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

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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 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* (Garcia-Alvarez et al. 2011, Shore et al., 2011). This *mec* homologue was initially referred to as *mecA*$_{\text{LGA251}}$, 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

79   et al., 2014, Małyszko et al., 2014, MacFadyen et al., 2018b). Most recently, a plasmid-borne

80   *mecB* gene has also been identified in *S. aureus* (Becker et al., 2018).

81   *S. aureus* isolates harbouring the *mecC* gene have been isolated from livestock,

82   companion and wild animals as well as humans in different countries (Paterson et al., 2012,

83   Loncaric et al., 2013, Schwarz et al., 2018). In contrast, information on the presence of the

84   *mecC* gene in other staphylococcal species is limited. The *mecC* gene (including known

85   allotypes) was previously found in members of the *S. sciuri* group (i.e. *S. sciuri* and *S.*

86   *stepanovicii*), *S. xylosus*, *S. saprophyticus* and has recently been described in the new

87   staphylococcal species *S. edaphicus* (Harrison et al., 2013, Harrison et al., 2014, Małyszko et

88   al., 2014, Semmler et al., 2016, Srednik et al., 2017, Pantůček et al., 2018).

89   The aim of the present study was to characterize a collection of *mecC*-positive

90   coagulase-negative staphylococci isolated from different wild and domestic animals for their

91   molecular characteristics and their antimicrobial resistance phenotypes and genotypes.

92

93   **2   Material and Methods**

94   *2.1   Isolation of methicillin-resistant coagulase negative Staphylococcus spp. and detection*

95   *of the mecC gene*

96   Between 01.01.2013 and 01.01.2018, nasal swabs of 767 wild animals belonging to 27

97   distinct species, that were submitted to the Research Institute of Wildlife Ecology within the

98   framework of the Austrian wildlife health surveillance program, were examined for the

99   presence of the *mecC* gene (Table S1a). During the same period, 2809 staphylococci isolated

100   from domestic animals during diagnostic activities were examined. A total of 698 out of 2809

101   staphylococci were identified as methicillin-resistant and examined for the presence of the

102   *mecC* gene (Table S1b). In addition, 723 nasal swabs collected from ruminants, including

103   adult cattle (n=221), calves (n=143), goats (n=95) and sheep (n=134), as well as New World

104   camelids, i.e. Alpacas (n=99) and Llamas (n=31), were included in the present study. *S.*

105   *stepanovicii* isolate 3orsfiwi, wherefrom a small part of class E *mec* gene complex had already

106   been sequenced (Loncaric et al., 2013), was included in the present study for further analysis.

107   All examined animals originated from Austria. Examination of the animal samples was

108   carried out as part of the routine bacteriological diagnostic activities at the Institute of

109   Microbiology, University of Veterinary Medicine, Vienna, Austria. Therefore, according to

110   the Good Scientific Practice of the University of Veterinary Medicine, Vienna, these clinical

111   examinations were not subject to the University of Veterinary Medicine, Vienna, Ethics and

112   Animal Welfare Commission reporting obligations. Swabbing of ruminants and New World

113   camelids was approved by the institutional ethics and animal welfare committee in

114   accordance with Good Scientific Practice of the University of Veterinary Medicine, Vienna

115   GSP guidelines and national legislation.

116   Nasal swabs of wild animals, ruminants and New World camelids were incubated at

117   37°C overnight in trypticase soy broth (TSB) (Becton Dickinson (BD), Heidelberg, Germany)

118   with 6.5% NaCl, and then streaked on Mueller-Hinton agar (Oxoid, Basingstoke, United

119   Kingdom) supplemented with 2.5% NaCl, 2 mg/L oxacillin and 20 mg/L aztreonam

120   (MHOXA) and on Columbia CNA Improved II Agar with 5% (v/v) sheep blood (BD) with

121   subsequent passage on the same media until purified. From all isolates showing typical

122   staphylococcal colony appearance on MHOXA, the tube coagulase test was performed.

