Detection of methicillin-resistant Staphylococcus aureus (MRSA) carrying the mecC gene in wild small mammals in Spain

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Objectives: To determine the rate of Staphylococcus aureus faecal carriage in 101 wild small mammals in Spain and to characterize the isolates obtained.

Methods: Faecal samples were seeded on mannitol salt agar and ORSAB plates. The presence of the resistance genes mecA, mecC and blaZ and the new blaZ allotype associated with staphylococcal cassette chromosome mec (SCCmec) XI (blaZ-SCCmecXI) was studied by PCR. S. aureus isolates were characterized by spa typing, agr typing and multilocus sequence typing. The presence of immune evasion cluster (IEC) genes and virulence genes was analysed by PCR.

Results: S. aureus was detected in 13/101 studied faecal samples and one isolate per positive sample was further studied. Two S. aureus isolates were methicillin-resistant S. aureus (MRSA) (recovered from wood mice, Apodemus sylvaticus) and 11 were methicillin-susceptible S. aureus (MSSA). Both MRSA isolates harboured the mecC gene and the novel blaZ-SCCmecXI, were typed as spa-t1535/agrIII/ST1945(CC130)/SCCmecXI (where ST stands for sequence type and CC stands for clonal complex), carried the exfoliative toxin etd2 gene and were IEC type E. Eight different spa types were identified among the 11 MSSA isolates (five new) and six different sequence types were identified (two new). All MSSA strains were susceptible to the antibiotics tested except one blaZ-positive penicillin-resistant isolate (spa-t120/agrII/ST15). MSSA isolates were ascribed to the Ccs (number of strains) CCS (1), CC1956 (4) and singleton (6). Nine of 11 MSSA isolates carried the cna virulence gene. Only one MSSA isolate carried IEC genes (type C).

Conclusions: This is the first report of MRSA carrying mecC in faecal samples of wild small mammals in Spain. These resistant isolates carried genes of the IEC system, unusual in S. aureus from animals. Wild small mammals could be a reservoir of the mecC gene with important implications for public health.

Keywords: clonal complex 30, CC30, sequence type 1945, ST1945, wood mouse

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most important antibiotic-resistant pathogens of humans and different animal species. In recent years, several studies have described livestock and companion animals as carriers of some specific lineages of MRSA, and they are therefore considered to be potential zoonotic bacteria. Moreover, MRSA have also been found in wild animals,1–3 although their role as possible reservoirs remains unclear and needs more research.

The genetic determinant of methicillin resistance (the mecA gene) is located in the staphylococcal cassette chromosome mec (SCCmec). The mecA gene encodes a modified penicillin-binding protein (PBP) known as PBP2a, with a low affinity for β-lactams. The SCCmec structural organization and genetic content shows extensive variation among MRSA isolates. In contrast, the sequence of the mecA gene is highly conserved.4

In 2011, a novel mecA homologue was described in MRSA isolates from the UK, Denmark and Ireland.5,6 This new variant has 70% identity with mecA and was initially named mecAIgA2515 and renamed mecC.7 The mecC gene encodes a PBP with 63% identity at the amino acid level with PBP2a, showing more affinity for oxacillin than for cefoxitin.8 This gene is associated with a new SCCmec classified as SCCmecXI.6 The mecC gene is not detected by the PCR method established for the detection of mecA and consequently isolates harbouring this new variant can be
misidentified and reported as methicillin-susceptible S. aureus (MSSA). A new mecC2 variant has been described recently in Staphylococcus saprophyticus from a common shrew.8

To date, strains carrying the mecC gene are predominantly ascribed to the clonal complex (CC) 130 and sequence type (ST) 425, although they can also belong to other CCs.3 Some authors suggested that the CC130 in humans may be a result of zoonotic processes.9,10 MRSA with the genotype mecC have been detected previously in animals and humans in different European countries, as reviewed,3 but, to our knowledge, never before in wild animals in Spain. The objective of the present study was to determine the prevalence of S. aureus and MRSA in faecal samples from wild small mammals in Spain and to characterize the isolates obtained.

