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Short communication

Diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from Austrian ruminants and New World camelids

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

[a] University Clinic for Ruminants, University of Veterinary Medicine, Vienna, Austria
[b] Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna, Austria
[c] Abbott Rapid Diagnostics (Alere Technologies GmbH), Jena, Germany
[d] InfectoGnostics Research Campus, Jena, Germany
[e] Institute for Medical Microbiology and Hygiene, Technische Universität Dresden, Dresden, Germany
[f] Institute of Microbiology and Epizootics, Centre of Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany
[g] Institute of Microbiology, University of Veterinary Medicine, Vienna, Austria

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ABSTRACT

The aim of this study was to determine the prevalence, the antimicrobial resistance patterns and the genetic diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) from Austrian ruminants and New World camelids that were treated at the University of Veterinary Medicine, Vienna. Between April 2014 and January 2017, 723 nasal swabs originating from ruminants and New World camelids were examined. MRSA isolates were characterized by *mecA/mecA1/mecC* PCRs and by DNA microarray analysis. They were genotyped by *spa* typing, *dru* typing, MLST and MLVA. Glycopolymer fingerprinting by FTIR spectroscopy was also performed. Antimicrobial susceptibility testing was conducted by agar disk diffusion. Twelve MRSA isolates were *mecA*-positive, whereas three were *mecC*-positive. The MRSA isolates carried five different SCC*mec* elements, and belonged to three sequence types (ST45, ST130, ST398). The MRSA isolates displayed seven different resistance phenotypes. The present study describes for the first time *mecC*-carrying MRSA isolates originating from domesticated animals in Austria. More systematic studies are needed to unravel the role of ruminants and New World camelids as reservoirs for MRSA as a potential risk for zooanthropogenic transmission.

1. Introduction

To date, methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important multi-drug resistant pathogens worldwide (Köck et al., 2010). So far, two different *mec* genes, *mecA* and *mecC* are known to occur in *S. aureus* from humans and animals. Although the presence of MRSA carrying *mecA* or *mecC*, has been documented in companion and wild animals from Austria (Loncaric et al., 2013b, 2014), data describing the presence of MRSA in Austrian ruminants are scarce. A recent study describing a suspected goat-to-human transmission of MRSA ST398 was the first one in Austria that detected MRSA in Austrian goats (Loncaric et al., 2013a). So far, there are no data about MRSA in New World camelids, i.e., South American camelids, neither from South America nor from Austria or other European countries. New World camelids are a group of animals, which include llamas (*Lama glama*), guanacos (*Lama guanicoe*), vicunas (*Lama vicugna*) and alpacas (*Lama pacos*). Breeding and production of New World camelids has become increasingly popular in recent years. Since these animals are often kept as hobby companion animals, they become important as a potential source of different pathogens that might be transmitted from them to humans and *vice versa*. Therefore, the aim of the present study was to gain deeper insight into the presence and the types of MRSA from Austrian ruminants and New World camelids, to determine their antimicrobial resistance properties and to provide a molecular and phenotypic characterization of the respective isolates. Since there is no information available on the presence of MRSA in the abovementioned animals, sampling was performed solely on animals presented for treatment at the Clinic for Ruminants at the University of Veterinary Medicine in Vienna, Austria.

* Corresponding author.
E-mail address: igor.loncaric@vetmeduni.ac.at (I. Loncaric).
[1] Both authors contributed equally to this study.

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2. Materials and methods

2.1. Bacterial isolates and estimation of confidence intervals

From spring 2014 until January 2017, 723 nasal swabs were collected from ruminants including cattle (n = 221), calves (n = 143), goats (n = 96) and sheep (n = 134), as well as New World camelids, including alpacas (n = 99) and llamas (n = 30), presented as patients in the Clinic for Ruminants at the University of Veterinary Medicine in Vienna, Austria. The study was discussed and approved by the institutional ethics and animal welfare committee in accordance with Good Scientific Practice guidelines of the University of Veterinary Medicine, Vienna and national legislation. All nasal swabs were incubated in tryptic soy broth (Beckton Dickinson (BD); Heidelberg, Germany) with 6.5% (w/v) NaCl overnight and subsequently streaked onto BBL™ CHROMagar™ MRSA II (BD), Mueller Hinton agar with 2.5 % (w/v) NaCl, 2 mg/L oxacillin and 20 mg/L aztreonam (MHoxa, Oxoid; Basingstoke, UK) and BD™ Columbia CNA Agar with 5 % Sheep Blood, Improved II (CNA). Colonies of *S. aureus* which showed the typical colony appearance of MRSA after incubation on BBL™ CHROMagar™ MRSA II were selected and re-cultivated on CNA agar. Cefoxitin resistance was confirmed by agar disk diffusion as well as by PCRs for *mecA* and *mecC* (CLSI, 2017; Loncaric et al., 2013b). Colonies suspected to be methicillin-resistant coagulase-negative staphylococci were preserved and will be analysed within the framework of a separate study. Strains were kept frozen at −80 °C for further analysis.

95% confidence intervals for the proportion of individuals tested MRSA positive per species were estimated using the function binom.test in the software R (R Core Team, 2016)

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by agar disk diffusion according to CLSI standards (CLSI 2015, 2017) for the following antimicrobial agents (µg/disk): cefoxitin (30), ciprofloxacin (5), amikacin (30), gentamicin (10), tetracycline (30), erythromycin (15), clindamycin (2), chloramphenicol (30), trimethoprim-sulfamethoxazole (1.25/23.75), nitrofurantoin (300), rifampicin (5), linezolid (30), and teicoplanin (30) (Beckton Dickinson (BD); Heidelberg, Germany).