123   Coagulase-negative isolates were spotted onto BD™ Oxacillin Screen Agar (BD), and

124   cefoxitin resistance was confirmed by agar disk diffusion (CLSI, 2018). All isolates suspected

125   to be methicillin-resistant staphylococci were examined by a *mecC*-specific PCR (Harrison et

126   al., 2014, Małyszko et al., 2014) and, if positive, they were further analysed. Whole cell DNA

127   for this approach was extracted as previously described (Loncaric et al., 2013). Fifteen

128 methicillin-resistant CoNS obtained during diagnostic activities from all clinical sites and

129 different domestic animals as well as the abovementioned staphylococci from other examined

130 animals, were *mecC*-positive and were stored at -80°C until further examination.

131

132 *2.2 Identification of staphylococcal isolates*

133 Isolates were identified as a staphylococcal species by matrix-assisted laser desorption-

134 ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik, Bremen,

135 Germany) and confirmed by *rpoB* sequencing (Mellmann et al., 2006).

136

137 *2.3 Antimicrobial susceptibility testing*

138 Agar disk diffusion was performed according to CLSI document M100 (28th ed.) (CLSI,

139 2018). The following antimicrobial agents were tested: penicillin (PEN, 10 IU), gentamicin

140 (GEN, 10 µg), erythromycin (ERY, 15 µg), clindamycin (CLI, 2 µg), tetracycline (TET, 30

141 µg), ciprofloxacin (CIP, 5 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg),

142 chloramphenicol (CHL, 30 µg), and linezolid (LZD, 30 µg). Additionally, the oxacillin MICs

143 were determined by E-test (bioMérieux, Marcy l'Étoile, France). The reference strain *S.*

144 *aureus* ATCC® 29523 served as quality control strain.

145

146 *2.4 Molecular characterization of staphylococcal isolates*

147 In addition to the *mecC* gene, all isolates were screened with primers targeting *mecA*

148 and *mecA1* as described elsewhere (Harrison et al., 2014). A further approach comprised four

149 PCRs for the detection of almost the entire class E *mec* gene complex (*blaZ-mecC-mecR1-*

150 *mecI*). The primers for this approach have been previously described (García-Álvarez et al.,

151 2011, Małyszko et al., 2014) or were designed based on previously described sequence

152 alignments of *mecC* positive *Staphylococcus* spp. available in GenBank. Prior to DNA

153   sequencing, PCR amplicons were cleaned using the GeneJET PCR Purification kit (Thermo

154   Fisher Scientific, Waltham, MA, USA). The obtained DNA sequences were assembled using

155   the CAP3 program (Huang and Madan, 1999). PCR amplification of *ccrA* and *ccrB*

156   recombinase genes was conducted as previously described (García-Álvarez et al., 2011).

157   Primer sequences are listed in Table S2. All PCR amplicons were sequenced. Nucleotide

158   sequences of almost the entire class E *mec* gene complex as well as the *ccrA* and *ccrB* genes

159   were aligned with the accessible corresponding sequences of *mecC*-positive staphylococci

160   deposited in GenBank using ClustalW in MEGA X (Kumar et al., 2018). A maximum

161   likelihood tree was generated using the same software. Tree topologies were estimated using

162   bootstrap analyses with 1000 replicates to accomplish confidence intervals as indicated on

163   each tree node. The distance between the gene *mecI* and the damage inducible gene G (*dinG*)

164   downstream of the class E *mec* complex in *S. stepanovicii* isolates AC983 and Z904, was

165   investigated by PCR (a product of 1138 bp length) which was designed based on known

166   sequences (KR732654 and in isolate 3orsfiwi). The amplicons were sequenced for

167   confirmatory reasons. In *S. sciuri* isolates, the presence of *attR*, *attL* and *attL2* repeats were

168   examined by PCR using combinations of primers P1+P2, P3+P4, and P5+P6, followed by

169   sequence analysis of the amplicons (Harrison et al., 2014). In order to identify more than 300

170   virulence and resistance genes in all isolates, a DNA microarray (*S. aureus* Genotyping Kit

171   2.0, Alere, Jena, Germany) was used (Monecke et al., 2008). For whole genome sequencing

172   (WGS) high quality genomic DNA (gDNA) was isolated from overnight cultures using the

173   MagAttract HMW DNA Kit (Qiagen, Hilden, Germany) and quantified on a Qubit® 2.0

174   Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) using the dsDNA BR Assay Kit

