethoprim.
with interpretation performed using The Clinical and Laboratory Standards Institute criterion (2015) for coagulase-negative staphylococci.

3.2. Whole-Genome Sequencing

Whole-genome sequencing was performed by Microbes NG (University of Birmingham, Birmingham UK) using Illumina technology with 2 × 250 bp paired-end reads. Genomic DNA was purified with solid-phase reversible immobilization beads, and libraries were prepared using Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer’s protocol with the following modifications: two nanograms of DNA instead of one were used as input, and PCR elongation time was increased to 1 min from 30 seconds. Reads were trimmed using Trimmomatic version 0.30 [20], using a sliding window quality cut-off of 15. Genome assembly was done de novo using SPAdes, version 3.7, with default parameters for 250 bp Illumina reads [21] and annotated by the NCBI Prokaryotic Genome Annotation Pipeline [22].

3.3. Genome Analysis

Genome-based identification was done using the Type Strain Genome Server [17] (https://tygs.dsmz.de/). Acquired resistance genes were identified using ResFinder-3.1 [23] employing the thresholds of 60% for percentage identity and minimum length of 60%. Visual inspection and formatting of the genome for Figure 1 was performed using Artemis 17.0.1 [24]. Schematic comparison of mecB regions was performed using EasyFig 2.2.5 [25]. Plasmid features were searched for using PlasmidFinder 2.0 [26] with the thresholds of 60% for percentage identity and minimum length of 60%. The phylogenetic relationship of ccr genes was assessed in MEGA X [27] using a single representative of each type (nucleotide accession numbers provided in Figure 2), aligning the nucleotide sequences with MUSCLE and producing a maximum-likelihood tree using a general time-reversible (GTR) model. The final tree was produced using the Interactive Tree of Life (iTOL) [28].

4. Nucleotide Accession Numbers

The whole-genome sequencing reads and annotated assembly of M. bohemicus H889678/16/1 are available under the GenBank accession numbers SRR12266687 and JACEGF000000000, respectively.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mazhar, S.; Hill, C.; McAuliffe, O. The genus Macroccocus: An insight into its biology, evolution, and relationship with Staphylococcus. Adv. Appl. Microbiol. 2018, 105, 1–50. [PubMed]
2. Schwendener, S.; Cotting, K.; Perreten, V. Novel methicillin resistance gene mecD in clinical Macroccocus caseolyticus strains from bovine and canine sources. Sci. Rep. 2017, 7, 43797. [CrossRef] [PubMed]
3. Schwendener, S.; Nigg, A.; Collaud, A.; Overesch, G.; Kittl, S.; Phumthanakorn, N.; Perreten, V. Typing of mecD Islands in genetically diverse methicillin-resistant Macroccocus caseolyticus strains from cattle. Appl. Environ. Microbiol. 2019, 85, e01496-19. [CrossRef] [PubMed]
4. Cotting, K.; Strauss, C.; Rodriguez-Campos, S.; Rostaher, A.; Fischer, N.M.; Roosje, P.J.; Favrot, C.; Perreten, V. Macroccocus canis and M. caseolyticus in dogs: Occurrence, genetic diversity and antibiotic resistance. Vet. Dermatol. 2017, 28, 559-e133. [PubMed]
5. Li, G.; Du, X.; Zhou, D.; Li, C.; Huang, L.; Zheng, Q.; Cheng, Z. Emergence of pathogenic and multiple-antibiotic-resistant Macrococcus caseolyticus in commercial broiler chickens. *Transbound. Emerg. Dis.* 2018, 65, 1605–1614. [CrossRef] [PubMed]

6. De la Fuente, R.; Suarez, G.; Ruiz Santa Quiteria, J.A.; Meugnier, H.; Bes, M.; Freney, J.; Fleurette, J. Identification of coagulase negative staphylococci isolated from lambs as *Staphylococcus caseolyticus*. *Comp. Immunol. Microbiol. Infect. Dis.* 1992, 15, 47–52. [CrossRef]

7. Hansen, C.M.; Meixell, B.W.; Van Hemert, C.; Hare, R.F.; Hue, T.; Præstegaard, T.; Skov, E.; Tvede, M.; Wilse, V.; Christiansen, H. Novel DNA from dairy cattle in England and Wales. *Antimicrob. Agents Chemother.* 2012, 56, 3284–3289. [CrossRef] [PubMed]

8. Baba, T.; Kuwahara-Arai, K.; Uchiyama, I.; Takeuchi, F.; Ito, T.; Hiramatsu, K. Complete genome sequence of *Macrococcus caseolyticus* strain JSCS5402, reflecting the ancestral genome of the human-pathogenic *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 2015, 59, 4577–4583. [CrossRef] [PubMed]

