Detection of livestock-associated meticillin-resistant Staphylococcus aureus CC398 in retail pork, United Kingdom, February 2015

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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 many countries. Here, we describe the isolation of LA-MRSA CC398 from retail meat samples of United Kingdom (UK) farm origin. Our findings indicate that this lineage is probably established in UK pig farms and demonstrate a potential pathway for the transmission of LA-MRSA CC398 from livestock to humans in the UK.

A survey was conducted in February 2015 to detect meticillin-resistant *Staphylococcus aureus* (MRSA) in retail meat products obtained from supermarkets in the United Kingdom (UK). A total of 103 (52 pork and 51 chicken) pre-packaged fresh meat products, labelled as being of UK farm origin, were purchased from supermarkets in five different locations (Locations A-E) in the UK. All meat products were frozen (-20 °C) and sent to the Department of Veterinary Medicine, University of Cambridge, for testing.

Preparation and testing of meat samples

The preparation of meat samples followed the European standard ISO 6887–2:2003 [1]. After thawing, the exterior packaging was disinfected before the meat was removed. A 10 g sample of meat was excised, mixed with 225 ml of 6% w/v NaCl Nutrient Broth (P and O laboratories, UK) and homogenised using a Stomacher (Stomcher80 Laboratory System, Seward Ltd, UK) for two minutes. Enrichment for *S. aureus* was performed as previously described [2]. Identification of potential MRSA colonies (blue colour) was confirmed by subculture on MRSA Brilliance 24 plates (Oxoid, Baskingstoke, UK) which were subsequently screened for *mecA*, *mecC* and *femB* by multiplex PCR as described previously [3]. Potential MRSA colonies subjected to PCR testing initially yielded two *mecA* positive cultures (samples C7 and D8). Three colonies from subcultures from each of these original samples were *spa* typed as described previously [4] which yielded a single *spa* type from one sample and two different *spa* types from the other.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of all three isolates was analysed using the VITEK 2 system (bioMérieux, Basingstoke, UK) in accordance with the manufacturer's instructions using a Staph AST-P635 card with results interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [5]. Antimicrobial susceptibility results (Table 1) showed that all three isolates were phenotypically MRSA and were additionally resistant to tetracycline and trimethoprim.

Genomic analyses

Genomic DNA of all three *S. aureus* isolates was extracted from overnight cultures grown in TSB at 37 °C using the MasterPure Gram Positive DNA Purification Kit (Cambio, Cambridge, UK). Illumina library preparation was carried out as described by Quail et al. [6] and Mi-Seq sequencing was carried out following the manufacturer's standard protocols (Illumina, Inc., San Diego, CA, US). Genomes were assembled de novo from Fastq files with Velvet [7]. The draft sequences for C7–1, C7–2 and D8 had a total of 38, 22 and 31 contigs, respectively. Comparative genomics were carried out using WebACT and viewed with the Artemis comparison tool (ACT) [8]. The presence of antibiotic resistance genes was identified using the ResFinder-1.3 Server [9] and by BLAST [10] against the assemblies. Nucleotide sequences of isolates C7–1, C7–2 and D8 have been

Table 1

Antimicrobial susceptibility characteristics of meticillin-resistant *Staphylococcus aureus* CC398 from retail pork samples, United Kingdom, February 2015 (n = 3)

Isolate	Benzylpenicillin	Cefoxitin	Oxacillin	Ciprofloxacin	Clindamycin	Erythromycin	Tetracycline	Trimethoprim
C7–1	R	R	R	R	S	S	R	R
C7–2	R	R	R	S	R	S	R	R
D8	R	R	R	R	S	R	R	R

R: resistant; S: susceptible.

Results of testing using a VITEK 2 system (bioMérieux, Basingstoke, UK) using a Staph AST-P635 card (testing for susceptibility to cefoxitin, benzylpenicillin, oxacillin, gentamycin, ciprofloxacin, clindamycin, erythromycin, linezolid, daptomycin, teicoplanin, vancomycin, tetracycline, fusidic acid, mupirocin, chloramphenicol, rifampicin, and trimethoprim). All three isolates were susceptible to gentamycin, linezolid, daptomycin, teicoplanin, vancomycin, fusidic acid, mupirocin, chloramphenicol and rifampicin. Breakpoints were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

deposited in the European short read archive with accession numbers ERR902083, ERR902084 and ERR902085, respectively.