175   (Thermo Fisher Scientific, Waltham, MA, USA). Nextera XT DNA Library Preparation Kit

176   (Illumina, San Diego, CA, USA) was used for library preparation and paired-end sequenced

177   with a read length of 2×300 base pairs on a MiSeq instrument according to the instructions

178   of the manufacturer (Illumina, San Diego, CA, USA). SPAdes version 3.11 and SeqSphere+

179   version 5.1.0 (Ridom, Münster, Germany) were used for read assembly. MLST (multilocus

180   sequence type), resistance genes and virulence genes were extracted from WGS data using

181   SeqSphere+ version 5.1.0 as described (Leopold, et al., 2014, Lepuschitz et al., 2017,

182   Lepuschitz et al. 2018). Antimicrobial resistance and virulence genes were identified in WGS

183   data using the AlereMicroarray data (Strauß et al., 2016), the Comprehensive Antibiotic

184   Resistance Database (CARD) (Jia et al., 2017) and the ResFinder tool-version 3.0 (Zankari et

185   al., 2012) (https://cge.cbs.dtu.dk/services/ResFinder/) with default settings for each database.

186   The presence of virulence genes was extracted from WGS data using AlereMicroarray data

187   (Strauß et al, 2016). The structure of SCC*mec* element in isolate LP600 was determined using

188   CLC Genomics Workbench 10.1.1. (Qiagen, Hilden, Germany) by mapping raw reads against

189   the recently published hybrid SCC*mec-mecC* reference sequence (Accession HG515014)

190   (Harrison et al. 2014).

191

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 72h 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).

217

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 (Figure 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%,

241 93.8% and 92.5% nucleotide sequence identity with the corresponding sequence of *S.*

242 *pseudintermedius* strain KM241 (AM904731).

243   As for the *ccrA* gene, the *ccrB* gene in the *S. caprae* strain Z111 and in the *S. warneri*

244 strain 2800 shared high DNA sequence similarities of 99.9% and 100% with the

245 corresponding sequences of *ccrB* of *mecC*-positive *S. aureus* strains LGA251, M10/0061,

246 ST425, CFSAN064037, ZTA09/03698-9ST, and CMFT540. The *ccrB* gene of the *S. sciuri*

247 isolates LP122, LP254, LP396, LP498 and LP600 shared >99.8% identity with the *ccrB* gene

248 in the *S. sciuri* strain GVGS2. The *ccrB* gene of the *S. sciuri* strain LP187 shared 97.2%

249 nucleotide sequence identity with the *ccrB* gene of the *S. cohnii* strain WC28 (GU370073).

250 The *S. sciuri* isolates LP211 and LP643 shared 93.1% and 93.2% nucleotide sequence identity

251 with the corresponding sequences of *ccrB* in the *S. equorum* strain KS1039 (CP013114).

252 Phylogenetic trees for the *ccrA* and *ccrB* sequences are shown in Figure 1b and c,

253 respectively.

254   PCR amplification of the part of genes the *mecI* and *dinG* downstream of the class E

255 *mec* complex in the *S. stepanovicii* isolates AC983 and Z904 yielded amplicons of the

256 expected size which shared >99.7% nucleotide sequence identity with the corresponding

257 sequences in *mecC*-positive *S. stepanovicii* strains IMT28705 and 3orsfiwi. By using the

258 primer combination for the detection of the *attR* site in the *mecC*-positive *S. sciuri* strain

259 GVGS2, corresponding homologous sequences were detected in the *S. sciuri* isolates LP112,

260 LP254, LP396, LP498 and LP600. The *attL* homologous sequence was detected in all nine

261 examined *mecC*-positive *S. sciuri* isolates. The *attL2* site was detected in all *S. sciuri* isolates

262 except strain LP498.

263   DNA microarray analysis revealed that all three examined *S. stepanovicii* isolates, as

264 well as the single *S. warneri, S. caprae, S. xylosus* isolates carried none of the non-β-lactam

265 resistance genes present on the array. None of the non-β-lactam resistance genes could be

266   detected in the *S. sciuri* isolates LP372, LP396 and LP600. Among the remaining *S. sciuri*

267   isolates, the macrolide-lincosamide-streptogramin B resistance gene *erm*(B) and the phenicol

268   exporter gene *fexA* (n=4, each) were most frequently detected resistance markers. In two *S.*

269   *sciuri* isolates (LP211, LP643), the rRNA methylase gene *cfr*, conferring resistance to

270   phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A, was detected.

