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Characterization of methicillin-resistant *Staphylococcus* spp. carrying the *mecC* gene, isolated from wildlife

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Objectives: A recently identified *mecA* homologue, *mecC*, in methicillin-resistant *Staphylococcus aureus* (MRSA) has been isolated from humans and different animal hosts. The aim of this study was to determine antimicrobial resistance and provide molecular characterization of MRSA and methicillin-resistant non-*Staphylococcus aureus* staphylococci (MRnSA) isolated from wildlife that carried the gene *mecC*.

Methods: Five *S. aureus* and one coagulase-negative *Staphylococcus* isolate displaying phenotypic oxacillin resistance, but not recognized with conventional PCR for *mecA*, were further characterized by a polyphasic approach. The presence of *mecC* in all isolates was determined using specific PCR. PCR targeting Panton–Valentine leucocidin (PVL) genes of MRSA was performed. MRSA isolates were genotyped by *spa* typing and multilocus sequence typing. All isolates were genotyped by staphylococcal cassette chromosome *mec* (SCC*mec*) typing. 16S rDNA sequence analysis for MRnSA identification was performed. Antimicrobial susceptibility testing was performed for all isolates.

Results: All five MRSA isolates contained the *mecC* gene, were PVL negative, carried SCC*mec* type XI and belonged to ST130 (where ST stands for sequence type), with *spa* types t843, t10513 or t3256, or to ST2620, with *spa* type t4335. The MRnSA isolate, most closely related to *Staphylococcus stepanovicii*, carried *mecA* and *blaZ* genes related to SCC*mec* XI. MRSA isolates exhibited resistance to the β-lactams only.

Conclusions: The MRSA isolates described in this study represent the first detection of *mecC*-positive MRSA in a European otter (*Lutra lutra*) and a European brown hare (*Lepus europaeus*). The MRnSA isolate represents the first isolation of MRnSA from a Eurasian lynx (*Lynx lynx*).

Keywords: MRSA, antimicrobial resistance, MLST, *spa* typing

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a frequent pathogen of humans and many animal species. Another group of staphylococci, methicillin-resistant non-*Staphylococcus aureus* staphylococci (MRnSA), have long been recognized as important human and animal pathogens.[1,2] Both MRSA and MRnSA are of growing interest in human and animal health. The *mecA* gene, which mediates methicillin resistance, is carried by a large mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*), which integrates into the staphylococcal chromosome.[3] The SCC*mec* element is highly variable in various staphylococcal species. So far, 11 SCC*mec* types (I–XI) and numerous subtypes have been recognized in MRSA.[4–6] The SCC*mec* diversity in MRnSA is even higher.[4,7] Appreciable indirect evidence has shown transfer of SCC*mec* between MRnSA and *S. aureus*.[7–9]

In 2011, a new divergent *mecA* homologue, designated *mecC* (formerly known as $mecA_{LGA251}$), located in a new SCC*mec* cassette designated SCC*mec* type XI, was described in *S. aureus*.[6,10] It is not detectable using routine *mecA*-specific PCR approaches and penicillin binding protein 2a (PBP2a) slide agglutination tests. This divergent *mecA* homologue was identified in MRSA strains from humans and cattle.[6,10] Searching for *mecC* in MRSA, as well as in other staphylococcal species, has recently been performed in several countries.[11]

While studies on MRSA in humans, companion animals and livestock have been widely documented, there is still a scarcity of information on infections, carriage and role of this particular

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pathogen in wildlife. Here we report the presence of *mecC*-positive MRSA in European brown hare (*Lepus europaeus*) from the German island of Pellworm, as well as the detection of *mecC*-positive MRSA and MRnSA among Austrian wildlife.

