Veterinary Microbiology 230 (2019) 138–144

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Characterization of *mecC* gene-carrying coagulase-negative *Staphylococcus* spp. isolated from various animals

Igor Loncaric[a,*], Anna Kübber-Heiss[b], Annika Posautz[b], Werner Ruppitsch[c], Sarah Lepuschitz[c], Bernhard Schauer[a], Andrea T. Feßler[d], Reinhild Krametter-Frötscher[e], Ewan M. Harrison[f], Mark A. Holmes[g], Frank Künzel[h], Michael P. Szostak[a], Tomasz Hauschild[i], Amélie Desvars-Larrive[b], Dusan Misic[j], Renate Rosengarten[a], Chris Walzer[b,k], Peter Slickers[l], Stefan Monecke[m,n,o], Ralf Ehricht[m,n], Stefan Schwarz[d], Joachim Spergser[a]

[a] Institute of Microbiology, University of Veterinary Medicine, Vienna, Austria
[b] Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna, Austria
[c] Austrian Agency for Health and Food Safety (AGES), Institute of Medical Microbiology and Hygiene, Vienna, Austria
[d] Institute of Microbiology and Epizootics, Centre of Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany
[e] University Clinic for Ruminants, University of Veterinary Medicine, Vienna, Austria
[f] Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK
[g] Departement of Veterinary Medicine, University of Cambridge, Cambridge, UK
[h] Clinical Unit of Internal Medicine Small Animals, University of Veterinary Medicine, Vienna, Austria
[i] Department of Microbiology, Institute of Biology, University of Bialystok, Bialystok, Poland
[j] Department for Microbiology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia
[k] Wildlife Health Program, Wildlife Conservation Society, Bronx, NY, USA
[l] Alere Technologies GmbH), Jena, Germany
[m] InfectoGnostics research campus, Jena, Germany
[n] Leibniz Institute of Photonic Technology (IPHT), Jena, Germany
[o] Institute for Medical Microbiology and Hygiene, Technical University of Dresden, Dresden, Germany

ARTICLE INFO

Keywords:
mecA
mecC
Coagulase-negative *Staphylococcus* spp.
Animals
Antimicrobial resistance

ABSTRACT

The presence of the methicillin resistance gene *mecC* in coagulase-negative *Staphylococcus* spp. (CoNS) is scarce. The aim of this study was to characterize *mecC*-positive CoNS isolated from various wild and domestic animals. The presence of the *mecC* gene was screened in 4299 samples from wild animals and domestic animals. Fifteen coagulase-negative staphylococci, that displayed a cefoxitin-resistant phenotype, were tested *mecC*-positive by PCR. Antimicrobial susceptibility testing was performed for all isolates. The 15 isolates were genotyped by sequencing of the entire class E *mec* gene complex (*blaZ-mecC-mecR1-mecI*), the *ccrA* and *ccrB* recombinase genes and other determinants within the type XI SCC*mec* element. DNA microarray analysis was performed and five selected isolates were additionally whole genome sequenced and analyzed. *S. stepanovicii* (n = 3), *S. caprae* (n = 1), *S. warneri* (n = 1), *S. xylosus* (n = 1) and *S. sciuri* (n = 9) were detected. All but the *S. sciuri* isolates were found to be susceptible to all non-beta lactams. The entire class E *mec* gene complex was detected in all isolates but *ccrA* and *ccrB* genes were not identified in *S. stepanovicii* and *S. xylosus*. The genes *erm*(B) and *fexA* (n = 4, each) were the most predominant non-beta lactam resistance genes detected in the *S. sciuri* isolates. Even though the presence of the *mecC* gene among CoNS is a rare observation, this study further expands our knowledge by showing that the *mecC* gene, including its allotypes, are present in more staphylococcal species from different animal species than has been previously described.

1. Introduction

Staphylococci are part of the physiological microbiota of the skin and the mucous membranes of humans and animals. They are commonly associated with opportunistic infections, the impact of which is frequently enhanced by the often expanded antimicrobial resistance of

* Corresponding author.
E-mail address: igor.loncaric@vetmeduni.ac.at (I. Loncaric).

https://doi.org/10.1016/j.vetmic.2019.02.014
Received 20 December 2018; Received in revised form 2 February 2019; Accepted 5 February 2019

the respective isolates. For decades, methicillin-resistant staphylococci, especially *S. aureus*, are a leading cause of nosocomial infections and a variety of life-threatening syndromes worldwide (Schleifer and Bell, 2009; Becker et al., 2014; Lakhundi and Zhang, 2018). Methicillin resistance in staphylococci is caused by an alternate penicillin-binding protein (PBP2a) that is encoded predominantly by the *mecA* gene and has a low affinity to β-lactam antibiotics (Katayama et al., 2000). The gene *mecA* is part of a *mec* complex and is usually accompanied by intact or truncated inducer/repressor genes: *mecI-mecR1* (Shore and Coleman, 2013). The *mec* complex is located on mobile genetic elements called Staphylococcal Cassette Chromosome *mec* (SCC*mec*). SCC*mec* elements are highly diverse in their structural organization and to date, thirteen major SCC*mec* types as well as various subtypes have been described in *S. aureus* from humans and animals (Jiang et al., 2018; Lakhundi and Zhang, 2018). Besides the *mec* complex, every SCC*mec* element carries cassette chromosome recombinase genes (*ccr*). In 2011, a novel *mec* gene type was discovered in *S. aureus* which shares approximately 70% nucleotide sequence identity with *mecA* (García-Álvarez et al., 2011, Shore et al., 2011). This *mec* homologue was initially referred to as $mecA_{LGA251}$, but later re-designated as *mecC*. The *mecC* gene in *S. aureus* is a part of the class E *mec* gene complex (*blaZ-mecC-mecR1-mecI*) (www.sccmec.org) and is commonly located on type XI SCC*mec* elements. So far, three further *mecC* allotypes have been detected in coagulase-negative staphylococci *mecC1* (shares 93.5% nucleotide identity with *mecC* in *S. aureus* LGA251), *mecC2* (shares 92.9% nucleotide identity with the *mecC* in LGA251) and *mecC3* (shares 92.0% nucleotide identity with the *mecC* in LGA251) (Harrison et al., 2014; Małyszko et al., 2014; MacFadyen et al., 2018b).· Most recently, a plasmid-borne *mecB* gene has also been identified in *S. aureus* (Becker et al., 2018).