Material and methods

Sampling, microbial isolation and identification

A total of 101 faecal samples from free-ranging wild small mammals (54 common voles (Microtus arvalis), 29 wood mice (Apodemus sylvaticus), 6 Algerian mice (Mus spretus), 6 brown rats (Rattus norvegicus), 5 greater white-toothed shrews (Crocidura russula) and 1 garden dormouse (Eliomys quercinus)) were analysed. Wild animals included in this study were a subsample of the small mammals captured in the framework of different projects headed by researchers at the Spanish Wildlife Research Institute (IREC) from 2011 to 2013, and these samples come from two different Spanish regions (north-central, 74 samples; southern, 27 samples).

Faecal samples were suspended in saline solution, and 100 µL was inoculated in brain heart infusion broth with 6.5% NaCl and incubated at 37°C for 24 h. Then, 100 µL was seeded on mannitol salt agar (MSA) plates and ORSAB plates (Oxoid) supplemented with oxacillin (2 mg/L). A maximum of four colonies per plate with morphology compatible with S. aureus were recovered and initially identified by microbiological conventional methods (Gram staining, catalase test, coagulase and DNase production). S. aureus identification was performed by amplification of the species-specific nuc gene.11 Only S. aureus strains showing different phenotypes of antimicrobial resistance of each sample were further studied.

Antimicrobial susceptibility testing and detection of antimicrobial resistance mechanisms

Susceptibility to 16 antimicrobial agents was determined by the agar dilution method according to the recommendations of CLSI (penicillin, oxacillin, cefoxitin, kanamycin, gentamicin, tobramycin, tetracycline, chloramphenicol, trimethoprim/sulfamethoxazole, erythromycin, clindamycin, ciprofloxacin, linezolid and vancomycin) and SFM (mupirocin and fusidic acid).12,13 The MICs of oxacillin and cefoxitin were also determined for MRSA isolates by the broth microdilution method.12

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Characterization of MRSA isolates

Both MRSA showed a negative result for mecA PCR and harboured the new mecC gene. For both MRSA, the MICs of oxacillin and cefoxitin were 16 and 8 – 16 mg/L, respectively. They were typed as spa-t1535/ST1945 (ascribed to CC130)/agr-III, and presented SCCmecX1: mecC-positive S. aureus isolates typed as ST1495 or t1535 have been detected previously in humans in France, Germany, Denmark and the UK,15,18,19 and in a domestic cat in Germany.18 Detection of mecC-positive S. aureus of ST1495 was reported very recently in human clinical isolates in Spain, but they were of different spa types (t6220 and t6220).19,20 Other spa types and STs associated with CC130 have been detected among mecC-positive S. aureus isolates recovered from farm and wild animals in different countries.3

The novel blaZ-SCCmecX1 was detected in both mecC-positive isolates and β-lactamase activity was confirmed by the Cefinase™ disc test. This blaZ allotype presents 67% amino acid identity with the previously known S. aureus blaZ gene;6 other authors have also reported mecC-positive isolates showing penicillinase activity.21

These MRSA isolates showed susceptibility to the remaining tested antimicrobial agents, a characteristic observed in other studies of mecC-positive S. aureus isolates of CC130.5,18,20 The two mecC-positive MRSA isolates carried the exfoliative toxin etd2 gene as well as the genes scn and sak of the IEC system, and consequently were ascribed to IEC type E.

The origin of the mecC gene is unclear, although it has been detected in staphylococci from humans and animals.3 To our knowledge, few investigations have determined the presence of IEC genes in mecC-positive isolates and all of them found isolates lacking sak, cpa and inlA genes.4,10,15 which supports the
The detection of sak and scn genes in our animal mecC-positive isolates is relevant and raises questions about the potential origin of these isolates. MRSA were detected in 2% of animals tested and in ≏7% of wood mice analysed. It is interesting that these isolates were recovered in faecal samples and it is possible that the rate of recovery would be higher if a more appropriate sample were taken (nasal samples). Thus, the real prevalence could be underestimated.

mecC-positive isolates were recovered from MSA plates but not ORSAB plates. Other authors already noted that mecC-carrying isolates are only able to grow weakly on commercially available selective agar plates for MRSA. In addition, b-lactam resistance levels can be low and isolates can even appear susceptible, particularly to oxacillin.