271   Virulence genes were rarely observed. The antimicrobial resistance patterns and the resistance

272   and virulence genes detected are summarized in Table 1. The complete results of the

273   microarray analysis are shown in Table S3.

274   Five isolates were subjected to whole-genome sequencing: *S. stepanovicii* 3orsfiwi, *S.*

275   *caprae* Z111, *S. warneri* 2800, *S. xylosus* AD10b and *S. sciuri* LP600. The SCC*mec* element

276   found in strain 3orsfiwi is located between the chromosomal staphylococcal core genes *orfX*

277   and *dusC*. This SCC*mec* element comprised a typical class E *mec* gene cluster consisting of

278   *blaZ, mecC, mecR* and *mecI*. It also comprised the gene *dinG*, which encodes a fusion protein

279   between a helicase and a nuclease (KR732654), the genes of which are also present next to

280   each other in SCC*mec* IX elements of *S. aureus*. The SCC*mec* element in LGA251 comprises

281   twelve genes between the *mec* class E gene cluster and the *dinG* homologue, among them the

282   cassette chromosome recombinase genes *ccrB, ccrA* and cassette chromosome helicase *cch*.

283   Cassette chromosome recombinase and their homologues is completely missing in 3orsfiwi

284   and the gene *dinG* is located immediately downstream of *mecI*. *S. caprae* Z111 and *S. warneri*

285   2800 contain the complete and nearly identical SCC*mec* element as *S. aureus* LGA251. WGS

286   revealed no further non-β-lactam and virulence genes known from *S. aureus* in *S. stepanovicii*

287   3orsfiwi, *S. caprae* Z111, and *S. warneri* 2800. The SCC*mec* element of *S. xylosus* AD10b

288   corresponded to that described in *S. xylosus* strain S04009 (HE993884). Cassette chromosome

289   recombinase and their homologues could not be detected in isolate AD10b. Analysis of the

290   genome sequence identified a *tet*(B) tetracycline resistance gene as only non-beta lactam

291 resistance gene.. The LP600 SCC*mec* element shows the same structure as the reference

292 hybrid SCCmec-mecC sequence, while mecA1 was part of the chromosomal locus as reported

293 in GVGS2. WGS analysis of *S. sciuri* LP600 identified the streptomycin resistance gene *str*

294 and the pleuromutilin-lincosamide-streptogramin A resistance gene *sal*(A) as only non-β-

295 lactam resistance genes. No further virulence genes were detected with the described methods

296 in the investigated isolates.

297

298 **4   Discussion**

299   In the present study, fifteen non-repetitive *mecC*-positive CoNS obtained from various

300 animals were analysed. In Austria, the presence of the *mecC* gene was previously detected in

301 *S. aureus* and *S. stepanovicii* (3orsfiwi) from wildlife as well as in *S. aureus* from goats

302 (Loncaric et al., 2013, Schauer et al., 2018). The presence of *mecC*-positive staphylococci from

303 other animals in Austria has not been described yet. In this study, we have identified two

304 additional staphylococcal species of animal origin, namely *S. caprae* and *S. warneri*, that

305 harbour the *mecC* gene.

306   No major phenotypic and genotypic differences in terms of resistance genes were seen

307 between the three examined *S. stepanovicii* isolates and the recently published *mecC*-positive

308 *S. stepanovicii* IMT28705 (Semmler et al., 2016). *S. caprae* Z111 and *S. warneri* 2800

309 harboured almost identical SCC*mec* elements as described in *mecC*-positive MRSA (Garcia-

310 Alvarez et al. 2011, Shore et al., 2011). So far, two different *mecC*-positive *S. xylosus* isolates

311 have been obtained from bovine mastitis and milk, respectively. Harrison et al. (2013)

312 described a highly related *mecC* homologue present in *S. xylosus* strain S04009, named

313 *mecC1*, which shared 93.5% nucleotide identity with the original *mecC* in *S. aureus* LGA251.