S. aureus isolates harbouring the *mecC* gene have been isolated from livestock, companion and wild animals as well as humans in different countries (Paterson et al., 2012; Loncaric et al., 2013; Schwarz et al., 2018). In contrast, information on the presence of the *mecC* gene in other staphylococcal species is limited. The *mecC* gene (including known allotypes) was previously found in members of the *S. sciuri* group (i.e. *S. sciuri* and *S. stepanovicii*), *S. xylosus*, *S. saprophyticus* and has recently been described in the new staphylococcal species *S. edaphicus* (Harrison et al., 2013, 2014; Małyszko et al., 2014; Semmler et al., 2016; Srednik et al., 2017; Pantůček et al., 2018).

The aim of the present study was to characterize a collection of *mecC*-positive coagulase-negative staphylococci isolated from different wild and domestic animals for their molecular characteristics and their antimicrobial resistance phenotypes and genotypes.

2. Material and methods

2.1. Isolation of methicillin-resistant coagulase negative Staphylococcus spp. and detection of the mecC gene

Between 01.01.2013 and 01.01.2018, nasal swabs of 767 wild animals belonging to 27 distinct species, that were submitted to the Research Institute of Wildlife Ecology within the framework of the Austrian wildlife health surveillance program, were examined for the presence of the *mecC* gene (Table S1a). During the same period, 2809 staphylococci isolated from domestic animals during diagnostic activities were examined. A total of 698 out of 2809 staphylococci were identified as methicillin-resistant and examined for the presence of the *mecC* gene (Table S1b). In addition, 723 nasal swabs collected from ruminants, including adult cattle (n = 221), calves (n = 143), goats (n = 95) and sheep (n = 134), as well as New World camelids, i.e. Alpacas (n = 99) and Llamas (n = 31), were included in the present study. *S. stepanovicii* isolate 3orsfiwi, wherefrom a small part of class E *mec* gene complex had already been sequenced (Loncaric et al., 2013), was included in the present study for further analysis. All examined animals originated from Austria. Examination of the animal samples was carried out as part of the routine bacteriological diagnostic activities at the Institute of Microbiology, University of Veterinary Medicine, Vienna, Austria. Therefore, according to the Good Scientific Practice of the University of Veterinary Medicine, Vienna, these clinical examinations were not subject to the University of Veterinary Medicine, Vienna, Ethics and Animal Welfare Commission reporting obligations. Swabbing of ruminants and New World camelids was approved by the institutional ethics and animal welfare committee in accordance with Good Scientific Practice of the University of Veterinary Medicine, Vienna GSP guidelines and national legislation.

Nasal swabs of wild animals, ruminants and New World camelids were incubated at 37 °C overnight in trypticase soy broth (TSB) (Becton Dickinson (BD), Heidelberg, Germany) with 6.5% NaCl, and then streaked on Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom) supplemented with 2.5% NaCl, 2 mg/L oxacillin and 20 mg/L aztreonam (MHOXA) and on Columbia CNA Improved II Agar with 5% (v/v) sheep blood (BD) with subsequent passage on the same media until purified. From all isolates showing typical staphylococcal colony appearance on MHOXA, the tube coagulase test was performed. Coagulase-negative isolates were spotted onto BD™ Oxacillin Screen Agar (BD), and cefoxitin resistance was confirmed by agar disk diffusion (CLSI, 2018). All isolates suspected to be methicillin-resistant staphylococci were examined by a *mecC*-specific PCR (Harrison et al., 2014; Małyszko et al., 2014) and, if positive, they were further analysed. Whole cell DNA for this approach was extracted as previously described (Loncaric et al., 2013). Fifteen methicillin-resistant CoNS obtained during diagnostic activities from all clinical sites and different domestic animals as well as the abovementioned staphylococci from other examined animals, were *mecC*-positive and were stored at −80 °C until further examination.

2.2. Identification of staphylococcal isolates

Isolates were identified as a staphylococcal species by matrix-assisted laser desorption-ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik, Bremen, Germany) and confirmed by *rpoB* sequencing (Mellmann et al., 2006).

2.3. Antimicrobial susceptibility testing

Agar disk diffusion was performed according to CLSI document M100 (28th ed.) (CLSI, 2018). The following antimicrobial agents were tested: penicillin (PEN, 10 IU), gentamicin (GEN, 10 µg), erythromycin (ERY, 15 µg), clindamycin (CLI, 2 µg), tetracycline (TET, 30 µg), ciprofloxacin (CIP, 5 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), chloramphenicol (CHL, 30 µg), and linezolid (LZD, 30 µg). Additionally, the oxacillin MICs were determined by E-test (bioMérieux, Marcy l'Étoile, France). The reference strain *S. aureus* ATCC® 29523 served as quality control strain.

2.4. Molecular characterization of staphylococcal isolates

In addition to the *mecC* gene, all isolates were screened with primers targeting *mecA* and *mecA1* as described elsewhere (Harrison et al., 2014). A further approach comprised four PCRs for the detection of almost the entire class E *mec* gene complex (*blaZ-mecC-mecR1-mecI*). The primers for this approach have been previously described (García-Álvarez et al., 2011; Małyszko et al., 2014) or were designed based on previously described sequence alignments of *mecC* positive *Staphylococcus* spp. available in GenBank. Prior to DNA sequencing, PCR amplicons were cleaned using the GeneJET PCR Purification kit (Thermo Fisher Scientific, Waltham, MA, USA). The obtained DNA sequences were assembled using the CAP3 program (Huang and Madan, 1999). PCR amplification of *ccrA* and *ccrB* recombinase genes was conducted as previously described (García-Álvarez et al., 2011). Primer sequences are listed in Table S2. All PCR amplicons were sequenced. Nucleotide

Table 1
Summarized characteristics of the 15 *mecC* positive coagulase-negative *Staphylococcus* spp.

