Veterinary Microbiology 254 (2021) 109005

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Diversity of methicillin-resistant coagulase-negative *Staphylococcus* spp. and methicillin-resistant *Mammaliicoccus* spp. isolated from ruminants and New World camelids

B. Schauer [a,b,1], M.P. Szostak [b,1], R. Ehricht [c,d,e], S. Monecke [c,d,f], A.T. Feßler [g], S. Schwarz [g], J. Spergser [b,2], R. Krametter-Frötscher [a,2], I. Loncaric [b,*]

[a] University Clinic for Ruminants, University of Veterinary Medicine, 1210, Vienna, Austria
[b] Institute of Microbiology, University of Veterinary Medicine, 1210, Vienna, Austria
[c] Leibniz Institute of Photonic Technology (IPHT), 07743, Jena, Germany
[d] InfectoGnostics Research Campus, Philosophenweg 7, 07749, Jena, Germany
[e] Friedrich Schiller University Jena, Institute of Physical Chemistry, 07743, Jena, Germany
[f] Institute for Medical Microbiology and Hygiene, Technical University of Dresden, 01307, Dresden, Germany
[g] Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, 14163, Berlin, Germany

ARTICLE INFO

Keywords:
Coagulase-negative
Staphylococcus spp.
Mammaliicoccus spp.
Mammaliicoccus sciuri group
Methicillin-resistance
Ruminants
New World camelids
MLST

ABSTRACT

Information about livestock carrying methicillin-resistant coagulase-negative staphylococci and mammaliicocci (MRCoNS/MRM) is scarce. The study was designed to gain knowledge of the prevalence, the phenotypic and genotypic antimicrobial resistance and the genetic diversity of MRCoNS/MRM originating from ruminants and New World camelids. In addition, a multi-locus sequence typing scheme for the characterization of *Mammaliicoccus* (formerly *Staphylococcus*) *sciuri* was developed. The study was conducted from April 2014 to January 2017 at the University Clinic for Ruminants and the Institute of Microbiology at the University of Veterinary Medicine Vienna. Seven hundred twenty-three nasal swabs originating from ruminants and New World camelids with and without clinical signs were examined. After isolation, MRCoNS/MRM were identified by MALDI-TOF, *rpoB* sequencing and typed by DNA microarray-based analysis and PCR. Antimicrobial susceptibility testing was conducted by agar disk diffusion. From all 723 nasal swabs, 189 MRCoNS/MRM were obtained. Members of the *Mammaliicoccus* (*M.*) *sciuri* group were predominant (*M. sciuri* (n = 130), followed by *M. lentus* (n = 43), *M. fleurettii* (n = 11)). In total, 158 out of 189 isolates showed phenotypically a multi-resistance profile. A seven-loci multi-locus sequence typing scheme for *M. sciuri* was developed. The scheme includes the analysis of internal segments of the house-keeping genes *ack*, *aroE*, *ftsZ*, *glpK*, *gmk*, *pta1* and *tpiA*. In total, 28 different sequence types (STs) were identified among 92 selected *M. sciuri* isolates. ST1 was the most prevalent ST (n = 35), followed by ST 2 (n = 15), ST3 and ST5 (each n = 5), ST4 (n = 3), ST6, ST7, ST8, ST9, ST10 and ST11 (each n = 2).

1. Introduction

Staphylococci and mammaliicocci are a group of Gram-positive cocci. The genus *Staphylococcus* is the type genus of the family *Staphylococcaceae* (Madhaiyan et al., 2020). This family comprises currently ten genera, including *Abyssicoccus, Aliicoccus, Auricoccus, Corticicoccus, Jeotgalicoccus, Macrococcus, Mammaliicoccus, Nosocomiicoccus, Salinicoccus* and *Staphylococcus* (Madhaiyan et al., 2020). Very recently, five well know staphylococcal species belonging to *Staphylococcus* (*S.*) *sciuri* group (*S. sciuri, S. fleurettii, S. lentus, S. stepanovicii* and *S. vitulinus*) were reassigned to the novel genus *Mammaliicoccus* with *Mammaliicoccus* (*M.*) *sciuri* as the type species (Madhaiyan et al., 2020).

Staphylococci and mammaliicocci are considered to be opportunistic pathogens, frequently appearing as colonizers but also being able to cause a wide range of severe infections (Nemeghaire et al., 2014a; Lakhundi and Zhang, 2018). *S. aureus* is a common cause of both,

* Corresponding author.
E-mail address: igor.loncaric@vetmeduni.ac.at (I. Loncaric).
[1] Shared first author.
[2] Shared last author.

https://doi.org/10.1016/j.vetmic.2021.109005
Received 6 December 2020; Accepted 29 January 2021
Available online 4 February 2021

community acquired and nosocomial infections in humans as well as in animals worldwide (Lakhundi and Zhang, 2018). Members of the *M. sciuri* group are often detected in animals, humans and the environment, generally considered harmless, but also found in diseased animals (Nemeghaire et al., 2014a; Loncaric et al., 2019b). *M. sciuri* was isolated from several cases of bovine mastitis, from sick goats, piglets and even from cases of canine dermatitis (Nemeghaire et al., 2014a).

