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First detection of livestock-associated meticillin-resistant *Staphylococcus aureus* CC398 in bulk tank milk in the United Kingdom, January to July 2012

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Livestock-associated meticillin-resistant *Staphylococcus aureus* belonging to clonal complex 398 (LA-MRSA CC398) is an important cause of zoonotic infections in several countries, but there is only a single published report of this lineage from the United Kingdom (UK). Here, we describe the isolation of LA-MRSA CC398 from bulk tank milk from five geographically dispersed farms in the UK. Our findings suggest that LA-MRSA CC398 is established in livestock in the UK. Awareness of the potential occupational risks and surveillance in other food-producing animal species should be promoted.

Isolation of meticillin-resistant *Staphylococcus aureus* from dairy cattle

During a study, performed from January to July 2012, to detect *mecC* meticillin-resistant *Staphylococcus aureus* (MRSA) in dairy cattle in the United Kingdom (UK), ca. 1,500 bulk tank milk samples were supplied by National Milk Laboratories Ltd., (Chippenham, UK). These were collected aseptically by trained technicians for quality assurance purposes and stored at 4 °C for up to five days prior to testing. Enrichment for *S. aureus* was performed using a modification of a published technique [1] omitting the incubation in phenol red mannitol broth supplemented with 4 mg/L oxacillin (24 h at 37°C). Identification of potential MRSA colonies (blue colour) was confirmed by subculture on Staph Brilliance 24 plates (Oxoid, Basingstoke, UK) and these were subsequently screened for *mecA*, *mecC* and *femB* by multiplex PCR as described previously [2]. Approximately 300 potential MRSA colonies were identified and subjected to PCR testing, yielding a total of seven *mecA* MRSA isolates from five farms, including three isolates from the same farm. These isolates were found to be *mecA*, *femB*-positive by PCR (Table). All seven isolates were resistant to penicillin, meticillin and cefoxitin by disk diffusion according to the European Committee on

Antimicrobial Susceptibility Testing (EUCAST) guidelines [3].

Molecular and phenotypic characterisation of LA-MRSA CC398 from dairy cattle in the United Kingdom

Multi-locus sequence typing found all seven isolates belonged to sequence type ST398, and CC398-specific PCR based on the restriction-modification system *sau1-hsdS1* confirmed that all the isolates belonged to clonal complex CC398 [4]. Isolates from three farms exhibited *spa* type *to11* and carried a composite staphylococcal cassette chromosome *mec* (SCC*mec*) V(5C2&5)c element, whereas isolates from the remaining two farms had *spa* types *to11* and *t2546* and harboured SCC*mec* IVa. All isolates lacked the *lukS-PV* and *lukF-PV* genes encoding Panton-Valentine leukocidin and the *scn* gene (Table). Antimicrobial susceptibility testing using disk diffusion according to the EUCAST guidelines revealed that all isolates were resistant to tetracycline, and PCR [10] demonstrated the presence of the tetracycline resistance gene *tet(M)* in all seven, and of *tet(K)* in three isolates (Table).

Discussion

Here we describe the first isolation of LA-MRSA CC398 from dairy cattle in the UK. This is only the second published instance of LA-MRSA CC398 in this country following the report of isolates (*to11* and SCC*mec* IVa) from two horses in south-eastern England [11]. In many countries in continental Europe and elsewhere, LA-MRSA CC398 poses an occupational risk for those in close contact with livestock, particularly pigs and veal calves. For instance, significantly higher rates of MRSA nasal carriage by humans in contact with pigs (farm workers, abattoir workers, veterinarians) have been noted in several epidemiological studies, with the isolates typically belonging to CC398 [12-16]. Further

TABLE

Molecular and phenotypic characteristics of methicillin-resistant *Staphylococcus aureus* CC398 from bulk tank milk in the United Kingdom, January to July 2012 (n=7)

Farm	Location	Date of sampling	Strain	CC398 ^a	<i>spa</i> type	SCC <i>mec</i> type ^b	<i>lukS-lukF</i> ^c	Scn ^d	<i>tet(M)</i> ^e	<i>tet(K)</i> ^e	Resistance profile ^f
1	Dumfries and Galloway	Jan 2012	17-51	+	to11	IVa	-	-	+	-	TET, NOR, KAN
1	Dumfries and Galloway	Jul 2012	17-57	+	to11	IVa	-	-	+	-	TET, NOR, KAN
1	Dumfries and Galloway	Jul 2012	34-179	+	to11	IVa	-	-	+	-	TET, NOR, KAN
2	Worcestershire	May 2012	22-79	+	to11	V(5C2&5)c	-	-	+	+	TET, NOR
3	Berkshire	May 2012	25-26	+	to11	V(5C2&5)c	-	-	+	+	ERY, CLI, TET, NOR
4	Warwickshire	May 2012	30-59	+	t2346	IVa	-	-	+	-	TET, KAN
5	Wrexham	Jul 2012	31-07	+	to11	V(5C2&5)c	-	-	+	+	TET, NOR, KAN