9. MacFadyen, A.C.; Fisher, E.A.; Costa, B.; Cullen, C.; Paterson, G.K. Genome analysis of methicillin resistance in *Macrococcus caseolyticus* from dairy cattle in England and Wales. *Microb. Genom.* 2018, 4, e000191. [CrossRef] [PubMed]

10. Paterson, G.K.; Harrison, E.M.; Holmes, M.A. The emergence of mecC methicillin-resistant *Staphylococcus aureus*. *Trends Microbiol.* 2014, 22, 42–47. [CrossRef]

11. Tsubakihita, S.; Kuwahara-Arai, K.; Baba, T.; Hiramatsu, K. Staphylococcal Cassette Chromosome mec-like element in *Macrococcus caseolyticus*. *Antimicrob. Agents Chemother.* 2010, 54, 1469–1475. [CrossRef]

12. MacFadyen, A.C.; Fisher, E.A.; Costa, B.; Cullen, C.; Paterson, G.K. Genome analysis of methicillin resistance in *Macrococcus caseolyticus* from dairy cattle in England and Wales. *Microb. Genom.* 2018, 4, e000191. [CrossRef] [PubMed]

13. Gómez-Sanz, E.; Schwendener, S.; Thomann, A.; Gobeli Brawand, S.; Perreten, V. 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, 4577–4583. [CrossRef] [PubMed]

14. Chanchaithong, P.; Perreten, V.; Schwendener, S. *Macrococcus canis* contains recombinogenic methicillin resistance elements and the *mecB* plasmid found in *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 2019, 74, 2531–2536. [CrossRef] [PubMed]

15. Schlattmann, A.; von Lützau, K.; Kaspar, U.; Becker, K. The porcine nasal microbiota with particular attention to livestock-associated methicillin-resistant *Staphylococcus aureus* in Germany—a culturomic approach. *Microorganisms* 2020, 8, 514. [CrossRef]

16. Baba, T.; Kuwahara-Arai, K.; Uchiyama, I.; Takeuchi, F.; Ito, T.; Hiramatsu, K. Complete genome sequence of *Macrococcus caseolyticus* strain JSCS5402, reflecting the ancestral genome of the human-pathogenic staphylococci. *J. Bacteriol.* 2009, 191, 1180–1190. [CrossRef]

17. Becker, K.; van Alen, S.; Idelevich, E.A.; Schleimer, N.; Seggewiß, J.; Mellmann, A.; Kaspar, U.; Peters, G. Plasmid-encoded transferable *mecB*-mediated methicillin resistance in *Staphylococcus aureus*. *Emerg. Infect. Dis.* 2018, 24, 242–248. [CrossRef]

18. Mazhar, S.; Altermann, E.; Hill, C.; McAuliffe, O. Draft genome sequences of *Macrococcus caseolyticus*, *Macrococcus canis*, *Macrococcus bohemicus*, and *Macrococcus goetzii*. *Microbiol. Resour. Announc.* 2019, 8, e00344-19. [CrossRef]

19. Meier-Kolthoff, J.P.; Göker, M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat. Commun.* 2019, 10, 2182. [CrossRef]

20. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 2014, 30, 2114–2120. [CrossRef]

21. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Pyshulenski, A.D.; et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 2012, 19, 455–477. [CrossRef] [PubMed]

22. Seemann, T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* 2014, 30, 2068–2069. [CrossRef] [PubMed]

23. Zankari, E.; Hasman, H.; Cosentino, S.; Vestergaard, M.; Rasmussen, S.; Lund, O.; Aarestrup, F.M.; Larsen, M.V. Identification of acquired antimicrobial resistance genes. *J. Antimicrob. Chemother.* 2012, 67, 2640–2644. [CrossRef] [PubMed]
24. Rutherford, K.; Parkhill, J.; Crook, J.; Horsnell, T.; Rice, P.; Rajandream, M.-A.; Barrell, B. Artemis: Sequence visualization and annotation. *Bioinformatics* 2000, 16, 944–945. [CrossRef]

25. Sullivan, M.J.; Petty, N.K.; Beatson, S.A. Easyfig: A genome comparison visualizer. *Bioinformatics* 2011, 27, 1009–1010. [CrossRef]

26. Carattoli, A.; Zankari, E.; García-Fernández, A.; Voldby Larsen, M.; Lund, O.; Villa, L.; Møller Aarestrup, F.; Hasman, H. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob. Agents Chemother.* 2014, 58, 3895–3903. [CrossRef]

27. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics Analysis across computing platforms. *Mol. Biol. Evol.* 2018, 35, 1547–1549. [CrossRef]

28. Letunic, I.; Bork, P. Interactive Tree Of Life (iTOl) v4: Recent updates and new developments. *Nucleic Acids Res.* 2019, 47, W256–W259. [CrossRef]

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