Bacterial isolates classified as multi-resistant are non-susceptible to at least one antimicrobial agent from three or more antibiotic classes (Sweeney et al., 2018). Some multi-resistant staphylococcal species have been thoroughly investigated (e.g. methicillin-resistant *S. aureus* and methicillin-resistant *S. pseudintermedius*), but comparatively little is known about the clinical importance of methicillin-resistant coagulase-negative staphylococci and mammaliicocci (MRCoNS/MRM). Nevertheless, these MRCoNS/MRM are carriers of antimicrobial resistance genes, possibly able to act as donors to transferring such genes to other staphylococci, like *S. aureus* (Nemeghaire et al., 2014a). MRCoNS/MRM developed resistance to most beta-lactam antibiotics via the expression of the acquired *mecA* or *mecC* genes, which encode alternative penicillin-binding proteins with a low affinity for beta-lactams (Schwarz et al., 2018; Lakhundi and Zhang, 2018). A second resistance mechanism against penicillin is commonly mediated via enzymatic inactivation of the drug by the *blaZ*- or bla_{ARL}-encoded β-lactamases (Schwarz et al., 2018). It was reported that members of the *M. sciuri* group can carry homologues of the methicillin resistance gene *mecA,* however, these homologues do not confer clinical methicillin resistance (Nemeghaire et al., 2014a), and are – unlike the *mecA* gene – also not located on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*) (Schwarz et al., 2018). On the other hand, methicillin resistance-mediating *mecA* or *mecC* gene variants carried on SCC*mec* elements were also described in members of *M. sciuri* group (Schwarz et al., 2018; Loncaric et al., 2019a).

Information about MRCoNS/MRM in Austrian ruminants and New World camelids is scarce. Loncaric et al. (2019a) described ruminants and Alpacas to be the host of MRCoNS and *M. sciuri* carrying the *mecC* gene. In recent years, the numbers of New World camelids have continuously increased in Austria, including the smaller Alpaca (*Lama pacos*) as well as the heavier Llama (*Lama glama*), Guanaco (*Lama guanicoe*) and Vicuña (*Lama vicugna*). As these animals are kept in close contact to humans, they could be considered as a source of human infections with *M. sciuri* (Shittu et al., 2004) or as sources of mobile genetic elements carrying resistance genes that might be transferred to human colonizers and pathogens.

The aims of the present study were: i) to detect the prevalence of different MRCoNS/MRM associated with Austrian ruminants and New World camelids, which were presented to the Clinic for Ruminants at the University of Veterinary Medicine in Vienna, Austria (passive surveillance); ii) to investigate phenotypic and genotypic resistance of identified strains, and iii) to develop a multi-locus sequence typing (MLST) scheme for *M. sciuri* to analyze the clonal relatedness among isolates.

2. Materials and methods

2.1. Bacterial isolates

The study was conducted from April 2014 to January 2017 at the University Clinic for Ruminants at the University of Veterinary Medicine in Vienna, Austria. In total, 723 nasal swabs originating from ruminants including cattle (n = 221), calves (n = 143), goats (n = 96) and sheep (n = 134) and New World camelids including llamas (n = 30) and alpacas (n = 99), which were patients or companion animals of patients, were collected as previously described by Schauer et al. (2018). One nasal swab per animal was taken. The study was discussed and the nasal swabbing was approved by the institutional ethics and animal welfare committee in accordance with the Good Scientific Practice guidelines of the University of Veterinary Medicine, Vienna and Austrian national legislation.

Nasal swabs were transferred to the Institute of Microbiology and incubated in tryptic soy broth (Beckton Dickinson (BD); Heidelberg, Germany) with 6.5 % (w/v) NaCl overnight and subsequently streaked onto Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom) with 2.5 % (w/v) NaCl, 2 mg/L oxacillin and 20 mg/L aztreonam (MHOXA) and BD™ Columbia CNA Agar with 5 % Sheep Blood, Improved II (CNA). Colonies showing the typical colony appearance of staphylococci or mammaliicocci after incubation were selected and re-cultivated on the same medium until purified and subsequently spotted onto BD™ Oxacillin Screen Agar BD. Isolates which grew on Oxacillin Screen Agar were saved at -80 C for further analyses.

2.2. Identification of staphylococcal and mammaliicoccal isolates

Isolates were identified to the species level by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik, Bremen, Germany) and rechecked using *rpoB* sequencing (Mellmann et al., 2006).

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by agar disk diffusion according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute (CLSI), 2018) for the following antimicrobial agents: cefoxitin (FOX 30 µg), ciprofloxacin (CIP 5 µg), amikacin (AMK 30 µg), gentamicin (GEN 10 µg), tetracycline (TET 30 µg), erythromycin (ERY 15 µg), clindamycin (CLI 2 µg), chloramphenicol (CHL 30 µg), trimethoprim-sulfamethoxazole (SXT 1.25/23.75 µg), nitrofurantoin (NIT 300 µg), rifampicin (RIF 5 µg), linezolid (LZD 30 µg) (Beckton Dickinson (BD); Heidelberg, Germany). Inducible clindamycin resistance was detected by agar disk diffusion using the D-zone test (Clinical and Laboratory Standards Institute (CLSI), 2018). Staphylococcal interpretative criteria have also been applied for mammaliicocci.

2.4. Detection of antimicrobial resistance genes

Isolates were recultivated overnight on BD Columbia III agar with 5 % sheep blood (Beckton Dickinson (BD); Heidelberg, Germany), staphylococcal or mammaliicoccal DNA was extracted following the recommendation of *S. aureus* Genotyping Kit 2.0 using a commercially available extraction kit (GenElute™ Mammalian Genomic DNA Miniprep Kits, Sigma-Aldrich, Vienna, Austria). Detection of antimicrobial resistance and virulence genes was conducted using a DNA microarray-based technology to detect 336 different target sequences, including antimicrobial resistance and virulence-associated genes, species markers and SCC*mec*-associated genes (*S. aureus* Genotyping Kit 2.0, Abbott (AlereTechnologies GmbH), Jena, Germany) (Monecke et al., 2008). For visualization of the diversity of the DNA microarray results (Coombs et al., 2010), the program SplitsTree4 on default settings (Huson and Bryant, 2006) was used. Further PCR assays were performed for following resistance genes which were not included in the DNA microarray: *tet*(L), *erm*(T), *erm*(43), *erm*(44), *lsa*(B), *vga*(C), *vga*(E), $vga(E)_v$, *sal*(A), *dfrD*, *dfrG, dfrK* (Hauschild et al., 2007; Argudín et al., 2011; Schwendener and Perreten, 2012; Li et al., 2014; Wipf et al., 2014; Wendlandt et al., 2015).

