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The newly described *mecA* homologue, $mecA_{LGA251}$, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species

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Objectives: A previously unidentified *mecA* homologue, $mecA_{LGA251}$, has recently been described in methicillin-resistant *Staphylococcus aureus* (MRSA) from humans and dairy cattle. The origin and epidemiology of this novel homologue are unclear. The objective of this study was to provide basic descriptive information of MRSA isolates harbouring $mecA_{LGA251}$ from a range of host animal species.

Methods: A number of *S. aureus* isolates from historical animal isolate collections were chosen for investigation based on their similarity to known $mecA_{LGA251}$ MRSA isolates. The presence of $mecA_{LGA251}$ was determined using a multiplex PCR and antimicrobial susceptibility testing performed by disc diffusion.

Results: MRSA harbouring $mecA_{LGA251}$ were found in isolates from a domestic dog, brown rats, a rabbit, a common seal, sheep and a chaffinch. All of the isolates were phenotypically MRSA, although this depended on which test was used; some isolates would be considered susceptible with certain assays. All isolates were susceptible to linezolid, rifampicin, kanamycin, norfloxacin, erythromycin, clindamycin, fusidic acid, tetracycline, trimethoprim/sulfamethoxazole and mupirocin. Five multilocus sequence types were represented (2273, 130, 425, 1764 and 1245) and six *spa* types (t208, t6293, t742, t6594, t7914 and t843).

Conclusions: The discovery of MRSA isolates possessing $mecA_{LGA251}$ from a diverse range of host species, including different taxonomic classes, has important implications for the diagnosis of MRSA in these species and our understanding of the epidemiology of this novel *mecA* homologue.

Keywords: animal infections, animal reservoirs, wildlife, MRSA

Introduction

Staphylococcus aureus causes a wide range of diseases in humans, from minor skin infections to severe illnesses such as septicaemia, toxic shock, endocarditis and pneumonia. The emergence and dissemination of methicillin-resistant *S. aureus* (MRSA) has posed a major challenge in the treatment of *S. aureus* infections. *S. aureus*, including MRSA, can colonize and infect a wide variety of other host species, including cats, dogs, pigs, cattle, poultry and horses. This is not only of veterinary significance, but has zoonotic importance, with animals acting as a potential source for the emergence of novel MRSA clones in human beings. Pig to human transmission of MRSA ST398 (where ST stands for sequence type) is suggested to explain the emergence and spread of this clone in humans.[1]

Methicillin resistance in *S. aureus* is conferred by the acquisition of one of several staphylococcal cassette chromosome *mec* (SCC*mec*) elements, which carry the *mecA* gene encoding

a penicillin-binding protein homologue (PBP2a) with low affinity for β-lactam antibiotics.[2] We have identified a novel *mecA* homologue, *mecA*$_{LGA251}$, encoded in a new SCC*mec* cassette designated type XI.[3] This *mecA* homologue exhibits only 70% identity at the DNA level and 63% identity at the protein level to the previously described *mecA* gene and is not detectable by routine *mecA*-specific PCR approaches and PBP2a slide agglutination tests. While *mecA*$_{LGA251}$ is present in MRSA isolates from humans and dairy cattle, its origin and epidemiology are currently unclear, with some evidence to suggest it may have spread from cattle to humans.[3] Here we describe *mecA*$_{LGA251}$-containing MRSA isolates from additional host species. This has important implications for the diagnosis of MRSA infections in these hosts, and for our understanding of the epidemiology and evolution of this *mecA* homologue and the MRSA lineages that carry it.

Materials and methods

Candidate isolates were identified through personal contacts, reports to the multilocus sequence typing (MLST) database (http://saureus.mlst.net) and scientific reports of phenotypically resistant but MRSA that were *mecA* negative or *S. aureus* isolates related by MLST to known *mecA*$_{LGA251}$-positive lineages [clonal complexes (CCs) 425, 130, 705 and 1943].[3,4] Not all requested isolates were obtainable. A total of 52 candidate isolates were tested from samples collected between 1993 and 2011.

The isolates identified were tested for the presence of *femB*, *mecA* and *mecA*$_{LGA251}$ by multiplex PCR using the following primers: *femB*, 1I 5′-CATGGTTACGAGCATCATGG-3′ and 1J 5′-AACGCCAGAAGCAAGGTTTA-3′, yielding a 533 bp product; *mecA*, 2W 5′-TGGTATGTGGAAGTTAGATTGG GAT-3′ and 2X 5′-CTAATCTCATATGTGTTCCTGTATTGGC-3′, as used by Nakagawa *et al.*,[5] yielding a 155 bp product; and *mecA*$_{LGA251}$, 1A 5′-CATTAAAATCAGAGCGAGGC-3′ and 1B 5′-TGGCTGAACCCATTT TTGAT-3′, yielding a 188 bp product. The specificity of each primer pair was confirmed in preliminary experiments, with product identity confirmed by sequencing. The presence of *mecA*$_{LGA251}$ in positive isolates was also confirmed by sequencing.