Species	Isolates	Host	Antimicrobial resistance profile			Virulence factors
			Phenotype	MIC* oxacillin mg/L	Genes detected**	detected
Staphylococcus stepanovicii	3orsfiwi	Eurasian lynx (*Lynx lynx*)	BLA	≥16	*blaZ-mecC-mecR1-mecI*	
	Z904	European otter (*Lutra lutra*)	BLA	≥16	*blaZ-mecC-mecR1-mecI*	*ssl10*
	AC983	Red fox (*Vulpes vulpes*)	BLA	≥16	*blaZ-mecC-mecR1-mecI*	*lukS, ssl10, ebpS*
Staphylococcus caprae	Z111	Beaver (*Castor fiber*)	BLA	≥16	*blaZ-mecC-mecR1-mecI*	
Staphylococcus xylosus	AD10b	Brown rat (*Rattus norvegicus*)	BLA	1	*blaZ-mecC-mecR1-mecI*	
Staphylococcus warneri	2800	Cat	BLA	≥16	*blaZ-mecC-mecR1-mecI*	
Staphylococcus sciuri	LP396	Cattle	BLA, SXT	≥16	*blaZ-mecC-mecR1-mecI, mecA, mecA1*	*sspP*
	LP122	Calf	BLA, GEN, TET, ERY, CLI, CHL, SXT	≥16	*blaZ-mecC-mecR1-mecI, mecA, mecA1, aacA-aphD, tet*(K), *erm*(C), *fexA*	*lukS, sspP, bbP, isaB, isdA*
	LP498	Calf	BLA	≥16	*blaZ-mecC-mecR1-mecI, mecA, mecA1*	*lukS, isdA*
	LP643	Sheep	BLA, TET, CIP, CHL	≥16	*blaZ-mecC-mecR1-mecI, mecA, mecA1, aadD, tet*(M), *erm*(B), *cfr, fexA*	*hysA2*
	LP187	Goat	BLA, TET, CIP, ERY, CLI, CHL	≥16	*blaZ-mecC-mecR1-mecI, mecA, mecA1, tet*(K), *tet*(M), *erm*(B), *fexA*	*hysA2*
	LP211	Goat	BLA, TET, CIP, ERY, CLI, CHL	≥16	*blaZ-mecC-mecR1-mecI, mecA, mecA1, aadD, tet*(M), *erm*(B), *cfr, fexA*	*sspP*
	LP372	Goat	BLA	≥16	*blaZ-mecC-mecR1-mecI, mecA, mecA1*	
	LP254	Alpaca	BLA	≥16	*blaZ-mecC-mecR1-mecI, mecA, mecA1*	*ssl10, hsdS3, hsdsX, hysA2*
	LP600	Alpaca	BLA	≥16	*blaZ-mecC-mecR1-mecI, mecA, mecA1, str**, *sal*(A) ***	*arcD-SCC, ssl10, hsdS3, hsdSX, hysA2*

BLA = β-lactams; CHL = Chloramphenicol; CIP = ciprofloxacin; CLI = clindamycin; ERY = erythromycin; GEN = gentamicin; SXT = trimethoprim-sulfamethoxazole; TET = tetracycline;
* MIC = Minimal Inhibitory Concentration.
** Gene associated with SCCmec and non-β lactamase resistant genes.
*** detected via whole genome sequencing.

sequences of almost the entire class E *mec* gene complex as well as the *ccrA* and *ccrB* genes were aligned with the accessible corresponding sequences of *mecC*-positive staphylococci deposited in GenBank using ClustalW in MEGA X (Kumar et al., 2018). A maximum likelihood tree was generated using the same software. Tree topologies were estimated using bootstrap analyses with 1000 replicates to accomplish confidence intervals as indicated on each tree node. The distance between the gene *mecI* and the damage inducible gene G (*dinG*) downstream of the class E *mec* complex in *S. stepanovicii* isolates AC983 and Z904, was investigated by PCR (a product of 1138 bp length) which was designed based on known sequences (KR732654 and in isolate 3orsfiwi). The amplicons were sequenced for confirmatory reasons. In *S. sciuri* isolates, the presence of *attR*, *attL* and *attL2* repeats were examined by PCR using combinations of primers P1 + P2, P3 + P4, and P5 + P6, followed by sequence analysis of the amplicons (Harrison et al., 2014). In order to identify more than 300 virulence and resistance genes in all isolates, a DNA microarray (*S. aureus* Genotyping Kit 2.0, Alere, Jena, Germany) was used (Monecke et al., 2008). For whole genome sequencing (WGS) high quality genomic DNA (gDNA) was isolated from overnight cultures using the MagAttract HMW DNA Kit (Qiagen, Hilden, Germany) and quantified on a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) using the dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) was used for library preparation and paired-end sequenced with a read length of 2 × 300 base pairs on a MiSeq instrument according to the instructions of the manufacturer (Illumina, San Diego, CA, USA). SPAdes version 3.11 and SeqSphere + version 5.1.0 (Ridom, Münster, Germany) were used for read assembly. MLST (multilocus sequence type), resistance genes and virulence genes were extracted from WGS data using SeqSphere + version 5.1.0 as described (Leopold et al., 2014; Lepuschitz et al., 2017, 2018). Antimicrobial resistance and virulence genes were identified in WGS data using the AlereMicroarray data (Strauß et al., 2016), the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al., 2017) and the ResFinder tool-version 3.0 (Zankari et al., 2012) (https://cge.cbs.dtu.dk/services/ResFinder/) with default settings for each database. The presence of virulence genes was extracted from WGS data using AlereMicroarray data (Strauß et al., 2016). The structure of SCC*mec* element in isolate LP600 was determined using CLC Genomics Workbench 10.1.1. (Qiagen, Hilden, Germany) by mapping raw reads against the recently published hybrid SCC*mec-mecC* reference sequence (Accession HG515014) (Harrison et al., 2014).