2.5. Development of multi-locus sequence typing (MLST) for *Mammaliicoccus sciuri*

Clonal relatedness among selected *M. sciuri* isolates was examined by a newly developed MLST scheme. For this, 92 *M. sciuri* isolates were selected from the pool of isolated *M. sciuri,* based on their pheno- and genotypic characteristics as well as of their origin, aiming for the highest possible genetic divergence. Several potential housekeeping gene

fragments, selected from loci that had been tested for other staphylococcal MLST schemes (https://pubmlst.org/organisms) and those from the *M. sciuri* (*S. sciuri*) NCTC12103 genome (GenBank accession no. LS483305.1). Internal segments of the seven house-keeping genes *ack*, *aroE*, *ftsZ*, *glpK*, *gmk*, *pta1* and *tpiA* were used for the MLST. Using Primer3Plus software tool (Untergasser et al., 2007), PCR primers were designed to amplify parts of target genes, which, as work progressed, revealed themselves to be highly conserved. The primer sequences, annealing temperatures, amplicon sizes and the position in the amplicon used for MLST are given in Table 1. The PCR reactions were performed starting with 94 °C for 300 s, followed by 30 cycles of 30 s at 94 °C, 30 s at the respective annealing temperatures (55 °C except for *ftsZ* (53 °C)), 1 min at 72 °C and a final extension at 72 °C for 5 min (Table 1). The amplicons were further purified and sequenced with the same primer pair used for amplification in both directions at LGC, Berlin, Germany. Sequences were analyzed using the commercial software package Geneious (Biomatters, New Zealand). An arbitrary allelic number was assigned to each locus sequence, with identical sequences being assigned the same allele number. Each isolate was then characterized on the basis of its combination of alleles at the seven loci and assigned to sequence types (STs). Clonal complexes (CC) were defined in which all STs within a clonal complex differ by no more than one allele from at least one other ST in the clonal complex (Holden et al., 2004). Allelic and ST diversity was analyzed by Simpson's index of diversity (Hunter and Gaston, 1988; Hunter, 1990). The relationship of STs was studied using goeBURST implemented in PHYLOViZ (Francisco et al., 2012). Founder STs were determined as the ST associated with the most single locus variants (SLVs). Minimum spanning (MS) trees were generated from the seven alleles using the same software.

All sequences were deposited in the *M. sciuri* MLST database. The database is developed in the frame of the present study and will be publicly available (https://pubmlst.org/organisms/mammaliicoccus-sciuri/). The database is hosted at PubMLST (Jolley et al., 2018).

3. Results

3.1. MRCoNS/MRM isolates

In total 189 (25.9 %) MRCoNS/MRM isolates were detected in the 723 nasal swabs. The species prevalence of calves colonized with MRCoNS/MRM isolates was 53.8 % (n = 77). Furthermore, species prevalence's of MRCoNS/MRM isolates were detected as followed from adult cattle 24.4 % (n = 54), breeding and companion goats 21.9 % (n = 21), sheep 14.2 % (n = 19), as well as New World camelids, i.e., alpacas 10.1 % (n = 10) and llamas 26.7 % (n = 8). The most commonly detected species was *M. sciuri* with 130 isolates which are divided into 51 isolates from calves, 32 isolates from cows, 17 sheep, 16 goats, seven alpacas and seven llamas. Followed by *M. lentus* (n = 43) which was found in 24 calves, eleven cows, four goats, three alpacas and once in a sheep. *M. fleurettii* (n = 11) was detected in eight cows, two calves and in one llama. *S. haemolyticus* (n = 5) was detected in three cows, an goat and in an sheep (Tables S1 and S2)

3.2. Antimicrobial susceptibility testing and detection of resistance genes

All isolates belonging to the *M. sciuri* group showed β-lactam resistance, which was mediated by (i) the *mecA* gene (n = 130, 100 %) located on a PseudoSCC*mec* [class A] element, SCC [*mec* complex class A+*ccr*AB1] element or an SCC*mec* III element (Table S2) and (ii) complete (n = 44, 33.8 %) or truncated (n = 2, 1.5 %) *bla*I/*bla*R/*blaZ* operons. Furthermore, 122 (93.8 %) *M. sciuri* isolates were phenotypically resistant to tetracycline, which is well reflected by the observation that these isolates carried the gene *tet*(M) and/or the genes *tet*(K) or *tet*(L) gene in various combinations (*tet*(M)(n = 83) only, *tet*(K)(n = 2) only, *tet* (L)(n = 14) only and *tet*(M)/*tet*(K)(n = 21) in combination) (Tables 2 and 3). Resistances to clindamycin (n = 81, 62.3 %) and erythromycin (n = 68, 52.3 %) (*lnu*(A), *sal*(A), *erm*(A), *erm*(B), *erm*(C), *erm*(44)), ciprofloxacin (n = 107, 82.3 %), chloramphenicol (n = 61, 46.9 %) (*fexA*, cat_{pC221}), trimethoprim-sulfamethoxazole (n = 51, 39.2 %) (*dfrD*, *dfrG*, *dfrK*), gentamicin (n = 35, 26.9 %) (*aacA-aphD*) and linezolid (n = 6, 4.6 %) (*cfr*) were also observed (Table S1). An inducible clindamycin resistance was detected in one *M. sciuri* isolate (LP544). All isolates were susceptible to rifampicin and nitrofurantoin. The majority of isolates displayed a multi-resistance phenotype (n = 114, 87.7 %) (Sweeney et al., 2018). Interestingly, two *M. sciuri* isolates yielded signals with *S. aureus*-specific probes for *lukS* (ST22 + ST45) and one for toxic shock syndrome toxin *tst* gene. Genes for the resistance to biocides were detected in three *M. sciuri* isolates carrying the *qacC* gene (Table S1).