Antimicrobial susceptibility testing was performed by disc diffusion (Oxoid, Basingtoke, UK) according to EUCAST methodology (www.eucast.org) for 12 antimicrobial agents: penicillin, cefoxitin, norfloxacin, erythromycin, clindamycin, kanamycin, tetracycline, linezolid, fusidic acid, rifampicin, trimethoprim/sulfamethoxazole and mupirocin. Growth on MRSA Brilliance 2 agar (Oxoid) was also assessed. All susceptibility results were interpreted according to EUCAST except for trimethoprim/sulfamethoxazole, for which interpretation was made according to CLSI guidelines. In addition, the MIC was determined for cefoxitin and oxacillin by microbroth dilution performed as described by EUCAST using Mueller–Hinton BBL II broth (Becton Dickinson, Heidelberg, Germany). An inoculum of 5×10^5 cfu of *S. aureus* ATCC 29213 was used for quality control.

Results

PCR testing identified *mecA*$_{LGA251}$ in MRSA isolated from four brown rats from Belgium (an identical strain from four different rats), one chaffinch from Scotland, one common seal from Scotland, three sheep from Denmark, one domestic dog from Scotland and one rabbit from Belgium (Table 1). The rat and sheep isolates were obtained from screening of apparently healthy individuals. The chaffinch and seal isolates were obtained from *post mortem* investigations of diseased animals, although it could not be determined if *S. aureus* was the primary cause of disease. The dog isolate was obtained from a clinical case, but further clinical details were not recorded. The rabbit isolate was obtained from a case of highly virulent staphylococcal disease.[6] The MIC of oxacillin for all isolates ranged from 0.125 to 16 mg/L, and the MIC of cefoxitin ranged from 4 to 32 mg/L (Table 1). Eight of the 11 isolates were phenotypically MRSA as assessed by growth on MRSA indicator agar, disc diffusion (cefoxitin) and oxacillin and cefoxitin MICs (Table 1). Strains MRSA 1390 and PI 41/95 were susceptible to cefoxitin by disc diffusion, did not grow on the above MRSA indicator agar and had MICs of oxacillin beneath the breakpoint of 2 mg/L. However, they were resistant to cefoxitin as assessed by MIC (Table 1). Strain 07.7672.A had an oxacillin MIC below the breakpoint, but was phenotypically MRSA using the other assays (cefoxitin MIC, cefoxitin disc diffusion and growth on MRSA agar, although it produced small colonies). All isolates were susceptible to linezolid, rifampicin, kanamycin, norfloxacin, erythromycin, clindamycin, fusidic acid, tetracycline, trimethoprim/sulfamethoxazole and mupirocin. Five multilocus STs were represented (2273, 130, 425, 1764 and 1245) and six *spa* types (t208, t6293, t742, t6594, t7914 and t843) (Table 1 and Figure 1). DNA sequencing confirmed that the *mecA*$_{LGA251}$ in all these isolates was identical to that originally reported[3] (data not shown).

Discussion

We have previously identified a divergent *mecA* homologue, *mecA*$_{LGA251}$, in MRSA strains from humans and dairy cattle.[3] This homologue is not detected by routine PCR and PBP2a slide agglutination assays, which prevented its earlier detection, and its epidemiology and evolution are currently unclear. Initially *mecA*$_{LGA251}$ was only reported in MRSA from humans and/or dairy cattle from the UK, Denmark, Ireland and Germany.[3,4,7,8] Using PCR, we have now identified this *mecA* homologue in MRSA isolates from several new host species (brown rat, rabbit, common seal, domestic dog, chaffinch and sheep) and from one new country, Belgium. Five STs were found among the 11 *mecA*$_{LGA251}$-positive isolates. Four of these STs (130, 425, 1764 and 1245) have previously been associated with *mecA*$_{LGA251}$. However, the four brown rat MRSA isolates from Belgium are of a new ST, ST2273, belonging to CC49, which has not previously been found among *mecA*$_{LGA251}$ MRSA. *S. aureus* belonging to CC49 have previously been isolated from Switzerland, the UK and Denmark from humans, pigs and red squirrels and include isolates of MRSA and methicillin-susceptible *S. aureus* (http://saureus.mlst.net, accessed May 2012). Of the six *spa* types identified in our isolates, *mecA*$_{LGA251}$ has been reported previously from three (t6293, t742 and t843),[3] but not from the other three (t208, t6594 and t7914).