314 A frameshift mutation close to the 5′ end of the *mecC1* gene in S04009 resulted in a truncated

315 64 amino acid (aa) product, which was unable to confer resistance to oxacillin and cefoxitin.

316 This frameshift mutation was also observed in *S. xylosus* AD10b analysed in the present

317 study, which may explain the low oxacillin MIC of this strain and its inability to grow on

318 oxacillin screening agar. Very recently, another *S. xylosus* (strain 47-83) was detected

319 (MacFadyen et al., 2018a), which encodes an intact prototype *mecC* as the one previously

320 found in LGA251. So far, *mecC*-positive *S. xylosus* has never been isolated from brown rat

321 (*Rattus norvegicus*). The predominant staphylococcal species that harboured the *mecC* gene

322 was *S. sciuri*. Besides the *mecC* gene, all *S. sciuri* in the present study harboured also *mecA*

323 and *mecA1* genes, which was also observed in *S. sciuri* GVGS2 (Harrison et al., 2014. Four

324 (LP112, LP254, LP396 and LP600) out of nine examined *S. sciuri* isolates shared almost

325 identical SCC*mec* features, i.e. *mec* gene complex E, *ccrA* and *ccrB* recombinase genes as

326 well as *attR, attL and attL2* repeats as observed in *S. sciuri* GVGS2 (Harrison et al., 2014.

327 While three of the *S. sciuri* isolates (LP187, LP211 and LP643) harboured almost intact *mec*

328 gene complexes of type E as described in *S. sciuri* GVGS2, their *ccrA* and *ccrB* recombinase

329 genes varied slightly from the corresponding genes in *S. sciuri* GVGS2. The *ccrA* genes were

330 most closely related to the respective genes in SCC*mec* type VII from *S. pseudintermedius*

331 strain KM241. This has already been described for *S. sciuri* GVGS2 but could not be

332 observed for the *ccrB* genes in *S. sciuri* isolates LP187, LP211 and LP643. This observation

333 may suggest that these isolates potentially harbour slightly different SCC*mec* elements in

334 comparison to *S. sciuri* GVGS2.

335 Overall, the presence of *mecC* in the examined staphylococci is a rare observation

336 which is in agreement with other studies. Most of the *mecC*-carrying CoNS in the present

337 study originated from non-diseased animals (nasal colonisation), except the *S. warneri* strain,

338 which was from a tissue sample of a diseased cat. Thus, the clinical importance of *mecC*-

339 positive CoNS remains questionable. Interestingly, majority of examined isolates from wild

340 animals originated from predators which may suggest colonization due to consumption of

341   other animals, like small mammals, which are known to be carriers of antibiotic-resistant

342   staphylococci (Hauschild and Schwarz, 2010, Małyszko et al., 2014, Kmeť et al., 2018). On

343   the other hand, the brown rat as a ubiquitous omnivorous synanthrope could easily be

344   colonized with antibiotic-resistant bacteria from humans and other animals. Whether *mecC*-

345   positive CoNS, especially those isolates with almost indistinguishable type E *mec* gene

346   complexes, could function as a possible source of *mecC* for *S. aureus*, as proposed for *mecA*

347   (Couto et al., 1996), remains to be determined. The presence of *mecC* and *ccr* genes in *S.*

348   *caprae* and *S. warneri* isolates with significant similarity to those in *S. aureus* suggests that

349   transfer of these elements between these species could have occurred. In conclusion, this

350   study further expands our knowledge that the *mecC* gene including its allotypes occur in a

351   wider range of staphylococcal species originating from different animal species than has been

352   described previously.

353

354   **Nucleotide accession numbers**

355   Almost entire *mec* E element: MK330607-MK330621, *ccrA* and *ccrB*: MK445226-

356   MK445247. The genomes of two five whole-genome sequenced isolates were deposed under

357   no. PRJEB2655 (ERR599835 ERX556801), PRJNA517387 (SRX5299061-3) in the NCBI

358   BioProject database.

369

370   Conflict of interest statement

371   None to declare

372

373   References

374   Becker, K., Heilmann, C., Peters, G., 2014. Coagulase-negative staphylococci. Clin.

375   Microbiol. Rev. 27, 870-926.

376   Becker, K., van Alen, S., Idelevich, E.A., Schleimer, N., Seggewiß, J., Mellmann, A., Kaspar,

377   U., Peters, G., 2018. Plasmid-encoded transferable *mecB*-mediated methicillin resistance

378   in *Staphylococcus aureus*. Emerg. Infect. Dis. 24, 242-248.