Materials and methods

According to hunting records, the hare population on Pellworm experienced a continuous decrease from 759 in 2000 to 151 in 2009. Therefore, a thorough health assessment programme was implemented. In the course of the annual hare hunt during the years 2010, 2011 and 2012, a total of 152 hares were collected (56, 54 and 42, respectively). Detailed post mortem, pathohistological, bacteriological and parasitological examinations were carried out. Initial macroscopic evaluation indicated problems in the gastrointestinal tract. Therefore, a piece of small intestine was collected from each hare for bacterial examination, as well as from any other organ showing pathological changes. In total, six non-repetitive staphylococcal isolates were obtained [in 2011, one *S. aureus* (3544/11) and three coagulase-negative intestinal isolates; in 2012, two *S. aureus* (3268/12 and 3269/12)]. Another study was performed during winter 2012/13 to determine the presence of oxacillin-resistant *Staphylococcus* spp. in Austrian wildlife. Nasal and perineal swabs of 40 different wild animals presented to the Research Institute of Wildlife Ecology for pathological examinations were screened. *S. aureus* from a European otter (*Lutra lutra*) (isolate 11mrsafiwi) and a European hedgehog (*Erinaceus europaeus*) (isolate AC 104/13), as well as a coagulase-negative *Staphylococcus* species isolate from a Eurasian lynx (*Lynx lynx*) (isolate 3orsfiwi), were detected (Table 1).

Preliminary antimicrobial susceptibility testing was performed by agar disc diffusion according to guidelines of the CLSI[12] for oxacillin and cefoxitin. Staphylococcal isolates that were susceptible to oxacillin/cefoxitin were excluded. Isolates 3544/11, 3268/12, 3269/12, 11mrsafiwi, AC 104/13 and 3orsfiwi were examined in more detail. The respective colonies of *S. aureus* isolates showed the typical colony appearance of MRSA after incubation on BBL™ CHROMagar™ MRSA II (Becton Dickinson, Heidelberg, Germany). The production of β-lactamase was confirmed by nitrocefin assay using BBL™ DrySlide™ Nitrocefin (Becton Dickinson, Heidelberg, Germany). Isolates that tested negative in the phenotypical detection of PBP2a and conventional *mecA* PCR[13] were further examined using primers for PCR detection of *mecC*.[14] All *mecC* amplicons were sequenced for confirmatory reasons. SCC*mec* typing with a multiplex PCR approach, which consisted of three PCRs for detecting *mecI*/*mecR1*, *ccrA*/*B* and *blaZ*[6] genes related to SCC*mec* XI, was conducted and, when positive, amplicons were sequenced. Since PCR amplification of *mecI*/*mecR1* and *ccrA*/*B* failed for isolate 3orsfiwi, SCC*mec* typing of this isolate was additionally performed as previously described.[13]

Further investigations of MRSA isolates were performed by PCR targeting Panton–Valentine leucocidin (PVL) genes, *spa* typing and multilocus sequence typing (MLST) as described previously.[13] For species characterization of the 3orsfiwi isolate, 16S rDNA sequence analysis was performed.[15] The isolate was identified using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/). All obtained sequences of 3orsfiwi have been deposited in the GenBank database under accession numbers KC594032, KC601652 and KC601653.

Antimicrobial susceptibility testing was performed by agar disc diffusion according to standards of the CLSI[12] for penicillin, cefquinome, tetracycline, ciprofloxacin, gentamicin, chloramphenicol, erythromycin, clindamycin, teicoplanin, trimethoprim/sulfamethoxazole, linezolid and rifampicin (all from Becton Dickinson, Heidelberg, Germany except cefquinome, Oxoid Ltd, Basingstoke, UK). A susceptibility interpretation for cefquinome (Merck Animal Health) interpretation was made according to the manufacturer's recommendation. Additionally, MIC was determined by Etest (bioMérieux, Marcy l'Étoile, France) for cefoxitin and by M.I.C. Evaluator

Table 1. Characteristics and origin of the isolates investigated in this study

Isolate	Host species	Species	ST	*spa* type	MIC (mg/L) OXA, FOX	Resistance phenotype	Additional notes
3544/11	European brown hare	*S. aureus*	130	t843	16, 32	β-lactams	emaciated; moderate, acute to subacute, necro-suppurative inflammation in various organs (lung, spleen, kidney, liver)
3268/12	European brown hare	*S. aureus*	130	t10513	8, 32	β-lactams	emaciated; multifocal pyogranulomatous lesions in various tissues (e.g. lung, spleen, liver, lymph nodes)
3269/12	European brown hare	*S. aureus*	130	t843	16, 32	β-lactams	emaciated; multifocal pyogranulomatous lesions in various tissues (e.g. lung, spleen, liver, lymph nodes)
11mrsafiwi	European otter	*S. aureus*	2620 (new)	t4335	16, 16	β-lactams	severe purulent pneumonia and leptomeningitis
AC 104/13	European hedgehog	*S. aureus*	130	t3256	16, 24	β-lactams	moderate enteritis, moderate suppurative bronchopneumonia and splenitis
3orsfiwi	Eurasian lynx	*S. stepanovicii*	NA	NA	4, 4	β-lactams	starvation—no other pathological changes were observed

OXA, oxacillin; FOX, cefoxitin; NA, not applicable.