The MIC values for these isolates are similar to the range identified for cefoxitin in the original identification of *mecA*$_{LGA251}$ (4–64 mg/L).[3] However, in the case of oxacillin, the MIC for strain PI 41/95, 0.125 mg/L is lower than previously described for *mecA*$_{LGA251}$-positive strains.[3] No sequence diversity was discovered in the *mecA*$_{LGA251}$ gene, thus the basis for variation in the antimicrobial susceptibilities of these isolates is unclear (data not shown).

Our findings indicate that *mecA*$_{LGA251}$-carrying MRSA strains are present in diverse host species and can be responsible for clinical disease in species other than man and cattle. This has important implications for understanding the epidemiology and dissemination of *mecA*$_{LGA251}$. The ubiquitous status of the

Table 1. Characteristics of $mecA_{LGA251}$-positive MRSA strains from this study

Strain name	Host species	Country of isolation	Year of isolation	ST	*spa* type	Cefoxitin MIC (mg/L)	Oxacillin MIC (mg/L)	Resistance (disc diffusion)[a]	Additional notes
MRSA 1390	brown rat (*Rattus norvegicus*)	Belgium	2008–09	2273 (new)	t208	8	0.5	penicillin	isolated from nasal mucosa of wild rats caught in River Demer basin
MRSA 1410	brown rat (*Rattus norvegicus*)	Belgium	2008–09	2273 (new)	t208	8	4	cefoxitin and penicillin	isolated from nasal mucosa of wild rats caught in River Demer basin
MRSA 1421	brown rat (*Rattus norvegicus*)	Belgium	2008–09	2273 (new)	t208	8	4	cefoxitin and penicillin	isolated from nasal mucosa of wild rats caught in River Demer basin
MRSA 1467	brown rat (*Rattus norvegicus*)	Belgium	2008–09	2273 (new)	t208	16	4	cefoxitin and penicillin	isolated from nasal mucosa of wild rats caught in River Demer basin
B307063	chaffinch (*Fringilla coelebs*)	Scotland	2011	130	t6293	16	4	cefoxitin and penicillin	isolated at *post mortem* examination from the liver and intestines of a wild bird with severe necrotic esophagitis resulting from a *Trichomonas gallinae* infection
PI 41/95	rabbit (*Oryctolagus cuniculus*)	Belgium	1995	425	t742	4	0.125	penicillin	reported in a paper describing an isolate that caused a highly virulent infection in a rabbit[6]
M1472/93/01	common seal (*Phoca vitulina*)	Scotland	1993	1764	t6594	16	4	cefoxitin and penicillin	isolated from a male seal pup with brain disease, Cromarty, Scottish Highlands
07.7672.A	domestic dog (*Canis lupus familiaris*)	Scotland	2007	1245	t7914	8	1	cefoxitin and penicillin	clinical isolate, but details not available
Får 2	sheep (*Ovis aries*)	Denmark	2011	130	t843	16	16	cefoxitin and penicillin	nasal swab from an apparently healthy animal
Får 7	sheep (*Ovis aries*)	Denmark	2011	130	t843	16	8	cefoxitin and penicillin	nasal swab from an apparently healthy animal
Får 9	sheep (*Ovis aries*)	Denmark	2011	130	t843	32	8	cefoxitin and penicillin	nasal swab from an apparently healthy animal

[a]Antibiotics tested: penicillin, cefoxitin, linezolid, rifampicin, kanamycin, norfloxacin, erythromycin, clindamycin, fusidic acid, tetracycline, trimethoprim/sulfamethoxazole and mupirocin.