3. Results

3.1. Bacterial isolates

In total, fifteen non-repetitive CoNS carrying the *mecC* gene and belonging to five different staphylococcal species were identified. The highest *rpoB* gene sequence similarities observed in the examined isolates were with the respective type strains of *S. stepanovicii* (3orsfiwi 99.8%, AC983 100%, and Z904 99.8%), *S. caprae* (Z111 99.4%), *S. warneri* (2800 99.4%), *S. xylosus* (AD10b 98.3%) and *S. sciuri* (LP122 99.8%, LP187 99.6%, LP211 99.8%, LP254 99.8%, LP372 99.6%, LP396 99.8%, LP498 99.8%, LP600 99.8% and LP643 99.8%). The three *S. stepanovicii* isolates [from a red fox (*Vulpes vulpes*), an European otter (*Lutra lutra*), and an Eurasian lynx (*Lynx lynx*)], the *S. caprae* isolate Z111 [from a European beaver (*Castor fiber*)], and the *S. xylosus* isolate AD10b [from a brown rat (*Rattus norvegicus*)] originated from wild animals. The single *mecC*-positive *S. warneri* 2800 was detected in a clinical sample from the wound of a cat. Nine *S. sciuri* isolates originated from adult cattle (L396), calves (LP112, LP498), sheep (LP643), goats (LP187, LP211, LP372), and alpacas (LP254, LP600). The *S. xylosus* isolate AD10b showed a very weak growth on MHOXA only after prolonged incubation for 72 h and did not grow on BD™ Oxacillin Screen Agar (BD). All other examined isolates grew well after inoculation on the same medium.

3.2. Antimicrobial susceptibility testing

All but the *S. sciuri* isolates were found to be susceptible to all non-β-lactams. All the *S. sciuri* isolates were susceptible to amikacin and linezolid. In addition to the antimicrobial agents stated above, the predominant phenotypic resistance properties of the *S. sciuri* isolates included resistance to ciprofloxacin, tetracycline, and chloramphenicol. All but the *S. xylosus* isolate showed oxacillin MICs of ≥16 mg/L. The oxacillin MIC of the *S. xylosus* isolate was 1 mg/L (Table 1).

3.3. Molecular characterization of staphylococcal isolates

In contrast to the other *mecC*-positive CoNS, the *S. sciuri* isolates tested positive not only for *mecC*, but also for *mecA* and *mecA1*. A set of PCRs covering almost the entire class E *mec* gene complex (*mecC* region) produced amplicons of the expected sizes and after assembly, a single sequence of approximately 5 kb was generated for each isolate. The entire *mecC* regions in all three *S. stepanovicii* isolates (3orsfiwi, AC983, Z904) shared between 99.6 and 99.8% nucleotide sequence identity with the *mecC* region of the *mecC*-positive *S. stepanovicii* strain IMT28705 (KR732654). The corresponding regions of the *S. caprae* isolate Z111 and the *S. warneri* isolate 2800 shared > 99.8% identity with the *mecC* region of the *S. aureus* strain LGA251 (FR821779). The *mecC* region of the *S. xylosus* isolate AD10b shared > 99.7% with the respective homologue in the *S. xylosus* strain S04009 (HE993884). All *S. sciuri* isolates (LP122, LP187, LP211, LP254, LP372, LP396, LP498, LP600 and LP643) exhibited nucleotide sequence identities of their *mecC* regions of > 99.6% with that of the *S. sciuri* strain GVGS2 (HG515014). These relationships are very well reflected by the phylogenetic analysis (Fig. 1a).

PCR amplification of the *ccrA* and *ccrB* genes failed in the *S. stepanovicii* isolates as well as in the *S. xylosus* strain. The *ccrA* gene in the *S. caprae* isolates Z111 and in the *S. warneri* isolate 2800 exhibited 100% nucleotide sequence identity with the accessible corresponding sequences of *ccrA* of *mecC*-positive *S. aureus* (strains: LGA251, M10/0061, ST425, CFSAN064037, ZTA09/03698-9ST, CMFT540). The *S. sciuri* isolates LP122, LP254, LP396, LP498 and LP600 shared 100%, 99.7%, 100%, 99.7% and 100% nucleotide sequence identity with the *ccrA* gene of the *S. sciuri* strain GVGS2 (HG515014). In contrast, the *ccrA* gene of *S. sciuri* isolates LP187, LP211 and LP643 exhibited best matches of 93.5%, 93.8% and 92.5% nucleotide sequence identity with the corresponding sequence of *S. pseudintermedius* strain KM241 (AM904731).

As for the *ccrA* gene, the *ccrB* gene in the *S. caprae* strain Z111 and in the *S. warneri* strain 2800 shared high DNA sequence similarities of 99.9% and 100% with the corresponding sequences of *ccrB* of *mecC*-positive *S. aureus* strains LGA251, M10/0061, ST425, CFSAN064037, ZTA09/03698-9ST, and CMFT540. The *ccrB* gene of the *S. sciuri* isolates LP122, LP254, LP396, LP498 and LP600 shared > 99.8% identity with the *ccrB* gene in the *S. sciuri* strain GVGS2. The *ccrB* gene of the *S. sciuri* strain LP187 shared 97.2% nucleotide sequence identity with the *ccrB* gene of the *S. cohnii* strain WC28 (GU370073). The *S. sciuri* isolates LP211 and LP643 shared 93.1% and 93.2% nucleotide sequence identity with the corresponding sequences of *ccrB* in the *S. equorum* strain KS1039 (CP013114). Phylogenetic trees for the *ccrA* and *ccrB* sequences are shown in Fig. 1b and c, respectively.

