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Case Report
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Isolation of *Staphylococcus sciuri* from horse skin infection

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Abstract
Staphylococcus sciuri is known as an opportunistic pathogen colonizing domesticated animals and has also been associated with wound infections in humans. Particularly over the last decade, oxacillin (methicillin) resistant strains had been emerged, which now increase the medical relevance of this species. This report describes the identification of an oxacillin-resistant *S. sciuri* isolate from a wound infection of a horse. We determined the absence of coagulase and hyaluronidase activity and analysed the antibiotic resistance profile.
Keywords: Colonization, Horse, Oxacillin resistance, *Staphylococcus sciuri*.

Introduction

Staphylococcus sciuri is a member of the *S. sciuri*-species group composed of coagulase-negative and novobiocin-resistant bacteria (Nemeghaire *et al*., 2014a). This group includes *S. sciuri* (with three subspecies), *S. lentus*, *S. vitulinus*, *S. fleurettii* and *S. stepanovicii* (Becker *et al*., 2014a), which are in general considered as commensal animal-associated species (Kloos *et al*., 1976). *S. sciuri* possesses a certain pathogenic potential and is able to induce infections in both, animals (Frey *et al*., 2013; Dos Santos *et al*., 2015) and humans (Stepanovic *et al*., 2003). Some isolates of the *S. sciuri* group are known to carry different homologues of the methicillin resistance genes *mecA*, B and C and display methicillin/oxacillin resistance (Becker *et al*., 2014a,b; Harrison *et al*., 2014).

In the present study, we report the identification of an oxacillin-resistant *S. sciuri* isolate from a purulent skin lesion of a horse, determined activity of coagulase and hyaluronidase and characterized the antibiotic resistance profile.

Case Details

A "Hannoveraner Hengst" at the age of ten presented a purulent skin lesion on the right forehand pastern. The medical prehistory claimed repeated episodes of purulent skin infections, foremost on the bridge of the nose, which poorly healed untreated within a couple of weeks.

One year later, a closed, swollen abscess-like structure was developed on the right forehand pastern. The abscess erupted within two to three days and presented a bloody skin lesion of ~4 cm in length and ~1 cm in width (Fig. 1).

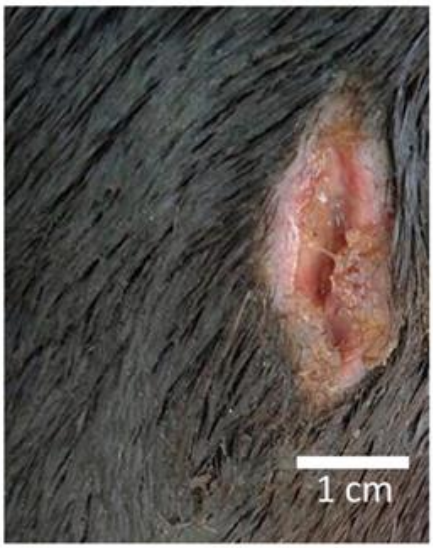

Fig. 1. Erupted abscess-like skin lesion of ~4 cm in length and ~1 cm in width on the right forehand pastern of the horse.

The skin lesion became purulent and was treated with non-antibiotic zinc-containing ointment. No systemic clinical signs were detected.

Culture-based analyses/Identification of S. sciuri

A swab specimen was taken from the purulent skin lesion and cultured onto Columbia blood agar plates (Becton Dickenson) containing 5% sheep blood at 37°C and 5% CO_2. Morphological analyses revealed growth of uniform, non-hemolytic, white opaque colonies after 24 h at 37 °C (Fig. 2A, B). Light microscopy and Gram-stain indicated a pure culture of Gram positive cocci clustered in grape-like aggregates (Fig. 2C).