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test (Thermo Fisher, Basingstoke, UK) for oxacillin and vancomycin. *S. aureus* ATCC 29213 was used as a quality control.

Results

PCR amplification of the *mecC* produced amplicons of the expected sizes in all six cases. The sequences of the *mecC* amplicons of all isolates shared highest similarity scores [MRSA isolates 100%, and isolate 3orsafiwi 99.6% (KC601653)] with the corresponding sequence of MRSA strain LGA251 (GenBank accession number FR821779). PCRs of MRSA detecting *mecI*/*mecR1*, *ccrA*/*B* and *blaZ* indicated that *mecC* was located within SCC*mec* XI elements, which was confirmed by sequencing data for all amplicons showing >99% identity with the MRSA strain LGA251. In isolate 3orsfiwi, only the PCR for *blaZ* produced an amplicon of the correct size, of which the sequence (KC601652) shared highest identity (97.3%) with the *blaZ* sequence in MRSA strain LGA251 and identities <91% with other staphylococcal *blaZ* sequences reported so far. Using a multiplex PCR SCC*mec* typing approach, PCR amplicons for the *ccr* and *mec* gene complexes could not be obtained. All MRSA isolates were PVL negative. Among the MRSA isolates, two multilocus sequence types (STs) were present (ST130 and ST2620, a single-locus variant of ST130 with one nucleotide difference in the *pta* gene) and four *spa* types (t843, t10513, t4335 and t3256) (Table 1).

The highest 16S rDNA gene sequence similarity observed for isolate 3orsfiwi (KC594032) was 99.8% with the type strain of *Staphylococcus stepanovicii* (GQ222244).

All isolates exhibited resistance to the β-lactams only. The MICs of oxacillin for all isolates ranged from 4 to 16 mg/L and the MIC of cefoxitin ranged from 4 to 32 mg/L (Table 1).

Discussion

Based on the results presented in this study, the *mecC*-positive *S. aureus* isolates belonged to ST130, which has previously been associated with *mecC*-positive isolates from different host species.[16] While *spa* type t843 is the most common *spa* type isolated from *mecC*-positive *S. aureus* of humans and cattle,[6,14] *spa* type t10513 has not previously been found among *mecC*-positive *S. aureus*. The *spa* type t4335 has two other entries in the Ridom spaserver database (http://spaserver.ridom.de/, 4 April 2013, date last accessed). These MRSA isolates (submitted in 2008 and 2013) also originated from Austria, but epidemiological metadata have not been provided. The *spa* type t3256 has three entries in the aforementioned database, but none of these originated from MRSA.

So far, *mecC*-positive MRSA strains have been isolated from wild brown rats, a chaffinch, a common seal[16] and a hedgehog.[17] To the authors' knowledge, the MRSA isolates described in this study represent the first detection of *mecC*-positive MRSA isolated from a European otter and a European brown hare.

The recently described *S. stepanovicii*, an oxidase-positive staphylococcal species, is closely related to the *Staphylococcus sciuri* group.[18] High 16S rDNA gene sequence similarity between isolate 3orsfiwi and the type strain of *S. stepanovicii* suggest the affiliation to this species. So far, *S. stepanovicii* has been isolated solely from wild small mammals.[18,19] Therefore, it is possible that the young lynx from which isolate 3orsfiwi originated became a carrier of the *mecC*-positive *S. stepanovicii* by eating small mammals. However, there are no reports concerning the normal bacterial flora of the Eurasian lynx. Hence, it is not clear whether *S. stepanovicii* is part of the normal bacterial flora of the Eurasian lynx or plays a role in the establishment of disease in this species.

Additionally, wildlife species, especially predators, could also be valuable indicator species in monitoring the presence of MRSA in the environment. Furthermore, our results suggest that genes closely related to SCC*mec* XI could also be present in MRnSA. Whether such strains could function as the evolutionary source of *mecC*, as suggested for classic mecA,[20] needs to be determined. To completely understand the epidemiology and evolution of the *mecC* in MRSA and MRnSA, extended surveillance in wildlife, companion animals, livestock and humans is required.

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Transparency declarations

None to declare.

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