The species *M. lentus* was detected in 43 nasal swabs. All of them showed a phenotypic resistance against β-lactams mediated by *mecA* carried either on a PseudoSCC*mec* [class A] element or on a SCC*mec* III element (Table S2). All except two were resistant to tetracycline (n = 41, 95.3 %) and carried the genes *tet*(K) (n = 35), *tet*(L) (n = 4), *tet*(M) (n = 1) or *tet*(K) + *tet*(M) (n = 1). Resistances against trimethoprim-sulfamethoxazole (n = 33, 76.7 %) (*dfrG* (n = 15), *dfrK* (n = 3), *dfrG* + *dfrK* (n = 15)), chloramphenicol (n = 26, 60.5 %) (*fexA* (n = 19), *cat* (n = 1), *fexA* + *cfr* (n = 6)), clindamycin (n = 25, 58.1 %) (*lnu*(A) (n = 8), *cfr* (n = 7), *erm*(B) (n = 2), *erm*(C) (n = 1), *erm*(A) + *lnu*(A) (n = 1), *erm* (B) + *lnu*(A) (n = 1) and *erm*(A) + *erm*(B) + *lnu*(A) (n = 2)), erythromycin (n = 17, 39.5 %) (*mph*(C) (n = 33), *erm*(A) + *mph*(C) (n = 2), *erm* (B) + *mph*(C) (n = 3), *erm*(C) + *mph*(C) (n = 1), *erm*(A) + *erm*(B) + *mph* (C) (n = 2)) and ciprofloxacin (n = 20, 46.5 %) was widespread (Tables 2, 3 and S1). Six isolates showed resistance to linezolid (n = 6, 14.0 %), all of them carried the *cfr* gene. All *M. lentus* isolates were susceptible

Table 1
Primers for PCR amplification of the seven loci for multi-locus sequence typing (MLST) of *Mammaliicoccus sciuri*.

Locus	Primer	Primer sequence (5′-3′)	Primer binding site in gene (*)	Product size (bp)	MLST region (*)	MLST region (size in bp)	Number of allels	Number of polymorphic sites
aroE1	aroE1_F	GCCATTCACTTTCACCGATT	32–51	728	136–642	507	6	46
	aroE1_R	AGCTTCTTGCCCAGTCCATA	759–740					
pta1	pta1_F	TATCGGCGACAATTGCTGTA	38–57	846	220–786	567	6	79
	pta1_R	GCATTAACGCACTACAAATAGAATG	883–859					
gmk	gmk_F	TAGTGCTCTCTGGCCCATCT	26–45	521	103–468	366	7	14
	gmk_R	GGCTTGAATACGCTCTTTCG	546–527					
tpiA	tpiA_F	CGCAGGTAACTGGAAAATGAA	18–38	691	154–630	477	11	12
	tpiA_R	GCCAACTAATGCGCCATC	708–691					
ack	ack_F	TCCAATTATTTGAGATGCCTGA	47–68	950	292–786	495	4	16
	ack_R	TTCCCCTACACCAGCTGTAAA	996–976					
glpK	glpK_F	TTGGTGCTAAGCCAGAAGAAA	194–214	872	280–990	711	10	53
	glpK_R	CGCCCTTTCACCAGCTAAAT	1065–1046					
ftsZ	ftsZ_F	ATTTGARCAAGGRTTTAATC	9–28	663	169–555	663	8	7
	ftsZ_R	GCWGAACCTTGGTTAGAC	671–654					

* Numbers indicate nucleotide positions inside each gene according to the Genbank entry for *M. sciuri* type strain NCTC12103 (NZ_LS483305.1).

Table 2
Resistance genes and their numbers and prevalence rates.

Gene	Explanation	Number	Percent
mecA	Alternative penicillin binding protein 2	189	100.00 %
blaZ	beta-lactamase gene	51	26.98 %
blaI	beta-lactamase repressor (inhibitor)	49	25.93 %
blaR	beta-lactamase regulatory protein	49	25.93 %
erm(A)	rRNA adenine N-&-methyltransferase, erythromycin/clindamycin resistance	11	5.82 %
erm(B)	rRNA adenine N-&-methyltransferase, erythromycin/clindamycin resistance	66	34.92 %
erm(C)	rRNA adenine N-&-methyltransferase, erythromycin/clindamycin resistance	12	6.35 %
erm (44)	rRNA adenine N-&-methyltransferase, erythromycin/clindamycin resistance	1	0.53 %
lnu(A)	Lincosamid-nucleotidyltransferase	30	15.87 %
msr(A)	macrolide efflux pump	2	1.06 %
mph(C)	Probable lysylphosphatidylglycerol synthetase, macrolide resistance	43	22.75 %
sal(A)	Target site protection (ribosome protective ABC-F protein)	6	3.17 %
aacA-aphD	Bifunctinal enzyme Aac/Aph, resistance towards gentamicin, tobramycin, kanamycin and amikacin	62	32.80 %
aadD	Aminoglycoside adenyltransferase, resistance towards tobramycin, kanamycin and neomycin	38	20.11 %
aphA3	3′5′-aminoglycoside phospho-transferase, resistance to neomycin, kanamycin and amikacin	6	3.17 %
sat4	Streptothricine-acetyltransferase	5	2.65 %
tet(K)	Tetracycline efflux protein	64	33.86 %
tet(M)	ribosomal protection protein associated with tetracycline resistance	108	57.14 %
tet(L)	Tetracycline resistance	18	9.52 %
cat	Chloramphenicol acetyltransferase	3	1.59 %
cfr	23S rRNA methyltransferase	21	11.11 %
fexA	Chloramphenicol/florfenicol exporter	86	45.50 %
dfrD	Target replacement (trimethoprim-resistant dihydrofolate reductase)	1	0.53 %
dfrG	Target replacement (trimethoprim-resistant dihydrofolate reductase)	45	23.81 %
dfrK	Target replacement (trimethoprim-resistant dihydrofolate reductase)	61	32.28 %
qacC	Quaternary ammonium compound resistance protein C	6	3.17 %