CC: clonal complex; ERY: erythromycin; CLI: clindamycin; TET: tetracycline; NOR: norfloxacin; KAN: kanamycin.

^a CC398 as determined by the method of Stegger et al. [4].

^b As determined by the method described in [5-7], V(5C2&5)c; SCC*mec* V harbouring the *czrC* gene in the J1 region.

^c As determined by the method described in [8].

^d As determined by the method described in [9].

^e As determined by the method described in [10].

^f Also tested (but all strains susceptible) were linezolid, rifampicin, fusidic acid, trimethoprim/sulfamethoxazole and mupirocin.

studies have shown an association between clinical disease resulting from LA-MRSA CC398 infection and contact with pigs or pig farms [16-20]. The impact of this can be significant locally, and this lineage can be imported into healthcare settings. For example, in a German hospital in an area with a large number of pigs, 22% of patients colonised with MRSA at admission carried ST398 [21]. Nosocomial transmission has also been reported [22]. LA-MRSA CC398, like other MRSA, may be responsible for life-threatening infections during long or frequent hospitalisations, or following wound or surgery site infections, and also increases healthcare costs resulting from screening, isolation of carriers, and decolonisation. Although pasteurisation of milk should ensure that CC398 MRSA will not enter the food chain, our finding of LA-MRSA CC398 in dairy cattle has clear public health implications for the UK. Workers on dairy farms, or individuals with regular contact with dairy cows, are likely to have a higher risk of colonisation or infection with LA-MRSA CC398 compared to the general population in the UK. LA-MRSA CC398 isolates from three of the farms where isolated were found carried SCC*mec* type IVa. The isolates from the other two farms carried SCC*mec* type V(5C2&5)c. Both of these SCC*mec* types have previously been found in LA-MRSA CC398 isolates [23].

Heterogeneity is seen in *S. aureus* CC398, with human and livestock-associated lineages being differentiated by the presence or absence of specific resistance and virulence-related genes [23-24]. In all of our isolates the absence of the *scn* gene, encoding the human-specific staphylococcal complement inhibitor, and the presence of *tet(M)* suggested that they were all livestock-associated, as opposed to *S. aureus* CC398 strains which circulate in the human population independent of a livestock reservoir [23-24]. Likewise, all seven isolates lacked the *lukS-PV* and *lukF-PV* genes encoding Panton-Valentine leukocidin which is absent in LA-MRSA CC398, but is present in some, but not all, human-associated CC398 isolates [23]. Three consecutive samples from the same farm over a seven-month period were positive for LA-MRSA CC398 isolates with identical *spa* (to11) and SCC*mec* types (IVa), suggesting that this strain is able to persist in dairy herds over prolonged periods. While there are relatively few reports of LA-MRSA CC398 from dairy cattle compared to pig farms, it has been found to cause bovine mastitis [25-27]. Our findings therefore have significance to veterinary medicine, in addition to public health. The relative absence of CC398 MRSA from the UK prior to this study, when it is widespread in the rest of Europe suggests that the geographical separation of the UK

from continental Europe may have delayed the spread of this lineage to the UK rather than there being any fundamental difference in husbandry or biosecurity in the UK. The authors are aware of unpublished surveys looking for potential LA-MRSA in UK dairy and pig herds that have been negative before now. These CC398-positive samples were not part of a formal prevalence study, and it is therefore unclear how common LA-MRSA CC398 isolates are in UK dairy farms or if they are present in other livestock. However, the five farms with positive samples were identified from a sample of ca. 1,500 farms, indicating a low prevalence currently.

Conclusions

This is the first description of LA-MRSA CC398 in food-producing animals in the UK. The ability of this lineage to colonise a wide range of host species, coupled with its zoonotic potential, make this finding of significance to both veterinary and human health. Future surveillance for this LA-MRSA CC398 strain in all food-producing animal species in the UK and the evaluation of occupational risk factors for MRSA carriage and infection should be considered.

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