2.3. Detection of antimicrobial resistance genes

Antimicrobial resistance genes were detected using a DNA microarray-based technology to detect over 300 different target sequences, including antimicrobial resistance and virulence-associated genes, species-specific genes, and SCC*mec*-associated genes (*S. aureus* Genotyping Kit 2.0, Alere, Jena, Germany) (Monecke et al., 2008). Resistance genes *ant(6′)la* and *str* which mediate streptomycin resistance and are not included in the DNA microarray, were screened by PCR (Hauschild et al., 2007).

2.4. Molecular typing and glycopolymer fingerprinting

DNA extraction was performed as previously described (Loncaric et al., 2014). The strains were genotyped by *spa* typing (Loncaric et al., 2014) and *dru* typing (Goering et al., 2008), Multi-Locus-Sequence-Typing (MLST) (Loncaric et al., 2014), and by Multiple-Locus Variable-number of tandem repeat Analyses (MLVA) using $MLVA_{-Orsey14}$ (Loncaric et al., 2014) scheme including two loci (Sa0387 and Sa2511) from $MLVA_{-Orsey16}$ scheme (Sobral et al., 2012) ($MLVA_{-Orsey14+2}$).

Using Fourier Transform Infrared (FTIR) spectroscopy, all strains were further phenotypically subtyped based on their surface glycostructural composition. That included the determination of the capsular polysaccharide (CP) expression (Grunert et al., 2013; Johler et al., 2016).

2.5. Virulence and other determinants

Staphylococcus-associated virulence genes were detected using the above-mentioned microarray. In addition, to provide more evidence of their origin, all MRSA strains were tested by PCR for the presence or absence of genes commonly located on mobile genetic elements (MGEs), such as φ3 *int*, φ6 *int*, φ7 *int*, *rep7*, *rep27*, and *cadDX* (Lekkerkerk et al., 2015). The genes *cadD, copB, qacAB, czr, smr, arsA* coding for heavy metal and disinfectant resistances were tested using simplex PCRs (Argudìn et al., 2016).

3. Results and discussion

3.1. Isolation of MRSA

MRSA were detected in 15 of 723 nasal swabs resulting in a carriage rate of 2.07% over all tested animal species. MRSA was most frequently isolated from goats (8.33%, 95%-confidence interval (CI) 3.67% – 15.76%) followed by sheep (2.99%, CI 0.82% – 7.47%), cattle (0.45% CI 0.01% – 2.90%), llamas (3.33%, CI 0.08% – 17.22%) and alpacas (1.01%,CI 0.03% – 5.50%). The methicillin resistance gene *mecA* was detected in 12 MRSA strains, whereas three strains, all from goats, carried the *mecC* gene

The isolation frequency of MRSA was low (0.51%) among the tested cattle. None of the examined nasal swabs from calves (age up to 6 months, veal and breeding) (n = 143) were MRSA-positive. This observation was surprising because the majority of the animals tested received antibiotic therapy that is a recognized risk factor for MRSA colonization. Previously observed MRSA prevalence rates of up to 28% in veal calves from the Netherlands (Graveland et al., 2010) were much higher than in the present study. However, it should be noted that passive surveillance as used in the present study cannot directly be compared with the studies that applied active surveillance. The highest MRSA colonization rate was seen in goats (8.33%) followed by sheep (2.99%). Both prevalence rates are high compared to a study from Denmark with a prevalence of 1.5% in sheep (Eriksson et al., 2013). However, Eriksson et al. (2013) sampled 81 living animals on farms and 115 dead animals at the slaughterhouse. This points towards the sampling of healthy animals in comparison to the diseased animals investigated in the present study, which might have an influence on the different prevalences. So far, there are no reports about MRSA colonization in New World camelids or about their nasal carriage rate of MRSA in any European country. We detected a nasal carriage rate of 1.01% in alpacas and 3.33% in llamas. As above mentioned direct comparison of MRSA prevalences with other studies cannot be perform. On the other hand, comparison of carriage rates between tested animals reveled that goats with 95% certainty will be at least 3.67% and not more than 15.76% MRSA positive. All other animals with 95% certainty will not reach the value of goats. This makes the difference significant. It is different with the lamas. There were only 3.33% positive, but due to the small sample this estimate is very inaccurate. The actual value of all the llamas which will ever been examined can be up to 17.22%. Thus, it is quite possible that llamas are actually more positive than goats.

3.2. Antimicrobial susceptibility testing and detection of resistance genes

The 15 strains showed seven different resistance phenotypes. Eleven strains (all ST398) were non-susceptible to tetracycline which was well reflected by carrying at least one of the tetracycline resistance genes *tet* (K) and/or *tet*(M). The macrolide-lincosamide-streptogramin B (MLS_B) resistance *erm*(C) gene was identified in four strains. Eight strains were categorized as multidrug-resistant (MDR) (Schwarz et al., 2010). However, all strains were susceptible to chloramphenicol, teicoplanin, linezolid, rifampicin, tigecycline, nitrofurantoin and trimethoprim/sulfamethoxazole (Table 1, Table S1).