to rifampicin, nitrofurantoin and all but five were susceptible to gentamicin (n = 5, 11.6 %). Four of these five *M. lentus* isolates harbored the gene *aacA-aphD*. The detected resistance genes reflected well the phenotypic finding (Table S1). Out of 43 *M. lentus* strains, 39 showed a multi-resistance phenotype (Sweeney et al., 2018). Among *M. lentus,* no virulence genes were detected, but three isolates were found to carry the *qacC* gene for resistance to quaternary ammonium compounds.

M. fleurettii was less frequently isolated (n = 11). All *M. fleurettii* isolates showed only phenotypic resistance to β-lactams (n = 11, 100 %) and carried the *mecA* gene (PseudoSCC*mec* [class A] element or SCC*mec* III element) (Table S1). Virulence genes or genes for biocide resistance were not detected in the *M. fleurettii* isolates.

The five detected *S. haemolyticus* isolates carried, in addition to the *mecA*, also the *blaZ/blaI/blaR* operon and the resistance genes *erm*(C), *aacA-aphD*, *aphA3*, *sat4*, *tet*(K), *dfrG* and *dfrK* in three isolates as well as *msr*(A), *mph*(C) and *fexA* in two isolates (Table S1). The phenotypic resistance patterns mirrored the genotypic findings. The five detected isolates of *S. haemolyticus* carried the SCC*mec* V/VT element. All five *S. haemolyticus* isolates were multi-resistant.

Analysis of the similarities between gene profiles via the SplitsTree software revealed that MRSH isolates fall into a separate cluster (Fig. 1). In contrast, the majority of *M. sciuri* isolates were separated into three clusters, the majority of *M. lentus* isolates were assigned to one cluster (Fig. 1).

Table 3
Occurrence of a combination of antimicrobial resistance gene in isolates examined in the present study.

β-Lactams	
mecA, blaZ, blaI, blaR	n = 49
mecA, blaZ	n = 2
Aminoglycosides	
aacA-aphD, aadD	n = 8
aacA-aphD, aphA3	n = 5
Tetracyclines	
tet(K), *tet*(M)	n = 24
Macrolides, lincosamides, streptogramin A and B, pleuromutilins	
erm(B), lnu(A)	n = 9
mph(C), lnu(A)	n = 11
erm(B), mph(C)	n = 5
erm(A), *erm*(B), *lnu*(A)	n = 2
erm(A), *erm*(B), *mph*(C), *lnu*(A)	n = 1
erm(B), *mph*(C), *lnu*(A)	n = 2
erm(A), *erm*(C)	n = 3
erm(A), *mph*(C), *lnu*(A)	n = 1
erm(44), *sal*(A)	n = 1
erm(A), *erm*(C), *lnu*(A)	n = 1
erm(C), *mph*(C)	n = 1
erm(C), *msr*(A), *mph*(C)	n = 2
erm(A), *mph*(C)	n = 1
Phenicols, lincosamides, oxazolidinones, pleuromutilins, streptogramin A	
cfr, fexA	n = 21
cat_{pC221}, *fexA*	n = 1
Trimethoprim	
dfrG, dfrK	n = 25

3.3. Multi-locus sequence typing (MLST)

All seven MLST loci were successfully amplified in all examined isolates and yielded amplicons of the expected sizes. The number of alleles per locus ranged from four (*ack*) to eleven (*tpiA*), while allelic diversity ranged between 0.2568 (*ack*) and 0.6964 (*tpiA*). Among all examined isolates, 28 different STs were identified, which resulted in a ST diversity of 0.8258 by Simpson's index. Among the 92 selected *M. sciuri* isolates, ST1 was the most prevalent ST (n = 35), followed by ST 2 (n = 15), ST3 and ST5 (each n = 5), ST4 (n = 3), ST6, ST7, ST8, ST9, ST10 and ST11 (each n = 2). In addition, seventeen singletons (ST12-ST28) were detected (Table 1). The MS tree of the 28 STs interfered by PHYLOViZ revealed five CCs. CC1 comprised eight STs: ST1 (founder), ST4, ST6, ST13, ST15, ST16, ST19, and ST23, CC2 (ST2 and ST7), CC5 (ST5 and ST12), CC11 (ST11 and ST20), and CC21 (ST21 and ST25) (Fig. 2).

4. Discussion

Comprehensive studies about MRCoNS/MRM are rare, especially those considering the nasal carriage rates as well as pheno- and genotypic characterization and analyses of antimicrobial resistance. Several studies were performed on the nasal carriage rates of CoNS and/or MRCoNS/MRM and their phenotypic antimicrobial susceptibility but without genotyping (Zhang et al., 2009; Huber et al., 2011; Vanderhaeghen et al., 2013). The colonization of small ruminants and New World camelids cannot be directly compared due to a lack of comparable data. The present study describes the presence of MRCoNS/MRM carrying the *mecA* genes from nasal swabs from cows, calves, goats, sheep, llamas and alpacas in Austria. MRM hosting the *mecC* gene have already been described in Austrian livestock by Loncaric et al. (2019a). All sampled animals did not show clinical signs that could be linked with the colonization by MRCoNS/MRM. Among the isolated MRCoNS/MRM,