PCR amplification of the part of genes the *mecI* and *dinG* downstream of the class E *mec* complex in the *S. stepanovicii* isolates AC983 and Z904 yielded amplicons of the expected size which shared > 99.7% nucleotide sequence identity with the corresponding sequences in *mecC*-positive *S. stepanovicii* strains IMT28705 and 3orsfiwi. By using the primer combination for the detection of the *attR* site in the *mecC*-positive *S. sciuri* strain GVGS2, corresponding homologous sequences were detected in the *S. sciuri* isolates LP112, LP254, LP396, LP498 and

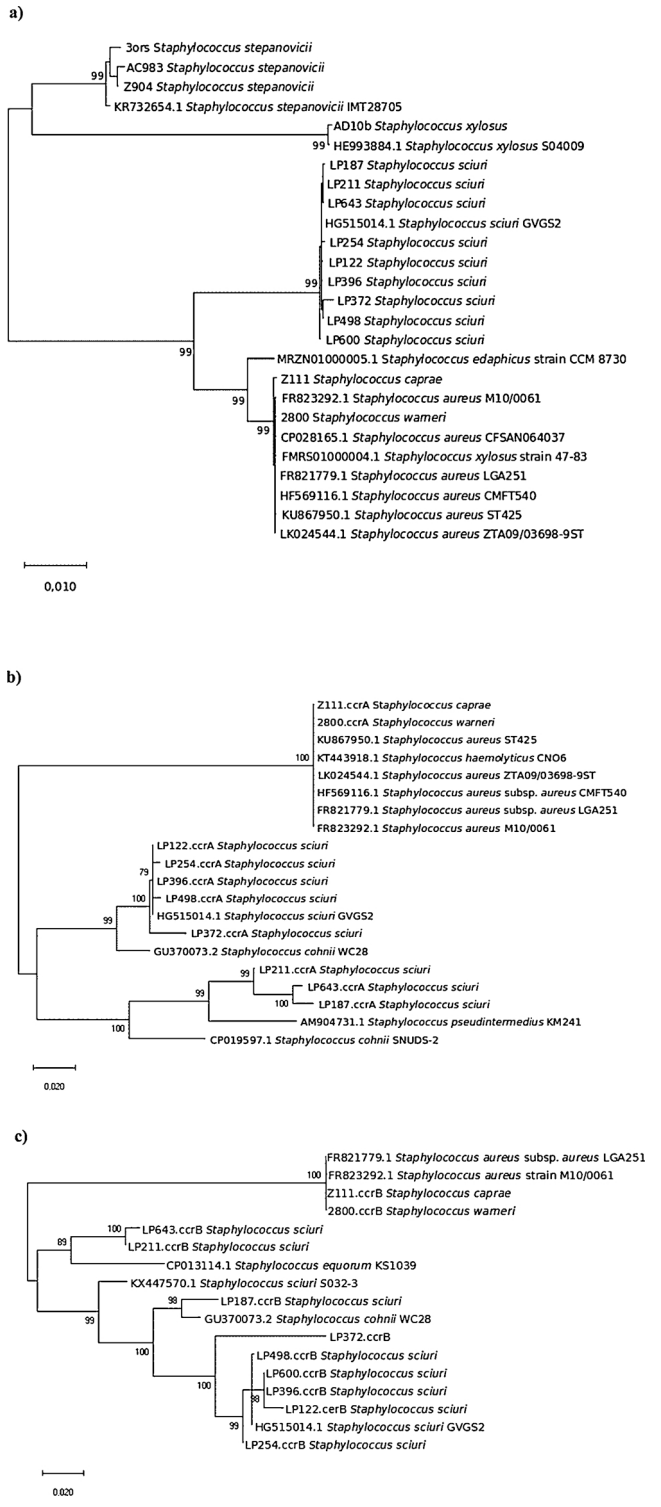

Fig. 1. Maximum Likelihood tree based on the E *mec* gene complex (*mecC* region) (a), *ccrA* gene (b) and *ccr*B (c) gene-sequences of examined *mecC* positive coagulase-negative *Staphylococcus* spp.: *S. stepanovicii* (3orsfiwi, AC983, Z904), *S. caprae* (Z111), *S. warneri* (2800), *S. xylosus* (AD10b) and *S. sciuri* (LP122, LP187, LP211, LP254, LP372, LP396, LP498, LP600 and LP643). Bootstrap values (%) < 75 based on 100 replicates are given at nodes. Bars indicate substitutions per nucleotide position.

LP600. The *attL* homologous sequence was detected in all nine examined *mecC*-positive *S. sciuri* isolates. The *attL2* site was detected in all *S. sciuri* isolates except strain LP498.

DNA microarray analysis revealed that all three examined *S. stepanovicii* isolates, as well as the single *S. warneri, S. caprae, S. xylosus* isolates carried none of the non-β-lactam resistance genes present on the array. None of the non-β-lactam resistance genes could be detected in the *S. sciuri* isolates LP372, LP396 and LP600. Among the remaining *S. sciuri* isolates, the macrolide-lincosamide-streptogramin B resistance gene *erm*(B) and the phenicol exporter gene *fexA* (n = 4, each) were most frequently detected resistance markers. In two *S. sciuri* isolates (LP211, LP643), the rRNA methylase gene *cfr*, conferring resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A, was detected. Virulence genes were rarely observed. The antimicrobial resistance patterns and the resistance and virulence genes detected are summarized in Table 1. The complete results of the microarray analysis are shown in Table S3.