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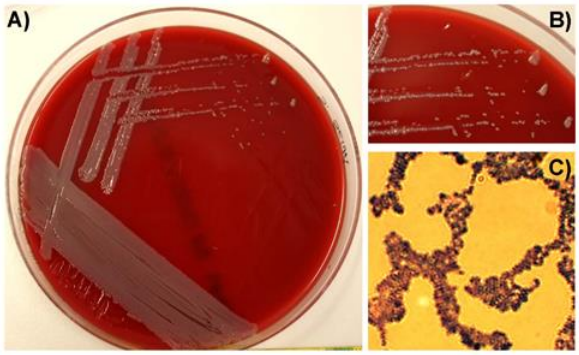

Fig. 2. (A): Morphology of *Staphylococcus sciuri* on Columbia blood agar plates. (B): The zoom in indicates white to light grey staphylococcal colonies without any haemolytic activity. (C): Gram-stain visualized Gram-positive, coccoid bacteria, clustered in grape-like structures.

Strain identification by sequencing of 16S rRNA was conducted from a single colony as described elsewhere (Weisburg, *et al*., 1991) using the following oligonucleotides: forward primer: 27f 5'-AGA GTT TGA TCM TGG CTC AG-3', reverse primer: 1492 r 5'-CGG TTA CCT TGT TAC GAC TT-3' and was repeated three times using colony material from the same culture plate. After purification of PCR-products using QIA quick PCR Purification Kit (Qiagen) sequencing procedure was performed by GATC-Biotech (Germany).

Blast® search (provided by NCBI, NLM, Bethesda, USA) identified *S. sciuri* in all three independent probes as primary infectious agent. The listed blast results presented in Table 1 confirm the high sequence identity with up to 98% query coverage to *S. sciuri* in all of the tested three independent probes. Results further point to the identification of *carnicatus* or *rodentium* as respective subspecies but the nominal difference to the results given for the third potential subspecies *sciuri* were not significant enough to allow a final determination of the subspecies.

A bacterial colonization analysis from swabs taken from the horse nostrils did not identified *S. sciuri* as constant colonizer of the horse, but identified typical members of horse microflora such as *Aeromonas viridans* and *S. vitulinus* by Maldi Tof.

Verification of strain-identification and MIC-determination

Species identification of the *S. sciuri* isolate was confirmed using the standardized API STAPH V5.0 system and revealed an ID value of 88.4%. The specification of the respective isolate was further confirmed using the bioMérieux VITEK®2 system (Germany) according to the manufacturer recommendations and also independently by the "National Reference Center of Staphylococci and Enterococci" of the Robert Koch Institute (RKI) in Wernigerode, Germany.

Table 1. 16SrRNA-based sequencing results from three independent culture probes as obtained by blast®-sequence alignment (NCBI; NLM).

sample	hit	S. sciuri			
		subsp.	carnicatus	rodentium	sciuri
1	1	Strain	GTC 1227	GTC 844	ATCC 29062
		Query cover	98%	98%	98%
		Ident	85%	85%	85%
		Accession	NR 041327.1	NR 041328.1	AJ421446.1
	2	Strain	GTC 1227	GTC 844	ATCC 29062
		Query cover	98%	98%	40%
		Ident	85%	85%	85%
		Accession	AB233331.1	AB233332.1	AY688097.1
	3	Strain	ATCC 700058	ATCC 70061	
		Query cover	40%	40%	
		Ident	82%	82%	
		Accession	AY688095.1	AY688096.1	
2	1	Strain	GTC 1227	GTC 844	ATCC 29062
		Query cover	96%	96%	95%
		Ident	86%	86%	86%
		Accession			
	2	Strain	GTC 1227	GTC 844	ATCC 29062
		Query cover	96%	96%	35%
		Ident	86%	86%	82%
		Accession	AB233331.1	AB233332.1	AY688097.1
	3	Strain	ATCC 700058	ATCC 70061	
		Query cover	35%	35%	
		Ident	82%	82%	
		Accession	AY688095.1	AY688096.1	
3	1	Strain	GTC 1227	GTC 844	ATCC 29062
		Query cover	95%	95%	95%
		Ident	86%	86%	86%
		Accession	NR 041327.1	NR 041328.1	AJ421446.1
	2	Strain	GTC 1227	GTC 844	ATCC 29062
		Query cover	95%	95%	37%
		Ident	86%	86%	82%
		Accession	AB233331.1	AB233332.1	AY688097.1
	3	Strain	ATCC 700058	ATCC 70061	
		Query cover	37%	37%	
		Ident	82%	82%	
		Accession	AY688095.1	AY688096.1	