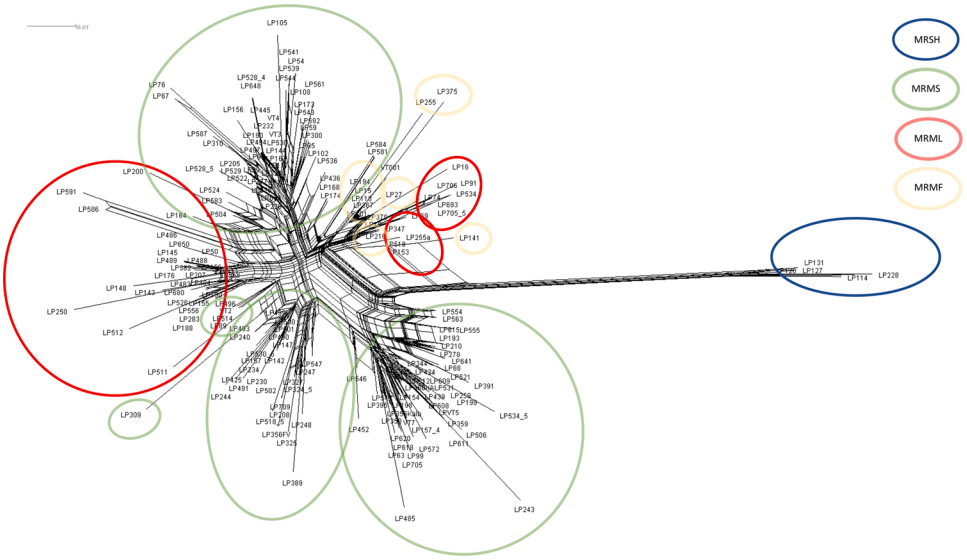

Fig. 1. SplitsTree network of methicillin-resistant coagulase negative *Staphylococcus* and methicillin-resistant *Mammaliicoccus* isolates investigated. MRSH, methicillin-resistant *Staphylococcus haemolyticus*; MRMS, methicillin-resistant *Mammaliicoccus sciuri*; MRML methicillin-resistant *Mammaliicoccus lentus*; MRMF, methicillin-resistant *Mammaliicoccus fleurettii.*

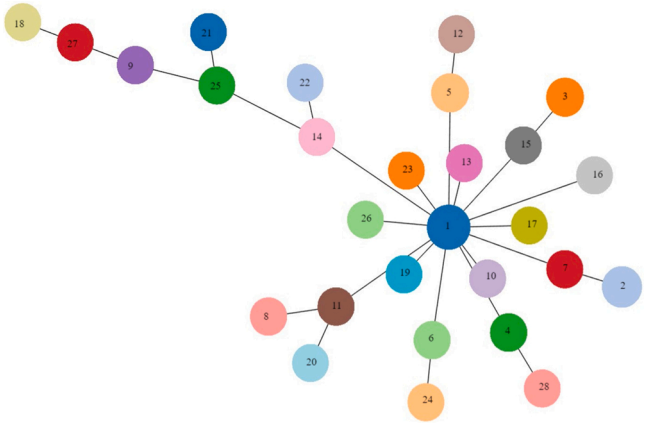

Fig. 2. Minimum spanning tree based on the MLST of *M. sciuri*. Colours and numbers correspond to the sequence types. Numbers (1-28) correspond to sequence types.

four different species were identified, *S. haemolyticus* and three species belonging to the genus *Mammaliicoccus*. In our study, *M. sciuri* was predominating, followed by *M. lentus*, *M. fleurettii* and *S. haemolyticus.*

Data of the present study revealed that the *M. sciuri* species group members are widely present in the examined population and carry a wide variety of antimicrobial genes. They were often detected in various combinations. The majority of these genes are also found in staphylococci. Beside *mecA* gene, the *blaZ-blaI-blaR1* resistance operon confers resistance to penicillins except isoxazolyl-penicillins (Schwarz et al., 2018), was detected solely in MR *M. sciuri,* which was already described in this particular species (Nemeghaire et al., 2014b). Phenotypic resistance to tetracyclines was observed in the majority of MR *M. sciuri* and MR *M. lentus* isolates and was mediated by *tet*(K), *tet*(M) and *tet*(L) alone or in combination. Studies have shown that the carriage of *tet* genes in *M. sciuri* group members is common (Bhargava and Zhang, 2012; Nemeghaire et al., 2014a, c). Interestingly the *tet*(K) gene was detected more often in MR *M. lentus* than in MR *M. sciuri*. Phenotypic resistance to erythromycin and clindamycin was often abundant among *M. sciuri* and *M. lentus*. Resistance to macrolides, lincosamides, and streptogramins (MLS) is based on various genes. MLS genes *erm*(A), *erm*(B), *erm*(C), *erm* (44), *lnu*(A), *sal*(A) and *mph*(C) could be detected in our study. All of them have already been observed in MR *M. sciuri* and MR *M. lentus* in the past (Huber et al., 2011; Vanderhaeghen et al., 2013; Nemeghaire et al., 2014c). *sal*(A) and *erm*(44) were detected solely in MR *M. sciuri*, whereas *mph*(C) gene was detected exclusively in MR *M. lentus*. Furthermore, it should be noted that the *mph*(C) gene was already detected in *M. lentus*, whose gene product does not confer macrolide resistance (Hauschild and Schwarz, 2010). The trimethoprim resistance genes *dfrD*, *dfrG* and *dfrK* were detected among MR *M. sciuri* and MR *M. lentus* isolates. The information about *dfr* genes among members of *M. sciuri* group is still scarce (reviewed in Nemeghaire et al., 2014a and Schwarz et al., 2018). Phenotypic resistance to gentamicin was observed in 35 MR *M. sciuri* and 5 MR *M. lentus* isolates. Interestingly, 21 isolates carried the *aacA-aphD* resistance gene without phenotypic gentamicin resistance, which was already reported in *S. pseudintermedius* (Perreten et al., 2010). The resistance genes *fexA*, cat_{pC221}, and *cfr* which mediated phenotypic resistance to all phenicols (*fexA*), chloramphenicol (cat_{pC221}) and all phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A (*cfr*) were detected in the present study supporting previous observations (Schwarz et al., 2018).