Five isolates were subjected to whole-genome sequencing: *S. stepanovicii* 3orsfiwi, *S. caprae* Z111, *S. warneri* 2800, *S. xylosus* AD10b and *S. sciuri* LP600. The SCC*mec* element found in strain 3orsfiwi is located between the chromosomal staphylococcal core genes *orfX* and *dusC*. This SCC*mec* element comprised a typical class E *mec* gene cluster consisting of *blaZ, mecC, mecR* and *mecI*. It also comprised the gene *dinG*, which encodes a fusion protein between a helicase and a nuclease (KR732654), the genes of which are also present next to each other in SCC*mec* IX elements of *S. aureus*. The SCC*mec* element in LGA251 comprises twelve genes between the *mec* class E gene cluster and the *dinG* homologue, among them the cassette chromosome recombinase genes *ccrB, ccrA* and cassette chromosome helicase *cch*. Cassette chromosome recombinase and their homologues is completely missing in 3orsfiwi and the gene *dinG* is located immediately downstream of *mecI*. *S. caprae* Z111 and *S. warneri* 2800 contain the complete and nearly identical SCC*mec* element as *S. aureus* LGA251. WGS revealed no further non-β-lactam and virulence genes known from *S. aureus* in *S. stepanovicii* 3orsfiwi, *S. caprae* Z111, and *S. warneri* 2800. The SCC*mec* element of *S. xylosus* AD10b corresponded to that described in *S. xylosus* strain S04009 (HE993884). Cassette chromosome recombinase and their homologues could not be detected in isolate AD10b. Analysis of the genome sequence identified a *tet*(B) tetracycline resistance gene as only non-beta lactam resistance gene. The LP600 SCC*mec* element shows the same structure as the reference hybrid SCCmec-mecC sequence, while mecA1 was part of the chromosomal locus as reported in GVGS2. WGS analysis of *S. sciuri* LP600 identified the streptomycin resistance gene *str* and the pleuromutilin-lincosamide-streptogramin A resistance gene *sal*(A) as only non-β-lactam resistance genes. No further virulence genes were detected with the described methods in the investigated isolates.

4. Discussion

In the present study, fifteen non-repetitive *mecC*-positive CoNS obtained from various animals were analysed. In Austria, the presence of the *mecC* gene was previously detected in *S. aureus* and *S. stepanovicii* (3orsfiwi) from wildlife as well as in *S. aureus* from goats (Loncaric et al., 2013; Schauer et al., 2018). The presence of *mecC*-positive staphylococci from other animals in Austria has not been described yet. In this study, we have identified two additional staphylococcal species of animal origin, namely *S. caprae* and *S. warneri*, that harbour the *mecC* gene.

No major phenotypic and genotypic differences in terms of resistance genes were seen between the three examined *S. stepanovicii* isolates and the recently published *mecC*-positive *S. stepanovicii* IMT28705 (Semmler et al., 2016). *S. caprae* Z111 and *S. warneri* 2800 harboured almost identical SCC*mec* elements as described in *mecC*-positive MRSA (Garcia-Alvarez et al. 2011, Shore et al., 2011). So far, two different *mecC*-positive *S. xylosus* isolates have been obtained from bovine mastitis and milk, respectively. Harrison et al. (2013) described a highly related *mecC* homologue present in *S. xylosus* strain S04009, named *mecC1*, which shared 93.5% nucleotide identity with the original *mecC* in *S. aureus* LGA251. A frameshift mutation close to the 5′

end of the *mecC1* gene in S04009 resulted in a truncated 64 amino acid (aa) product, which was unable to confer resistance to oxacillin and cefoxitin. This frameshift mutation was also observed in *S. xylosus* AD10b analysed in the present study, which may explain the low oxacillin MIC of this strain and its inability to grow on oxacillin screening agar. Very recently, another *S. xylosus* (strain 47–83) was detected (MacFadyen et al., 2018a), which encodes an intact prototype *mecC* as the one previously found in LGA251. So far, *mecC*-positive *S. xylosus* has never been isolated from brown rat (*Rattus norvegicus*). The predominant staphylococcal species that harboured the *mecC* gene was *S. sciuri*. Besides the *mecC* gene, all *S. sciuri* in the present study harboured also *mecA* and *mecA1* genes, which was also observed in *S. sciuri* GVGS2 (Harrison et al., 2014. Four (LP112, LP254, LP396 and LP600) out of nine examined *S. sciuri* isolates shared almost identical SCC*mec* features, i.e. *mec* gene complex E, *ccrA* and *ccrB* recombinase genes as well as *attR, attL and attL2* repeats as observed in *S. sciuri* GVGS2 (Harrison et al., 2014. While three of the *S. sciuri* isolates (LP187, LP211 and LP643) harboured almost intact *mec* gene complexes of type E as described in *S. sciuri* GVGS2, their *ccrA* and *ccrB* recombinase genes varied slightly from the corresponding genes in *S. sciuri* GVGS2. The *ccrA* genes were most closely related to the respective genes in SCC*mec* type VII from *S. pseudintermedius* strain KM241. This has already been described for *S. sciuri* GVGS2 but could not be observed for the *ccrB* genes in *S. sciuri* isolates LP187, LP211 and LP643. This observation may suggest that these isolates potentially harbour slightly different SCC*mec* elements in comparison to *S. sciuri* GVGS2.

Overall, the presence of *mecC* in the examined staphylococci is a rare observation which is in agreement with other studies. Most of the *mecC*-carrying CoNS in the present study originated from non-diseased animals (nasal colonisation), except the *S. warneri* strain, which was from a tissue sample of a diseased cat. Thus, the clinical importance of *mecC*-positive CoNS remains questionable. Interestingly, majority of examined isolates from wild animals originated from predators which may suggest colonization due to consumption of other animals, like small mammals, which are known to be carriers of antibiotic-resistant staphylococci (Hauschild and Schwarz, 2010; Małyszko et al., 2014; Kmeť et al., 2018). On the other hand, the brown rat as a ubiquitous omnivorous synanthrope could easily be colonized with antibiotic-resistant bacteria from humans and other animals. Whether *mecC*-positive CoNS, especially those isolates with almost indistinguishable type E *mec* gene complexes, could function as a possible source of *mecC* for *S. aureus*, as proposed for *mecA* (Couto et al., 1996), remains to be determined. The presence of *mecC* and *ccr* genes in *S. caprae* and *S. warneri* isolates with significant similarity to those in *S. aureus* suggests that transfer of these elements between these species could have occurred. In conclusion, this study further expands our knowledge that the *mecC* gene including its allotypes occur in a wider range of staphylococcal species originating from different animal species than has been described previously.