379   Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial

380   Susceptibility Testing: Twenty-eight Informational Supplement M100-28, 2018 Wayne,

381   PA, USA CLSI.

382   Couto, I., de Lencastre, H., Severina, E., Kloos, W., Webster, J.A., Hubner, R.J., Sanches,

383   I.S., Tomasz, A., 1996. Ubiquitous presence of a *mecA* homologue in natural isolates of

384   *Staphylococcus sciuri*. Microb. Drug Resist. 2, 377-391.

385   García-Álvarez, L., Holden, M.T., Lindsay, H., Webb, C.R., Brown, D.F., Curran, M.D.,

386   Walpole, E., Brooks, K., Pickard, D.J., Teale, C., Parkhill, J., Bentley, S.D., Edwards,

387   G.F., Girvan, E.K., Kearns, A.M., Pichon, B., Hill, R.L., Larsen, A.R., Skov, R.L.,

388   Peacock, S.J., Maskell, D.J., Holmes, M.A., 2011. Methicillin-resistant *Staphylococcus*

389   *aureus* with a novel *mecA* homologue in human and bovine populations in the UK and

390   Denmark: a descriptive study. Lancet Infect. Dis.11, 595-603.

391 Harrison, E.M., Paterson, G.K., Holden, M.T.G., Morgan, F.J., Larsen, A.R., Petersen, A.,

392   Leroy, S., De Vliegher, S., Perreten, V., Fox, L.K., Lam, T.J., Sampimon, O.C., Zadoks,

393   R.N., Peacock, S.J., Parkhill, J., Holmes, M.A., 2013. A *Staphylococcus xylosus* isolate

394   with a new *mecC* allotype. Antimicrob. Agents Chemother. 57, 1524–1528.

395 Harrison, E.M., Paterson, G.K., Holden, M.T., Ba, X., Rolo, J., Morgan, F.J., Pichon, B.,

396   Kearns, A., Zadoks, R.N., Peacock, S.J., Parkhill, J., Holmes, M.A., 2014. A novel

397   hybrid SCC*mec-mecC* region in *Staphylococcus sciuri*. J. Antimicrob. Chemother. 69,

398   911-918.

399 Hauschild, T., Schwarz, S., 2010. Macrolide resistance in *Staphylococcus* spp. from free-

400   living small mammals. Vet. Microbiol. 144, 530-531.

401 Huang, X., Madan, A. 1999. CAP3: A DNA sequence assembly program. Genome Res. 9,

402   868-877.

403 Jia, B., Raphenya, A.R., Alcock, B., Waglechner, N., Guo, P., Tsang, K.K., Lago, B.A., Dave,

404   B.M., Pereira, S., Sharma, A.N., Doshi, S., Courtot, M., Lo, R., Williams, L.E., Frye,

405   J.G., Elsayegh, T., Sardar, D., Westman, E.L., Pawlowski, A.C., Johnson, T.A.,

406   Brinkman, F.S., Wright, G.D., McArthur, A.G., 2017. CARD 2017: expansion and

407   model-centric curation of the comprehensive antibiotic resistance database. Nucleic

408   Acids Res. 45(D1), D566-D573.

409 Jiang, N., Li, J., Feßler, A.T., Wang, Y., Schwarz, S., Wu, C., 2018. Novel pseudo-

410   staphylococcal cassette chromosome *mec* element (φSCC*mec*T55) in MRSA ST9. J.

411   Antimicrob. Chemother. doi: 10.1093/jac/dky457. [Epub ahead of print].

412 Katayama, Y., Ito, T., Hiramatsu, K., 2000. A new class of genetic element, *Staphylococcus*

413   cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*.

414   Antimicrob. Agents Chemother. 44, 1549-1555.

415 Kmeť, V., Čuvalová, A., Stanko, M., 2018: Small mammals as sentinels of antimicrobial-

416   resistant staphylococci. Folia Microbiol. (Praha) 63, 665-668.