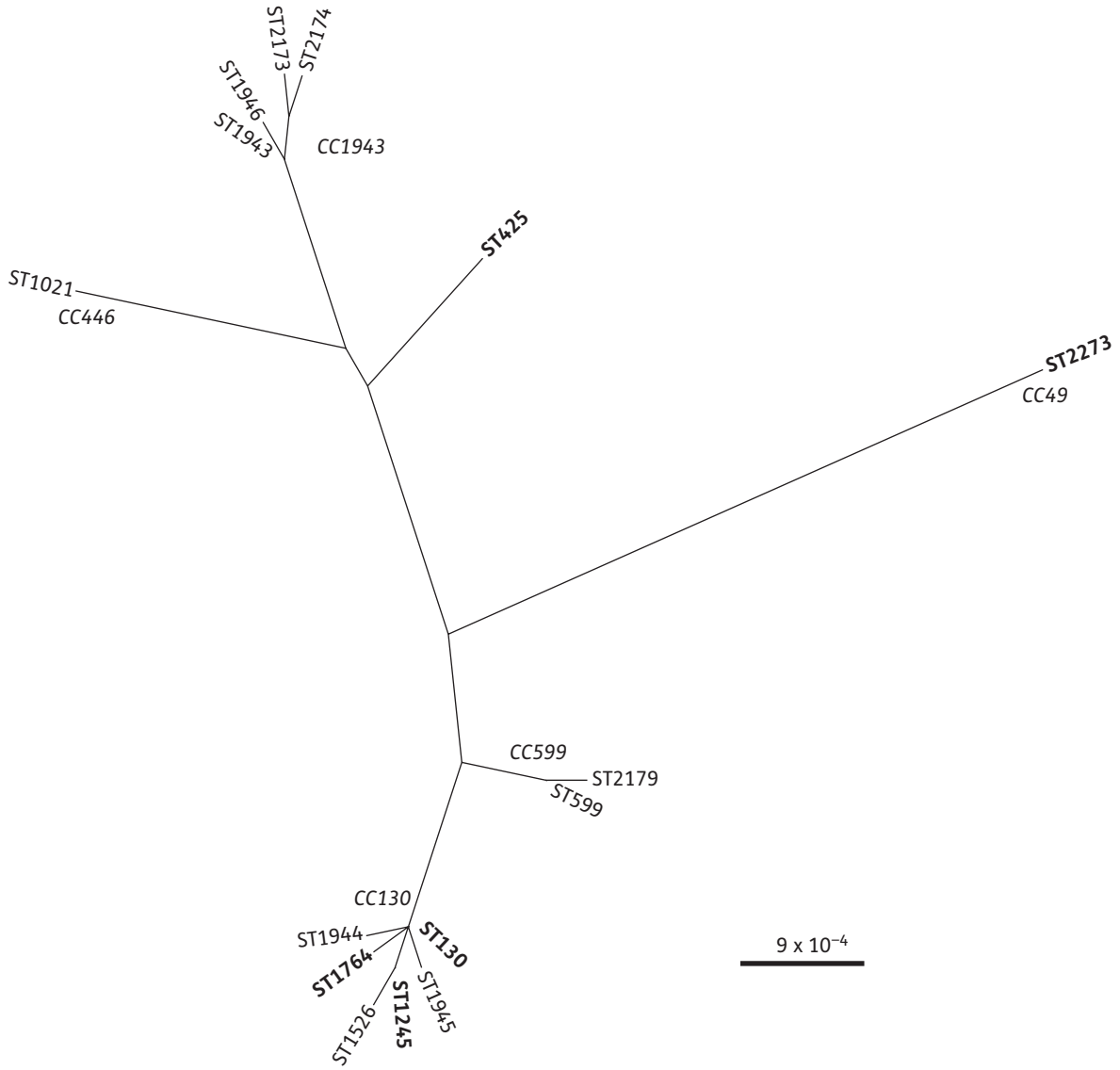

Figure 1. Phylogenetic relationships between MRSA isolates positive for *mecA*$_{LGA251}$. An unrooted phylogenetic tree using the concatenated MLST allele sequences showing the relationship between all STs in which the divergent *mecA* gene has been found. The CCs of related STs are indicated in italic text. The STs of isolates that are described in this study are indicated in bold text. The tree was generated using Geneious v5.6 (www.geneious.com) using the Tamura-Nei distance model for genetic distance and the tree built using neighbour-joining with no outgroup.

brown rat makes this species a strong candidate vector for the spread of *mecA*$_{LGA251}$, but it should be noted that to date only *mecA*$_{LGA251}$-positive CC49 strains of MRSA have been isolated from the brown rat, and CC49 *mecA*$_{LGA251}$-positive strains have not been found in other host species. The collection of isolates described in this report did not result from an exhaustive search of historical bacteriological collections or from a comprehensive survey of current clinical disease in animal species; however, *mecA*$_{LGA251}$-positive MRSA should be considered in the diagnosis of putative MRSA not only in the host species we highlight here, but also in additional hosts. Furthermore, our findings suggest that in addition to livestock and companion animal contact, wild animals and birds may pose a so far unregistered risk for transmission of MRSA between humans and animals.

To conclude, further MRSA surveillance in diverse host species including humans, companion animals, livestock and wildlife is required to fully understand *mecA*$_{LGA251}$ epidemiology and evolution, to evaluate its significance in disease and to implement control measures where necessary.

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Transparency declarations

None to declare.

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