Nucleotide accession numbers

Almost entire *mec* E element: MK330607-MK330621, *ccrA* and *ccrB*: MK445226- MK445247. The genomes of two five whole-genome sequenced isolates were deposed under no. PRJEB2655 (ERR599835 ERX556801), PRJNA517387 (SRX5299061-3) in the NCBI BioProject database.

Conflict of interest statement

None to declare.

Acknowledgements

We are grateful to Annett Reissig, Elke Müller (IPHT) and Darius Gawlik (all from Abbott) for technical assistance. We are grateful to Dennis Hanke (Institute of Microbiology and Epizootics, Centre of Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany) for helping us generating phylogenetic trees. This study was supported by internal funding of Institute of Microbiology and Research Institute of Wildlife Ecology from University of Veterinary Medicine Vienna, Austria and partially supported by the Austrian Buiatric association. The work conducted by ATF and SS was financially supported by the Federal Ministry of Education and Research (BMBF) under project number 01KI1727D as part of the Research Network Zoonotic Infectious Diseases.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.02.014.

References

Becker, K., Heilmann, C., Peters, G., 2014. Coagulase-negative staphylococci. Clin. Microbiol. Rev. 27, 870–926.
Becker, K., van Alen, S., Idelevich, E.A., Schleimer, N., Seggewiß, J., Mellmann, A., Kaspar, U., Peters, G., 2018. Plasmid-encoded transferable *mecB*-mediated methicillin resistance in *Staphylococcus aureus*. Emerg. Infect. Dis. 24, 242–248.
Clinical and Laboratory Standards Institute, 2018. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-eight Informational Supplement M100-28. CLSI, Wayne, PA, USA.
Couto, I., de Lencastre, H., Severina, E., Kloos, W., Webster, J.A., Hubner, R.J., Sanches, I.S., Tomasz, A., 1996. Ubiquitous presence of a *mecA* homologue in natural isolates of *Staphylococcus sciuri*. Microb. Drug Resist. 2, 377–391.
García-Álvarez, L., Holden, M.T., Lindsay, H., Webb, C.R., Brown, D.F., Curran, M.D., Walpole, E., Brooks, K., Pickard, D.J., Teale, C., Parkhill, J., Bentley, S.D., Edwards, G.F., Girvan, E.K., Kearns, A.M., Pichon, B., Hill, R.L., Larsen, A.R., Skov, R.L., Peacock, S.J., Maskell, D.J., Holmes, M.A., 2011. Methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. . Lancet Infect. Dis. 11, 595–603.
Harrison, E.M., Paterson, G.K., Holden, M.T.G., Morgan, F.J., Larsen, A.R., Petersen, A., Leroy, S., De Vliegher, S., Perreten, V., Fox, L.K., Lam, T.J., Sampimon, O.C., Zadoks, R.N., Peacock, S.J., Parkhill, J., Holmes, M.A., 2013. A *Staphylococcus xylosus* isolate with a new *mecC* allotype. Antimicrob. Agents Chemother. 57, 1524–1528.
Harrison, E.M., Paterson, G.K., Holden, M.T., Ba, X., Rolo, J., Morgan, F.J., Pichon, B., Kearns, A., Zadoks, R.N., Peacock, S.J., Parkhill, J., Holmes, M.A., 2014. A novel hybrid SCC*mec*-*mecC* region in *Staphylococcus sciuri*. J. Antimicrob. Chemother. 69, 911–918.
Hauschild, T., Schwarz, S., 2010. Macrolide resistance in *Staphylococcus* spp. from free-living small mammals. Vet. Microbiol 144, 530–531.
Huang, X., Madan, A., 1999. CAP3: A DNA sequence assembly program. Genome Res. 9, 868–877.
Jia, B., Raphenya, A.R., Alcock, B., Waglechner, N., Guo, P., Tsang, K.K., Lago, B.A., Dave, B.M., Pereira, S., Sharma, A.N., Doshi, S., Courtot, M., Lo, R., Williams, L.E., Frye, J.G., Elsayegh, T., Sardar, D., Westman, E.L., Pawlowski, A.C., Johnson, T.A., Brinkman, F.S., Wright, G.D., McArthur, A.G., 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 45 (D1), D566–D573.
Jiang, N., Li, J., Feßler, A.T., Wang, Y., Schwarz, S., Wu, C., 2018. Novel pseudo-staphylococcal cassette chromosome *mec* element (φSCC*mec*T55) in MRSA ST9. J. Antimicrob. Chemother. https://doi.org/10.1093/jac/dky457. [Epub ahead of print].
Katayama, Y., Ito, T., Hiramatsu, K., 2000. A new class of genetic element, *Staphylococcus* cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 44, 1549–1555.
Kmeť, V., Čuvalová, A., Stanko, M., 2018. Small mammals as sentinels of antimicrobial-resistant staphylococci. Folia Microbiol. (Praha) 63, 665–668.
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.
Lakhundi, S., Zhang, K., 2018. Methicillin-Resistant *Staphylococcus aureus*: Molecular characterization, evolution, and epidemiology. Clin. Microbiol. Rev. 31 (4), e00020–18 pii 12.
Leopold, S.R., Goering, R.V., Witten, A., Harmsen, D., Mellman, A., 2014. Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. J. Clin. Microbiol. 52, 2365–2370.
Lepuschitz, S., Mach, R., Springer, B., Allerberger, F., Ruppitsch, W., 2017. Draft genome sequence of a community-acquired methicillin-resistant *Staphylococcus aureus* USA300 isolate from a river sample. Genome Announ. 5. https://doi.org/10.1128/genomeA.01166-17. pii: e01166-17.
Lepuschitz, S., Huhulescu, S., Hyden, P., Springer, B., Rattei, T., Allerberger, F., Mach, R.L., Ruppitsch, W., 2018. Characterization of a community-acquired-MRSA USA300 isolate from a river sample in Austria and whole genome sequence based comparison to a diverse collection of USA300 isolates. Sci. Rep. 8, 9467.

