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 (SCCmec) 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 SCCmec 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 SCCmec 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 (SCCmec), which integrates into the staphylococcal chromosome.3 Two SCCmec elements are highly variable in various staphylococcal species. So far, 11 SCCmec types (I–XI) and numerous subtypes have been recognized in MRSA.4–6 The SCCmec diversity in MRnSA is even higher.6–7 Appreciable indirect evidence has shown transfer of SCCmec between MRnSA and *S. aureus*.7–9

In 2011, a new divergent mecA homologue, designated mecC (formerly known as mecA (GA253)), located in a new SCCmec cassette designated SCCmec 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
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 pathohistological 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 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 were further examined using primers for PCR detection of mecC. All mecC amplicons were sequenced for confirmatory reasons. SCCmec typing with a multiplex PCR approach, which consisted of three PCRs for detecting mecI/mecR1, ccrA/B and blaZ genes related to SCCmec XI, was conducted and, when positive, amplicons were sequenced. Since PCR amplification of mecI/mecR1 and ccrA/B failed for isolate 3orsfiwi, SCCmec typing of this isolate was additionally performed as previously described.

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. For species characterization of the 3orsfiwi isolate, 16S rDNA sequence analysis was performed. 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 for penicillin, cefoxitin, tetracycline, ciprofloxacin, gentamicin, chloramphenicol, erythromycin, clindamycin, teicoplanin, trimethoprim/sulfamethoxazole, linezolid and rifampicin (all from Becton Dickinson, Heidelberg, Germany except cefoxitin, Oxoid Ltd, Basingstoke, UK). A susceptibility interpretation for cefoxitin (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.
Results

PCR amplification of the mec produced amplicons of the expected sizes in all six cases. The sequences of the mec amplicons of all isolates shared highest similarity scores (MRSA isolates 100%, and isolate 3orsfiwi 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 mec was located within SCCmec 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 SCCmec 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 ST6260, 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. While spa type t843 is the most common spa type isolated from mecC-positive S. aureus of humans and cattle, 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 2011; 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 and a hedgehog. 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. 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. 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 SCCmec XI could also be present in MRnSA. Whether such strains could function as the evolutionary source of mecC, as suggested for classic mecA, 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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