Characterization of MSSA isolates

Ten of the 11 MSSA isolates showed susceptibility to all tested antimicrobials. Only one MSSA isolate (C6600, recovered from a common vole) was resistant to penicillin (containing the blaZ gene) and was typed as spa-t120/agr-III/MLST-ST15. One MSSA isolate (C6590) was resistant to penicillin (containing the mecC gene) and was typed as spa-t12363/agr-IV/MLST-ST1956. One MSSA isolate (C6592) was resistant to penicillin (containing the mecC gene) and was typed as spa-t12364/agr-IV/MLST-ST1956. One MSSA isolate (C6594) was resistant to penicillin (containing the mecC gene) and was typed as spa-t12863/agr-I/MLST-ST2767.

Regarding virulence factors, nine MSSA carried the cna gene. Only one of 11 MSSA (C6600) carried IEC genes (sak and chp) and this isolate was ascribed to IEC type C according to the criteria mentioned above.

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Table 1. Characteristics of the S. aureus isolates recovered from wild small mammals in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Animal origin</th>
<th>Year of sample collection</th>
<th>Molecular typing</th>
<th>SCCmec</th>
<th>IEC</th>
<th>Antimicrobial resistance phenotype</th>
<th>Antimicrobial resistance genes</th>
<th>Virulence genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6595</td>
<td>Apodemus sylvaticus</td>
<td>2013</td>
<td>spa-t1535 agr-III MLST-ST1945 CC130</td>
<td>X1</td>
<td>sak-sak (group E)</td>
<td>PEN-OXA-FOX meC-blaZ-SCCmecX1</td>
<td>etd2</td>
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<tr>
<td>C6596</td>
<td>Apodemus sylvaticus</td>
<td>2013</td>
<td>spa-t1535 agr-III MLST-ST1945 CC130</td>
<td>X1</td>
<td>sak-sak (group E)</td>
<td>PEN-OXA-FOX meC-blaZ-SCCmecX1</td>
<td>etd2</td>
<td></td>
</tr>
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<td>Microtus arvalis</td>
<td>2012</td>
<td>spa-t120 agr-II MLST-ST15 CC5</td>
<td>—</td>
<td>sak-chp (group C)</td>
<td>PEN-blaZ-SCCmecX1</td>
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<tr>
<td>C6589</td>
<td>Microtus arvalis</td>
<td>2011</td>
<td>spa-t12365 agr-IV MLST-ST1956 CC1956</td>
<td>—</td>
<td>sak-chor (group E)</td>
<td>PEN-OXA-FOX meC-blaZ-SCCmecX1</td>
<td>etd2</td>
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<td>Apodemus sylvaticus</td>
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<td>spa-t12365 agr-IV MLST-ST1956 CC1956</td>
<td>—</td>
<td>sak-chor (group E)</td>
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<td>spa-t12752 agr-IV MLST-ST1956 CC1956</td>
<td>—</td>
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<td>etd2</td>
<td></td>
</tr>
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<td>spa-t9303 agr-III MLST-ST2328 singleton</td>
<td>—</td>
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<td>PEN-OXA-FOX meC-blaZ-SCCmecX1</td>
<td>etd2</td>
<td></td>
</tr>
<tr>
<td>C6590</td>
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<td>2011</td>
<td>spa-t9303 agr-III MLST-ST2328 singleton</td>
<td>—</td>
<td>sak-chor (group E)</td>
<td>PEN-OXA-FOX meC-blaZ-SCCmecX1</td>
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<td>C6592</td>
<td>Microtus arvalis</td>
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</tr>
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<tr>
<td>C6597</td>
<td>Microtus arvalis</td>
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<td>spa-t12364 agr-IV MLST-ST2766 singleton</td>
<td>—</td>
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<td>etd2</td>
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<td>C6604</td>
<td>Mus musculus</td>
<td>2012</td>
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<td>—</td>
<td>sak-chor (group E)</td>
<td>PEN-OXA-FOX meC-blaZ-SCCmecX1</td>
<td>etd2</td>
<td></td>
</tr>
</tbody>
</table>

NT, non-typeable; PEN, penicillin; OXA, oxacillin; FOX, cefoxitin.

Table 1 illustrates the characteristics of the S. aureus isolates recovered from wild small mammals in this study.
Transparency declarations
None to declare.

References