Loncaric, I., Kubber-Heiss, A., Posautz, A., Stalder, G.L., Hoffmann, D., Rosengarten, R., Walzer, C., 2013. Characterization of methicillin-resistant *Staphylococcus* spp. carrying the *mecC* gene, isolated from wildlife. J. Antimicrob. Chemother. 14, 2222–2225.

MacFadyen, A.C., Harrison, E.M., Ellington, M.J., Parkhill, J., Holmes, M.A., Paterson, G.K., 2018a. A highly conserved *mecC*-encoding SCC*mec* type XI in a bovine isolate of methicillin-resistant *Staphylococcus xylosus*. J. Antimicrob. Chemother. 73, 3516–3518.

MacFadyen, A.C., Harrison, E.M., Drigo, I., Parkhill, J., Holmes, M.A., Paterson, G.K., 2018b. A *mecC* allotype, *mecC3*, in the coagulase-negative *Staphylococcus Staphylococcus caeli,* encoded within a variant SCC*mecC*. J. Antimicrob. Chemother. https://doi.org/10.1093/jac/dky502. [Epub ahead of print].

Małyszko, I., Schwarz, S., Hauschild, T., 2014. Detection of a new *mecC* allotype, *mecC2*, in methicillin-resistant *Staphylococcus saprophyticus*. J. Antimicrob. Chemother. 69, 2003–2005.

Mellmann, A., Becker, K., von Eiff, C., Keckevoet, U., Schumann, P., Harmsen, D., 2006. Sequencing and staphylococci identification. Emerg. Infect. Dis. 12, 333–336.

Monecke, S., Slickers, P., Ehricht, R., 2008. Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol. Med. Microbiol. 53, 237–251.

Pantůček, R., Sedláček, I., Indráková, A., Vrbovská, V., Mašlaňová, I., Kovařovic, V., Švec, P., Králová, S., Krištofová, L., Kekláková, J., Petráš, P., Doškař, J., 2018. *Staphylococcus edaphicus* sp. nov., isolated in Antarctica, harbors the *mecC* gene and genomic islands with a suspected role in adaptation to extreme environments. Appl. Environm. Microbiol. 84, e01746–17.

Paterson, G.K., Larsen, A.R., Robb, A., Edwards, G.E., Pennycott, T.W., Foster, G., Mot, D., Hermans, K., Baert, K., Peacock, S.J., Parkhill, J., Zadoks, R.N., Holmes, M.A., 2012. The newly described *mecA* homologue, *mecA*LGA251, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species. J. Antimicrob. Chemother. 67, 2809–2813.

Schauer, B., Krametter-Frötscher, R., Knauer, F., Ehricht, R., Monecke, S., Feßler, A.T., Schwarz, S., Grunert, T., Spergser, J., Loncaric, I., 2018. Diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from Austrian ruminants and New World camelids. Vet. Microbiol. 215, 77–82.

Schleifer, K.-H., Bell, J.A., 2009. Family VIII. *Staphylococcaceae* fam. nov. In: second ed. In: De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., Whitman, W.B. (Eds.), 2009. Bergey's Manual of Systematic Bacteriology, The Firmicutes, vol. 3. Springer, Dordrecht, pp. 392–433.

Schwarz, S., Feßler, A.T., Loncaric, I., Wu, C., Kadlec, K., Wang, Y., Shen, J., 2018. Antimicrobial resistance among staphylococci of animal origin. Microbiol. Spectr. 6 (4). https://doi.org/10.1128/microbiolspec.ARBA-0010-2017.

Semmler, T., Harrison, E.M., Lübke-Becker, A., Ulrich, R.G., Wieler, L.H., Guenther, S., Stamm, I., Hanssen, A.M., Holmes, M.A., Vincze, S., Walther, B., 2016. A look into the melting pot: the *mecC*-harboring region is a recombination hot spot in *Staphylococcus stepanovicii*. PLoS One 11, e0147150. https://doi.org/10.1371/journal.pone.0147150.

Shore, A.C., Coleman, D.C., 2013. Staphylococcal cassette chromosome *mec*: recent advances and new insights. Int. J. Med. Microbiol. 303, 350–359.

Shore, A.C., Deasy, E.C., Slickers, P., Brennan, G., O'Connell, B., Monecke, S., Ehricht, R., Coleman, D.C., 2011. Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA, mecI, mecR1, blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 55, 3765–3773.

Srednik, M.E., Archambault, M., Jacques, M., Gentilini, E.R., 2017. Detection of a *mecC*-positive *Staphylococcus saprophyticus* from bovine mastitis in Argentina. J. Glob. Antimicrob. Resist. 10, 261–263.

Strauß, L., Ruffing, U., Abdulla, S., Alabi, A., Akulenko, R., Garrine, M., Germann, A., Grobusch, M.P., Helms, V., Herrmann, M., Kazimoto, T., Kern, W., Mandomando, I., Peters, G., Schaumburg, F., von Müller, L., Mellmann, A., 2016. Detecting *Staphylococcus aureus* virulence and resistance genes: a comparison of whole-genome sequencing and DNA microarray technology. J. Clin. Microbiol. 54, 1008–1016.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67, 2640–2644.