Furthermore, automated antimicrobial susceptibility testing was performed at the "National Reference Center for Staphylococci and Enterococci" of the Robert Koch Institute in Wernigerode, Germany via

microbouillon-dilution, including the following antibiotics: ß-lactams (benzylpenicillin, oxacillin), macrolides (erythromcycin), lincosamides (clindamycin), oxazolidinone (linezolid), fucidanes (fusidic acid), aminoglycosides (gentamycin), ansamycins (rifampicin), tetracycline (oxytetracyclin), glycopeptides (vancomycin, teicoplanin), gylcylcyclins (tigecyclin), fluoroquinolons (ciprofloxacin, moxifloxacin), cyclic lipopeptides (daptomycin), the epoxid fosfomycin and the folate synthesis inhibitor cotrimoxazol. MIC value determination was evaluated according to the EUCAST standards for human medicine and revealed sensitivity to most of the tested antibiotics (Table 2).

Table 2. Analyses of MIC of different antibiotics by the "National Reference Center of Staphylococci and Enterococci" of the Robert Koch Institute in Wernigerode, Germany, evaluated by EUCAST-based interpretation.

Antibiotics	MIC (µg/ml)	interpretation
Benzylpenicillin	0.125	S
Oxacillin	1.0	R
Fosfomycin	8.0	R
Gentamycin	0.5	S
Linezolide	1.0	S
Erythromycin	0.5	S
Clindamycin	0.25	S
Oxytetracyclin	0.5	S
Tigecyclin	0.125	S
Vancomycin	1.0	S
Teicoplanin	4.0	R
Ciprofloxacin	0.5	S
Moxifloxacin	0.25	S
Daptomycin	1.0	S
Co-trimoxazole	0.5	S
Rifampicin	0.063	S
Fusidic acid	8.0	R

Interestingly, the antibiotic resistance profile covering 17 antibiotics indicated a resistance against fosfomycin, fusidic acid and teicoplanin (Table 2).

Teicoplanin resistance was additionally tested by plating a higher inoculum. In sum, the results point to a heterogenic teicoplanin resistant strain. Moreover, according to the EUCAST standards, the *S. sciuri* strain is resistant against oxacillin. This is remarkable since detection of the common resistance genes *mecA* and *mecC* via specific PCR by Reference Center of the RKI (Wernigerode, Germany) was negative.

Additionally, the particular *S. sciuri* was tested negative for coagulase and hyaluronidase activity, respectively. The activities of both virulence factors was analysed by tube test with human and rabbit plasma and by decapsulation test with *S. equi* as described by Essers and Radebold (1980).

Discussion

S. sciuri is mostly recovered from skin and mucous membrane of animals and has long been considered as a non-pathogenic commensal bacterium (Adegoke, 1986). During the last decade of years, it has been associated with several cases of bovine mastitis (Lüthje and Schwarz, 2006; Nam *et al*., 2010; Frey *et al*., 2013), as well as from goats suffering from peste des petites ruminants (PPR) (Ugochukwu and Agwu, 1991), from cases of canine dermatitis (Hauschild and Wójcik, 2007; Hauschild *et al.*, 2010), and from several outbreaks of fatal exudative epidermitis in piglets (Chen *et al*., 2007; Nemeghaire *et al.,* 2014c).