The presence of MR *S. haemolyticus* was 2.7 %. This result is in approximative accordance with the findings from other countries (Huber et al., 2011; Bhargava and Zhang, 2012; Vanderhaeghen et al., 2013), carriage rates in humans have been described to be higher (Faria et al., 2014).

The biocide resistance gene *qacC* was detected in MR *M. sciuri* and MR *M. lentus* isolates. The presence of this gene had already been observed in *M. sciuri* (Nemeghaire et al., 2014b) but not in *M. lentus*. Information about virulence and toxin genes in MR *M. sciuri* group is limited.

MLST is a well-established method for genotyping different bacteria based on the sequence variation of defined housekeeping genes. MLST provides data useful for worldwide epidemiological studies and studies on evolution and population genetics. Since there is no universal tool for interlaboratory comparison of different *M. sciuri* isolates, the 7-locus MLST scheme for *M. sciuri* was developed. To obtain more data about *M. sciuri*, more diverse *M. sciuri* population, the greater number of isolates originated from different geographical areas and more host species should be included in the studies to come. Nevertheless, we strongly believe that the data generated by *M. sciuri* MLST will be globally shared to obtain detailed information on this particular species.

5. Conclusion

The present study demonstrated that the MR members of *M. sciuri* group are common in ruminants and New World camelids. They contain a large exchangeable antimicrobial gene reservoir for other closely related bacterial species, mainly in staphylococci but also in other bacteria.

It is hoped that the new *M. sciuri* MLST scheme developed in the present will be expanded to include isolates originated from different animal species and humans as well as other countries to provide more details for this particular species. Taking into account the close proximity between domesticated ruminants/New World camelids and humans, our results undoubtedly identified a public health issue because of bidirectional interspecies transmission of MRCoNS and MRM.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The research work was supported by the Austrian Buiatric Association and by internal funding from the Institute of Microbiology, University of Veterinary Medicine Vienna, Austria. The contribution of ATF and SS was supported by the German 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.2021.109005.

References

Argudín, M.A., Tenhagen, B.-A., Fetsch, A., Sachsenröder, J., Käsbohrer, A., Schroeter, A., Hammerl, J.A., Hertwig, S., Helmuth, R., Bräunig, J., Mendoza, M.C., Appel, B., Rodicio, M.R., Guerra, B., 2011. Virulence and resistance determinants of German *Staphylococcus aureus* ST398 isolates from nonhuman sources. Appl. Environ. Microbiol. 77, 3052–3060.

Bhargava, K., Zhang, Y., 2012. Multidrug-resistant coagulase-negative *Staphylococci* in food animals. J. Appl. Microbiol. 113, 1027–1036.

Clinical and Laboratory Standards Institute (CLSI), 2018. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-eight Informational Supplement M100-28. CLSI supplement VET08. Wayne, PA, USA.

Coombs, G.W., Monecke, S., Ehricht, R., Slickers, P., Pearson, J.C., Tan, H.L., Christiansen, K.J., O'Brien, F.G., 2010. Differentiation of clonal complex 59 community-associated methicillin-resistant *Staphylococcus aureus* in Western Australia. Antimicrob. Agents Chemother. 54, 1914–1921.

Faria, N.A., Conceição, T., Miragaia, M., Bartels, M.D., de Lencastre, H., Westh, H., 2014. Nasal carriage of *methicillin resistant Staphylococci*. Microb. Drug Resist. 20, 108–117.

Francisco, A.P., Vaz, C., Monteiro, P.T., Melo-Cristino, J., Ramirez, M., Carriço, J.A., 2012. PHYLOViZ: phylogenetic inference and data visualization for sequence based typing methods. BMC Bioinform. 13, 87.

Hauschild, T., Schwarz, S., 2010. Macrolide resistance in *Staphylococcus spp*. from free-living small mammals. Vet. Microbiol. 144, 530–531.

Hauschild, T., Stepanović, S., Dakić, I., Djukić, S., Ranin, L., Ježek, P., Schwarz, S., 2007. Tetracycline resistance and distribution of *tet* genes in members of the *Staphylococcus sciuri* group isolated from humans, animals and different environmental sources. Int. J. Antimicrob. Agents 29, 356–358.

Holden, M.T.G., Feil, E.J., Lindsay, J.A., Peacock, S.J., Day, N.P.J., Enright, M.C., Foster, T.J., Moore, C.E., Hurst, L., Atkin, R., Barron, A., Bason, N., Bentley, S.D., Chillingworth, C., Chillingworth, T., Churcher, C., Clark, L., Corton, C., Cronin, A., Doggett, J., Dowd, L., Feltwell, T., Hance, Z., Harris, B., Hauser, H., Holroyd, S., Jagels, K., James, K.D., Lennard, N., Line, A., Mayes, R., Moule, S., Mungall, K., Ormond, D., Quail, M.A., Rabbinowitsch, E., Rutherford, K., Sanders, M., Sharp, S., Simmonds, M., Stevens, K., Whitehead, S., Barrell, B.G., Spratt, B.G., Parkhill, J., 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc. Natl. Acad. Sci. U.S.A. 101, 9786–9791.

Huber, H., Ziegler, D., Pflüger, V., Vogel, G., Zweifel, C., Stephan, R., 2011. Prevalence and characteristics of methicillin-resistant coagulase-negative *staphylococci* from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. BMC Vet. Res. 7, 6.

Hunter, P.R., 1990. Reproducibility and indices of discriminatory power of microbial typing methods. J. Clin. Microbiol. 28, 1903–1905.

Hunter, P.R., Gaston, M.A., 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. 26, 2465–2466.

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267.

