Infection, Genetics and Evolution 96 (2021) 105125

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Short communication

Methicillin-resistant *Macrococcus canis* in a human wound

Géraldine Jost [a], Sybille Schwendener [b], Nadia Liassine [a], Vincent Perreten [b,*]

[a] Dianalabs, Geneva, Switzerland
[b] Division of Molecular Epidemiology & Infectious Diseases, Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland

ARTICLE INFO

Keywords:
WGS
Antibiotic resistance
SCC*mec* element
Opportunistic pathogen
Dog
Human

ABSTRACT

A hemolytic *Macrococcus canis* strain (LI021) was isolated for the first time from a human skin infection. The complete genome of LI021 consisting of a 2,216,765-bp circular chromosome was obtained by *de novo* hybrid assembly of Illumina and Oxford Nanopore technology reads. Strain LI021 belonged to the new sequence type ST75 and was resistant to β-lactam antibiotics due to the presence of a methicillin resistance gene *mecB*. The *mecB* gene as well as putative hemolysin genes *hlgB* and *hlgC* were located on a novel composite pseudo (Ψ) SCC*mec* island. These findings show that a methicillin-resistant *M. canis* may be associated with human infection and indicate that this bacterium should be considered by human diagnostic laboratories.

Macrococcus canis is a new species first described from an infection site of a dog in 2017 (Gobeli Brawand et al., 2017). The genus *Macrococcus* is most closely related to *Staphylococcus* (Baba et al., 2009; Mazhar et al., 2019; Kloos et al., 1998). Members of both genera are widespread commensals of animals, some of them acting as opportunistic pathogens requiring antimicrobial treatment (Heilmann et al., 2019; Natsis and Cohen, 2018; Li et al., 2018, Cotting et al., 2017). So far, *M. canis* infections like otitis, rhinitis, mastitis, and dermatitis have only been reported in dogs, and some of the strains were resistant to antibiotics including β-lactams (Cotting et al., 2017). β-lactam resistance in *M. canis* was associated with the *mecB* gene, a related *mecA* homolog found on both plasmid and staphylococcal cassette chromosome *mec* (SCC*mec*) (Gómez-Sanz et al., 2015; Chanchaithong et al., 2019).

Here, we report the isolation and characterization of *M. canis* from a human specimen. A 52-year-old immunocompromised female outpatient, with a renal transplant and on dialysis, presented cutaneous maculopapular and impetigo lesions on the whole body. The patient owned two dogs. In dogs, *M. canis* may reside on the surface of healthy skin, but can also develop into dermatitis. Skin infections associated with *M. canis* are not well documented for dogs, and the lesions seen in the human patient have so far not been reported in animals. This suggests that the particular health conditions of this patient predisposed the human skin to a severe infection. A superficial swab (eSwab) was performed from face and arms lesions. A polymicrobial growth with colonies mainly showing β-hemolysis was obtained on sheep blood agar plate (bioMérieux, Marcy l'Etoile, France) after incubation at 37 °C under a 5% CO_2-enriched atmosphere for 48 h. Each colony exhibiting a different morphology on the plates was selected for identification by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) analysis (Bruker Daltonics, Bremen, Germany; MBT compass version 4.1 software and MBT 7854 MSP Library). MALDI-TOF MS identified *Acinetobacter ursingii* and *Enterococcus faecalis* within the polymicrobial growth, but not the predominant β-hemolytic colonies. They were identified as *M. canis* by 16S rRNA gene sequencing as this microorganism did not belong to the MBT 7854 MSP Library of the MALTI-TOF MS. Of note, the updated MBT 8468 MSP library which now contains reference spectra for *M. canis* confirmed the identification. Antimicrobial susceptibility testing was performed by microdilution using EUST sensititre plate (ThermoFisher, Massachusetts, United States) and following the CLSI standards. Interpretation of MIC was tentatively performed using resistance breakpoints recommended for *Staphylococcus* spp. from CLSI, except for fusidic acid for which breakpoints of EUCAST were used (Cotting et al., 2017). No breakpoints were available for streptomycin, tiamulin, and mupirocin. The *M. canis* strain LI021 was resistant to penicillin (MIC, >2 mg/L) and cefoxitin (>16 mg/L) and susceptible to all other antibiotics tested (chloramphenicol (≤4 mg/L), erythromycin (0.5 mg/L), ciprofloxacin (≤0.25 mg/L), kanamycin (≤4 mg/L), tetracycline (≤0.5 mg/L), clindamycin (≤0.12 mg/L), rifampicin (≤0.016 mg/L), fusidic acid (≤0.5 mg/L), gentamicin (≤1 mg/L), linezolid (≤1 mg/L), mupirocin (1 mg/L), tiamulin (>4 mg/L), streptomycin (≤4 mg/L), sulfamethoxazole (≤64 mg/L), trimethoprim (8 mg/L), quinupristin/dalfopristin (1 mg/L) and vancomycin (≤1 mg/L).