The recurrent manifestation of skin lesions monitored in the present case, initially suggested a permanent colonization of the horse with *S. sciuri.* In contrast to several reports pointing to nasal colonization of horses with *S. sciuri*, so far no data on permanent skin colonization has been reported for horses (Bagcigil *et al*., 2007; Aslantas *et al*., 2012; Karakulska *et al*., 2012). The lack of *S. sciuri* in cultures of nasal swabs in this case may point to the occurrence of a single colonization event or may suggest repeated episodes of temporary colonization.

Interestingly, a transmission in between healthy domestic animals colonized with *S. sciuri* was repeatedly observed (Moodley and Guardabassi, 2009; Aslantas *et al*., 2012). This transmission may be promoted by insects serving as transmission vectors. In this respect, a report also suggested that the possible source of *S. sciuri* colonization in surgical wounds may be flies perching on open wounds (Kolawole and Shittu, 1997).Thus, it is assumed that frequent contact with healthy domestic and farm animals may also contribute to an at least temporary colonization of the skin, and subsequently the wounds, by *S. sciuri* (Kloos *et al*., 1976; Nemeghaire *et al*., 2014b).

Despite the rare occurrence of *S. sciuri* in humans (Marsou *et al*., 1999; Couto *et al*., 2000; Nagase *et al*., 2002), some reports furthermore point to the role of *S. sciuri* as opportunistic pathogens isolated from various clinical specimen and causing serious infections in humans such as endocarditis, peritonitis, septic shock, and wound infections (Hedin and Widerstrom, 1998; Wallet *et al*., 2000; Horii *et al*., 2001; Stepanovic *et al*., 2002, 2003;). Moreover, despite the lack of data regarding *S. sciuri* colonization of the handler, a recurrent transmission from the handler to the horse

cannot be excluded. The isolated *S. sciuri* strain was tested negative for coagulase and hyaluronidase activity and the antibiotic profiling confirmed sensitivity against most of the tested antibiotics, which suggested a low general pathogenicity of this strain. Nevertheless, the *S. sciuri* strain revealed resistance against fosfomycin, fusidic acid and teicoplanin (Table 2). According to the information provided by the Reference Center of the RKI in Wernigerode, approximately 80% of the tested *S. sciuri* strains reveal resistance against fusidic acid.

Interestingly, based on the EUCAST definition, the present strain is also resistant against Oxacillin. The *S. sciuri* species cluster group is represented by three *S. siuri* subspecies and also contains the species *S. vitulinus*. This cluster group carries different *mecA* homologues and has been proposed as origin and reservoir of the *S. aureus mecA* gene (Becker *et al*., 2014a,b; Nemeghaire *et al*., 2014a). In the genome of the present *S. sciuri* isolate, neither a *mecA* nor a *mecC* gene mediating methicillin/oxacillin resistance could be amplified by specific PCR at the National Reference Center at the RKI in Wernigerode. Thus, it has been reported that phenotypic methicillin (and other β-lactam) -resistance in *Staphylococcaceae* members is conferred not only by *mecA*, but also by different *mecA* allotypes and also by homologous genes such as *mecB* and *mecC* (Becker *et al*., 2014a,b). Moreover, a hybrid SCC*mec* consisting of a *mecA*-encoding SCC*mec* type VII element and a separate *mecC* region in terms of a ΨSCC*mec* element was published for *S. sciuri* (Harrison *et al*., 2014; Becker *et al*., 2014a). These reports might suggest the presence of a further *mec*-homolog or a *mec* gene hybrid within the genome of the isolated *S. sciuri* strain, which could not be amplified by the *mecA* and *mecC*-specific oligonucleotides.

Nevertheless, based on genomic and plasmid encoded genes, multiresistant *S. sciuri* isolates carrying resistance genes against all major classes of antibiotics have already been reported (Li *et al*., 2016) and support the potential to temporarily serve as a "bacterial shuttle" e.g. by transmitting genetic information between other bacterial species of the horse´s skin microbiome.

In sum, these results suggest the identification of a coagulase-negative *Staphylococcus* exhibiting moderate virulence.

Conflict of interest

The Authors declare that there is no conflict of interest.

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