Jolley, K.A., Bray, J.E., Maiden, M.C.J., 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 3, 124.

Lakhundi, S., Zhang, K., 2018. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin. Microbiol. Rev. 31 e00020-18, /cmr/31/4/e00020-18.atom.

Li, J., Li, B., Wendlandt, S., Schwarz, S., Wang, Y., Wu, C., Ma, Z., Shen, J., 2014. Identification of a novel vga(E) gene variant that confers resistance to pleuromutilins, lincosamides and streptogramin A antibiotics in *staphylococci* of porcine origin. J. Antimicrob. Chemother. 69, 919–923.

Loncaric, I., Kübber-Heiss, A., Posautz, A., Ruppitsch, W., Lepuschitz, S., Schauer, B., Feßler, A.T., Krametter-Frötscher, R., Harrison, E.M., Holmes, M.A., Künzel, F., Szostak, M.P., Hauschild, T., Desvars-Larrive, A., Misic, D., Rosengarten, R., Walzer, C., Slickers, P., Monecke, S., Ehricht, R., Schwarz, S., Spergser, J., 2019a. Characterization of *mecC* gene-carrying coagulase-negative *Staphylococcus spp*. isolated from various animals. Vet. Microbiol. 230, 138–144.

Loncaric, I., Lepuschitz, S., Ruppitsch, W., Trstan, A., Andreadis, T., Bouchlis, N., Marbach, H., Schauer, B., Szostak, M.P., Feßler, A.T., Künzel, F., Licka, T., Springer, B., Allerberger, F., Monecke, S., Ehricht, R., Schwarz, S., Spergser, J., 2019b. Increased genetic diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from companion animals. Vet. Microbiol. 235, 118–126.

Madhaiyan, M., Wirth, J.S., Saravanan, V.S., 2020. Phylogenomic analyses of the *Staphylococcaceae* family suggest the reclassification of five species within the genus *Staphylococcus* as heterotypic synonyms, the promotion of five subspecies to novel species, the taxonomic reassignment of five *Staphylococcus* species to *Mammaliicoccus* gen. nov., and the formal assignment of *Nosocomiicoccus* to the family *Staphylococcaceae*. Int. J. Syst. Evol. Microbiol.

Mellmann, A., Becker, K., von Eiff, C., Keckevoet, U., Schumann, P., Harmsen, D., 2006. Sequencing and *Staphylococci* identification. Emerging 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.

Nemeghaire, S., Argudín, M.A., Feßler, A.T., Hauschild, T., Schwarz, S., Butaye, P., 2014a. The ecological importance of the *Staphylococcus sciuri* species group as a reservoir for resistance and virulence genes. Vet. Microbiol. 171, 342–356.

Nemeghaire, S., Argudín, M.A., Haesebrouck, F., Butaye, P., 2014b. Molecular epidemiology of methicillin-resistant *Staphylococcus sciuri* in healthy chickens. Vet. Microbiol. 171, 357–363.

Nemeghaire, S., Vanderhaeghen, W., Argudin, M.A., Haesebrouck, F., Butaye, P., 2014c. Characterization of methicillin-resistant *Staphylococcus sciuri* isolates from industrially raised pigs, cattle and broiler chickens. J. Antimicrob. Chemother. 69, 2928–2934.

Perreten, V., Kadlec, K., Schwarz, S., Gronlund Andersson, U., Finn, M., Greko, C., Moodley, A., Kania, S.A., Frank, L.A., Bemis, D.A., Franco, A., Iurescia, M., Battisti, A., Duim, B., Wagenaar, J.A., van Duijkeren, E., Weese, J.S., Fitzgerald, J.R., Rossano, A., Guardabassi, L., 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J. Antimicrob. Chemother. 65, 1145–1154.

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.

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.

Schwendener, S., Perreten, V., 2012. New MLS B Resistance Gene *erm* (43) in *Staphylococcus lentus*. Antimicrob. Agents Chemother. 56, 4746–4752.

Shittu, A., Lin, J., Morrison, D., Kolawole, D., 2004. Isolation and molecular characterization of multiresistant *Staphylococcus sciuri* and *Staphylococcus haemolyticus* associated with skin and soft-tissue infections. J. Med. Microbiol. 53, 51–55.

Sweeney, M.T., Lubbers, B.V., Schwarz, S., Watts, J.L., 2018. Applying definitions for multidrug resistance, extensive drug resistance and pandrug resistance to clinically significant livestock and companion animal bacterial pathogens. J. Antimicrob. Chemother. 73, 1460–1463.

Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., Leunissen, J.A.M., 2007. Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res. 35, W71–W74.

Vanderhaeghen, W., Vandendriessche, S., Crombé, F., Nemeghaire, S., Dispas, M., Denis, O., Hermans, K., Haesebrouck, F., Butaye, P., 2013. Characterization of methicillin-resistant non-*Staphylococcus aureus staphylococci* carriage isolates from different bovine populations. J. Antimicrob. Chemother. 68, 300–307.

Wendlandt, S., Kadlec, K., Feßler, A.T., Schwarz, S., 2015. Identification of ABC transporter genes conferring combined pleuromutilin–lincosamide–streptogramin A

resistance in bovine methicillin-resistant *Staphylococcus aureus* and coagulase-negative *staphylococci*. Vet. Microbiol. 177, 353–358.
Wipf, J.R.K., Schwendener, S., Perreten, V., 2014. The novel macrolide-lincosamide-Streptogramin B resistance gene *erm* (44) is associated with a prophage in *Staphylococcus xylosus*. Antimicrob. Agents Chemother. 58, 6133–6138.
Zhang, Y., Agidi, S., LeJeune, J.T., 2009. Diversity of *staphylococcal* cassette chromosome in coagulase-negative *staphylococci* from animal sources: diversity of SCC in CoNS. J. Appl. Microbiol. 107, 1375–1383.