417 Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular

418   Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35, 1547-

419   1549.

420 Lakhundi, S., Zhang, K., 2018. Methicillin-Resistant *Staphylococcus aureus*: Molecular

421   characterization, evolution, and epidemiology. Clin. Microbiol. Rev. 12, 31(4). pii:

422   e00020-18.

423 Leopold, S.R., Goering, R.V., Witten, A., Harmsen, D., Mellman, A., 2014. Bacterial whole-

424   genome sequencing revisited: portable, scalable, and standardized analysis for typing and

425   detection of virulence and antibiotic resistance genes. J. Clin. Microbiol. 52, 2365-2370.

426 Lepuschitz, S., Huhulescu, S., Hyden, P., Springer, B., Rattei, T., Allerberger, F., Mach, R.L.,

427   Ruppitsch, W., 2018. Characterization of a community-acquired-MRSA USA300 isolate

428   from a river sample in Austria and whole genome sequence based comparison to a

429   diverse collection of USA300 isolates. Sci. Rep. 8, 9467.

430 Lepuschitz, S., Mach, R., Springer, B., Allerberger, F., Ruppitsch, W., 2017. Draft genome

431   sequence of a community-acquired methicillin-resistant *Staphylococcus aureus* USA300

432   isolate from a river sample. Genome Announc. 5. pii: e01166-17. doi:

433   10.1128/genomeA.01166-17.

434 Loncaric, I., Kubber-Heiss, A., Posautz, A., Stalder, G.L., Hoffmann, D., Rosengarten, R.,

435   Walzer, C., 2013. Characterization of methicillin-resistant *Staphylococcus* spp. carrying

436   the *mecC* gene, isolated from wildlife. J. Antimicrob. Chemother. 14, 2222–2225.

437 MacFadyen, A.C., Harrison, E.M., Ellington, M.J., Parkhill, J., Holmes, M.A., Paterson, G.K.,

438   2018a. A highly conserved *mecC*-encoding SCC*mec* type XI in a bovine isolate of

439   methicillin-resistant *Staphylococcus xylosus*. J. Antimicrob. Chemother. 73, 3516-3518.

440 MacFadyen, A.C., Harrison, E.M., Drigo, I., Parkhill, J., Holmes, M.A., Paterson, G.K.,

441 2018b. A *mecC* allotype, *mecC3*, in the coagulase-negative *Staphylococcus*

442 *Staphylococcus caeli,* encoded within a variant SCC*mecC*. J. Antimicrob. Chemother.

443 doi: 10.1093/jac/dky502. [Epub ahead of print].

444 Małyszko, I., Schwarz, S., Hauschild, T., 2014. Detection of a new *mecC* allotype, *mecC2*, in

445 methicillin-resistant *Staphylococcus saprophyticus*. J. Antimicrob. Chemother. 69, 2003-

446 2005.

447 Mellmann, A., Becker, K., von Eiff, C., Keckevoet, U., Schumann, P., Harmsen, D., 2006.

448 Sequencing and staphylococci identification. Emerg. Infect. Dis. 12, 333-336.

449 Monecke, S., Slickers, P., Ehricht, R., 2008. Assignment of *Staphylococcus aureus* isolates to

450 clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol.

451 Med. Microbiol. 53, 237-251.

452 Pantůček, R., Sedláček, I., Indráková, A., Vrbovská, V., Mašlaňová, I., Kovařovic, V., Švec,

453 P., Králová, S., Krištofová, L., Kekláková, J., Petráš, P., Doškař, J., 2018.

454 *Staphylococcus edaphicus* sp. nov., isolated in Antarctica, harbors the *mecC* gene and

455 genomic islands with a suspected role in adaptation to extreme environments. Appl.

456 Environm. Microbiol. 84, e01746-17.

457 Paterson, G.K., Larsen, A.R., Robb, A., Edwards, G.E., Pennycott, T.W., Foster, G., Mot, D.,

458 Hermans, K., Baert, K., Peacock, S.J., Parkhill, J., Zadoks, R.N., Holmes, M.A., 2012.

459 The newly described *mecA* homologue, *mecA*LGA251, is present in methicillin-resistant

460 *Staphylococcus aureus* isolates from a diverse range of host species. J. Antimicrob.