Whole genome sequencing of *M. canis* strain LI021 was performed

* Corresponding author.
E-mail address: vincent.perreten@vetsuisse.unibe.ch (V. Perreten).

https://doi.org/10.1016/j.meegid.2021.105125
Received 21 May 2021; Received in revised form 1 September 2021; Accepted 22 October 2021
Available online 26 October 2021

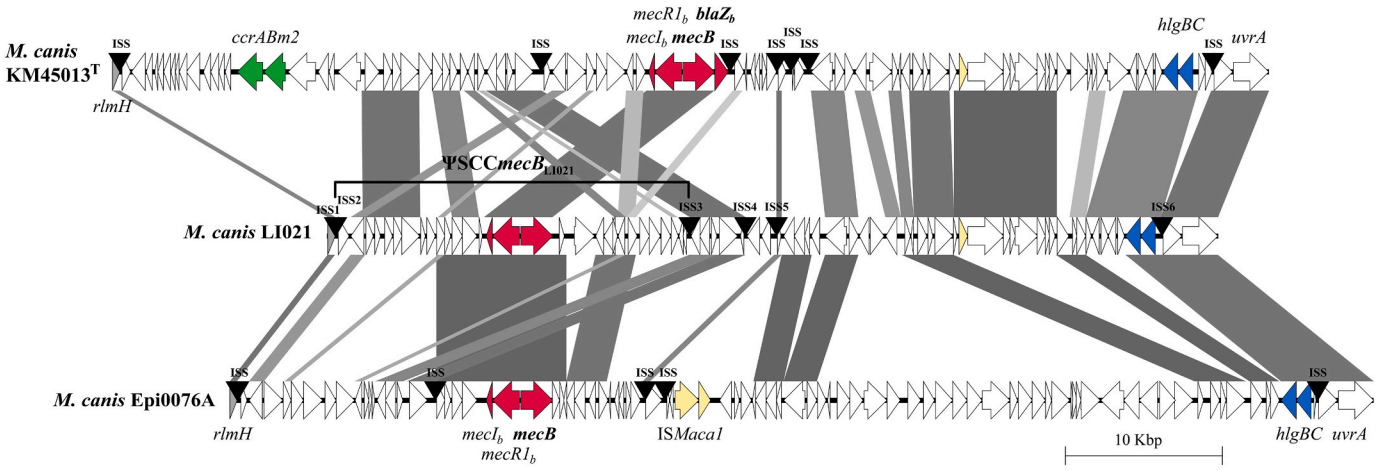

Fig. 1. Structure of SCC*mecB* elements in *Macrococcus canis*. The composite elements were integrated at the 3′ end of the chromosomal *rlmH* gene (gray) and subdivided by integration site sequence (ISS) for SCC. The comparison was generated with Easyfig software (Sullivan et al., 2011) using sequences of *M. canis* strains KM45013^{T} (GenBank: CP021059), LI021 (CP046590, position 1193986 to 1250870), and Epi0076A (CP047363). The ISS of LI021 have the following sequences: ISS1, 5′-GAAAGTTATCATAAGTGA; ISS2, 5′-CAAAGTTATCATAAATGA; ISS3, 5′-GAATCGTATCATAAGTGA; ISS4, 5′-GAGTCGTATCATAAATGA; ISS5, 5′-GAAAGTTACCACAAATAG; and ISS6, 5′-TGGGTATATCACAAATAA. Genes are represented by arrows and color-coded: red, *mec* operon genes; green, *ccr* genes; yellow, other recombinase genes; blue, putative hemolysin genes (*hlgBC*); uncolored, UvrABC system protein A gene (*uvrA*). Gray connections between the structures indicate regions with between 83% and 100% nucleotide sequence identity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

enabling identification of the methicillin resistance gene and SCC element, and multilocus sequence typing (MLST) (http://pubmlst.org/mcanis/). The complete 2,216,765 bp circular chromosome of LI021 (GenBank accession number CP046590) was generated by *de novo* assembling of Illumina NovaSeq reads and MinION Oxford Nanopore reads using Unicycler software (v0.4.4) (Wick et al., 2017). SCC*mec* features annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) service were manually edited in the genome. Strain LI021 belonged to a currently so far unique sequence type ST75 and carried the *mecB* gene in a novel 22,636 bp pseudo (Ψ) SCC*mec* element (ΨSCC*mecB*$_{LI021}$) downstream of the *orfX* (*rmlH*) gene (Fig. 1). The *mecB* gene was located in an operon that contains the regulators *mecR1*$_{m}$ and *mecI*$_{m}$ but lacks the *blaZ*$_{m}$ gene. Similar *blaZ*$_{m}$-deleted *mecB* gene complexes have been reported in *M. goetzii* CCM4927^{T} and *M. canis* Epi0076A (Chanchaithong et al., 2019). The ΨSCC*mecB*$_{LI021}$ shares high nucleotide identity (99%) to ΨSCC*mec* of Epi0076A in the 8290-bp region containing the *mecB* gene (Fig. 1). Downstream of ΨSCC*mecB*$_{LI021}$, three additional integration site sequences for SCC (ISS) segmented the region into ΨSCC subunits of 3553-bp (ISS3 to ISS4), 2000-bp (ISS4 to ISS5) and 24,592-bp (ISS5 to ISS6). At the 3′ end of this array, LI021 carries putative γ-hemolysin genes (*hlgB* and *hlgC*) (Fig. 1), which are likely to be responsible for the β-hemolysis observed on the blood plates. Overall, the composite ΨSCC*mec* island of LI021 shares several fragments with the composite SCC*mecB* element of *M. canis* KM45013^{T} suggesting relatedness and high plasticity in this region.

The skin infection in a human patient described here was associated with *M.canis* which probably originated from a dog. Unfortunately, no samples could be collected from the dogs living in the same household. The presence of a methicillin-resistant *M. canis* in a human infection site and previous reports of *Macrococcus caseolyticus* and the new species *M. goetzii*, *M. epidermidis,* and *M. bohemicus* in human clinical materials (Mašlaňová et al., 2018) emphasizes that more attention should be paid to *Macrococcus* as a new emerging opportunistic pathogen.

Data availability

The complete genome of *M. canis* strain LI021 has been deposited into the GenBank under accession number CP046590.

Declaration of Competing Interest

None.

Acknowledgments

We thank the team of microbiology from Dianalabs for their dedicated contributions to this work and Alexandra Rossano from the Institute of Veterinary Bacteriology, University of Bern, for technical assistance by WGS. This study was financed by internal funds of the Institute of Veterinary Bacteriology and Dianalabs.

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