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

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Received 17 January 2014; returned 4 February 2014; revised 17 February 2014; accepted 13 March 2014

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) CC5 (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 mecA1LGA2515 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.6 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).\(^3\) A new mecC2 variant has been described very 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) 42S, 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.\(^1\) 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).\(^1,2\) The MICs of oxacillin and cefoxitin were 16 and 8–16 mg/L, respectively. They were typed for MRSA isolates by the broth microdilution method.\(^12\) S. aureus ATCC 29213 was used as the quality control strain.

The presence of the resistance genes mecA, mecC and blaZ and the new blaZ-like allele associated with SCCmecXI (blaZ-SCCmecXI) was studied by PCR.\(^3,4,14\) The production of β-lactamase was tested in isolates harbouring blaZ-SCCmecXI using paper discs impregnated with chromogenic cephalosporin (Cefinase\(^\text{TM})\).

**Molecular typing of S. aureus isolates**

The S. aureus isolates obtained were characterized by spa typing (www.ridom.com), multi locus sequence typing (MLST) (www.mlst.net) and agr typing.\(^16\) The presence of SCCmecXI was tested by PCR using previously described primers.\(^6\)

**Virulence genotype and detection of immune evasion cluster (IEC) genes**

The presence of virulence genes ( lukS/lukF, tst, cna, eta, etb, etd and etdZ) and staphylococcal enterotoxin genes (sea, seb, sec, seg, sei, sen, see and sej) was studied by PCR.\(^10,15\) The detection of genes of the IEC system (scn, chp, sak, sea and sep) was performed as previously reported, which allows classification into seven different IEC types (A–G).\(^17\)

**Results and discussion**

S. aureus isolates were detected in 13 of the 101 faecal samples of wild small mammals tested in this study (13%, 95% CI 6.4 – 19.6), which corresponded to the following animal species: eight common voles (14.8%), four wood mice (13.8%) and one brown rat (16.7%). All S. aureus were recovered from MSA plates and none from ORSAB plates.

As all S. aureus isolates recovered from each positive sample presented the same antimicrobial resistance phenotype, only one S. aureus per positive sample was maintained and further characterized. Thus, we studied a collection of 13 S. aureus isolates recovered from the studied animals (Table 1). Two S. aureus isolates were MRSA, and they were recovered from two different wood mice (A. sylvaticus) trapped in the south of Spain in 2013. The remaining 11 S. aureus isolates were MSSA and were recovered from animals captured in the south and north-central regions of Spain in 2011 and 2012 (Table 1).

**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 the same antimicrobial resistance phenotype, only one S. aureus per positive sample was maintained and further characterized. Thus, we studied a collection of 13 S. aureus isolates recovered from the studied animals (Table 1). Two S. aureus isolates were MRSA, and they were recovered from two different wood mice (A. sylvaticus) trapped in the south of Spain in 2013. The remaining 11 S. aureus isolates were MSSA and were recovered from animals captured in the south and north-central regions of Spain in 2011 and 2012 (Table 1).

The novel blaZ-SCCmecXI was detected in both mecC-positive isolates and β-lactamase activity was confirmed by the Cefinase\(^\text{TM}) disc test. This blaZ allele type 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, chp and scn genes.\(^4,10,15\) which supports the
hypothesis of a possible animal origin of these isolates. 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, β-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-II/MLST-ST15. Five new spa types (t12363, t12364, t12365, t12752 and t12863) and two new STs (ST2766 and ST2767) were identified in this study. MSSA strains were ascribed to CC5 (ST15, one strain) and CC1956 (ST1956, four strains); the other six strains were not ascribed to any CC (ST2328, four strains; ST2766, one strain; and ST2767, one strain). The predominant agr types were IV and III. Regarding virulence factors, nine MSSA carried the cna gene. Only one of 11 MSSA (C6600) carried IEC genes (scn and chp) and this isolate was ascribed to IEC type C according to the criteria mentioned above.

Conclusions

In summary, wild small mammals, specifically the rodent A. sylvaticus, could be a reservoir of mecC-positive S. aureus isolates of CC130 lineage, which could be transmitted to domestic animals or to humans with important implications for public health. The detection of genes of the IEC system in these isolates raises questions about the possible origin of these apparently zoonotic isolates.

Acknowledgements

We acknowledge the collaboration of field technicians and other researchers from IREC in capturing the small mammals.

Funding

This work was supported by Projects SAF2012-35474 and CGL2011-30274 from the Ministerio de Economía y Competitividad (MINECO) of Spain and the Fondo Europeo de Desarrollo Regional (FEDER), and by EU FP7 EMIDA ERA-NET grant APHAEA on wildlife disease surveillance in Europe. P. G. has a pre-doctoral fellowship from the Universidad de La Rioja (Spain) and D. B. has a pre-doctoral fellowship from MINECO. F. R.-F. acknowledges funding from a Juan de la Cierva post-doctoral contract from MINECO.
Transparency declarations

None to declare.

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