461 Chemother. 67, 2809-2813.

462 Schauer, B., Krametter-Frötscher, R., Knauer, F., Ehricht, R., Monecke, S., Feßler, A.T.,

463 Schwarz, S., Grunert, T., Spergser, J., Loncaric, I., 2018. Diversity of methicillin-

464   resistant *Staphylococcus aureus* (MRSA) isolated from Austrian ruminants and New

465   World camelids. Vet. Microbiol. 215, 77-82.

466 Schwarz, S., Feßler, A.T., Loncaric, I. , Wu, C., Kadlec, K., Wang, Y., Shen, J., 2018.

467   Antimicrobial resistance among staphylococci of animal origin. Microbiol. Spectr. 6(4).

468   doi: 10.1128/microbiolspec.ARBA-0010-2017.

469 Schleifer, K.-H., Bell, J.A. (2009) Family VIII. *Staphylococcaceae* fam. nov. In: De Vos, P.,

470   Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H.,

471   Whitman, W.B. (Eds.), 2009. Bergey's Manual of Systematic Bacteriology, The

472   Firmicutes, vol. 3, second ed., Springer, Dordrecht, pp. 392–433.

473 Semmler, T., Harrison, E.M., Lübke-Becker, A., Ulrich, R.G., Wieler, L.H., Guenther, S.,

474   Stamm, I., Hanssen, A.M., Holmes, M.A., Vincze, S., Walther, B., 2016. A look into the

475   melting pot: the *mecC*-harboring region is a recombination hot spot in *Staphylococcus*

476   *stepanovicii*. PLoS One 11, e0147150. doi: 10.1371/journal.pone.0147150.

477 Shore, A.C., Deasy, E.C., Slickers, P., Brennan, G., O'Connell, B., Monecke, S., Ehricht, R.,

478   Coleman, D.C., 2011. Detection of staphylococcal cassette chromosome *mec* type XI

479   carrying highly divergent *mecA, mecI, mecR1, blaZ*, and *ccr* genes in human clinical

480   isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob.

481   Agents Chemother. 55, 3765–3773.

482 Shore, A.C., Coleman, D.C., 2013. Staphylococcal cassette chromosome *mec*: recent

483   advances and new insights. Int. J. Med. Microbiol. 303, 350-359.

484 Srednik, M.E., Archambault, M., Jacques, M., Gentilini, E.R., 2017. Detection of a *mecC*-

485   positive *Staphylococcus saprophyticus* from bovine mastitis in Argentina. J. Glob.

486   Antimicrob. Resist. 10, 261-263.

487 Strauß, L., Ruffing, U., Abdulla, S., Alabi, A., Akulenko, R., Garrine, M., Germann, A.,

488   Grobusch, M.P., Helms, V., Herrmann, M., Kazimoto, T., Kern, W., Mandomando, I.,

489   Peters, G., Schaumburg, F., von Müller, L., Mellmann, A., 2016. Detecting

490   *Staphylococcus aureus* virulence and resistance genes: a comparison of whole-genome

491   sequencing and DNA microarray technology. J. Clin. Microbiol. 54, 1008-1016.

492   Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup,

493   F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J.

494   Antimicrob. Chemother. 67, 2640-2644.

Table 1:

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

1   **Figure 1 - Maximum Likelihood tree based on the E** *mec* **gene complex (***mecC* **region)**

2   **(a),** *ccrA* **gene (b) and** *ccr*B **(c) gene-sequences of examined** *mecC* **positive coagulase-**

3   **negative** *Staphylococcus* **spp.:** *S. stepanovicii* **(3orsfiwi, AC983, Z904),** *S. caprae*

4   **(Z111),** *S. warneri* **(2800),** *S. xylosus* **(AD10b) and** *S. sciuri* **(LP122, LP187, LP211,**

5   **LP254, LP372, LP396, LP498, LP600 and LP643). Bootstrap values (%) <75 based**

6   **on 100 replicates are given at nodes. Bars indicate substitutions per nucleotide**

7   **position.**

8

9