Multilocus sequence typing using the assembly sequences found that all three isolates belonged to sequence type ST398 and carried a composite staphylococcal cassette chromosome *mec* (SCC*mec*) V(5C2 and 5)c element including the cadmium and zinc resistance gene *czrC* [11]. All isolates lacked the *lukS-PV* and *lukF-PV* genes encoding Panton-Valentine leukocidin and the human-associated immune evasion cluster genes *sak*, *scn* and *chp* (often carried by the phage φSa3) [12]. All three isolates carried an extra copy of the von Willebrand factor-binding protein (vWbp) gene, *vwb* previously found on pathogenicity island SaPIbov5 in a ST398 isolate which confers the ability to clot ruminant plasma [13]. Genomic analysis demonstrated the presence of the tetracycline resistance genes *tet*(M) and *tet*(K) in addition to *mecA*, in all three isolates, together with other resistance determinants which varied between isolates and matched their antimicrobial susceptibilities (Tables 1 and 2). Three canonical single nt polymorphisms (canSNP) shown by Stegger et al. [14] to distinguish between human and livestock clades of ST398 had the livestock associated nt in all three positions for all three of the isolates.

Discussion

Here we describe the first isolation of LA-MRSA ST398 from retail meat originating from farms in the UK. Recent reports of CC398 isolates from horses [15], dairy cattle [2], poultry [16], and pigs [17,18] indicate that this lineage is widely distributed in the UK. In many countries LA-MRSA CC398 represents an occupational risk for those in close contact with livestock,

Table 2

Molecular characteristics of meticillin-resistant *Staphylococcus aureus* CC398 from retail pork samples, United Kingdom, February 2015 (n = 3)

Isolate	Location	Meat type	MLST	*spa* Type	SCC*mec* type	φSa3	canSNP 748	canSNP 1002	canSNP 3737	*tet*(M)	*tet*(K)	Other
C7–1	C	Pork sausage	ST398	t011	V(5c2 and 5)c	Neg	LA	LA	LA	Pos	Pos	*blaZ* *dfrK*
C7–2	C	Pork sausage	ST398	t034	V(5c2 and 5)c	Neg	LA	LA	LA	Pos	Pos	*blaZ* *dfrG* *spc* *linB* *aad9*
D8	D	Pork mince	ST398	t034	V(5c2 and 5)c	Neg	LA	LA	LA	Pos	Pos	*blaZ* *dfrG* *aadD* *lnu(B)* *erm(C)* *linB* *cadR* *merR*

LA: livestock-associated; MLST: Multilocus sequence typing; Neg: negative; Pos: positive.

The φSa3 phage is associated with human ST398 isolates which carries a cluster of human immune evasion genes [14]. The columns headed canSNP748, canSNP1002 and canSNP3737 refer to canonical SNPs described by Stegger et al. [14] associated with human- or livestock-associated lineages. The antimicrobial resistance genes were identified using the ResFinder-1.3 Server [9].

particularly pigs and veal calves. For example, significantly higher rates of CC398 MRSA nasal carriage by humans in contact with pigs (farm workers, abattoir workers, veterinarians) have been shown in epidemiological studies [19-22]. Other studies have revealed an association between clinical disease resulting from LA-MRSA CC398 infection and recent contact with pigs or pig farms [23-27]. As with other MRSA, LA-MRSA CC398 may be responsible for serious illness following wound or surgery site infections. They may also contribute to increased healthcare costs due to screening, isolation of carriers, and decolonisation. Adequate cooking (heating above 71°C) and hygienic precautions during food preparation should minimise the likelihood of human colonisation via contaminated pork. Still our finding of LA-MRSA CC398 in pork identifies a potential pathway from farms to the wider population. Cuny et al. [28] identified thawing liquid of broiler chicken carcasses as having greater numbers of bacteria which may represent an increased risk for frozen meats. Our study did not examine the thaw water separately and also failed to find ST398 in poultry samples which suggests that this lineage may be present in the UK at lower rates than in continental Europe; however, further studies are required to establish this.

While human contamination of carcasses or meat products in the abattoir or at the meat packing plant may occur, there is evidence that the ST398 isolates are of animal origin. The isolates carried tetracycline resistance genes, lacked the human virulence phage, φSa3, possessed the three canonical SNPs previously shown to identify animal lineages and copies of the von Willebrand factor-binding protein (vWbp) gene associated with livestock [13,14]. The ST398 isolates all came from processed pork (sausages and minced pork) likely to comprise meat from multiple carcases. Testing of these meat products used a highly sensitive method of detection of bacterial contamination and so the numbers of MRSA present may be low. It cannot be ruled out that the meat packing plants from which the MRSA from this study originated also handle imported meat. If this were the case, it is conceivable that cross-contamination might have occurred between non-UK to UK sourced meat. Further phylogenetic studies are required to provide evidence to examine that possibility.

Conclusions

This is the first description of LA-MRSA CC398 in retail meat products in the UK. The presence of a lineage capable of colonising a wide range of host species with a zoonotic potential make this finding of significance for both human and animal health. Furthermore, the presence of LA-MRSA CC398 in the human food chain demonstrates in addition to the established risk through direct contact with animals a possible further pathway for the transmission of antimicrobial resistance from livestock to the broader human population, and not just via those with direct contact with farm animals.

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Conflict of interest

None declared.

Authors’ contributions

Nazreen F Hadjirin performed laboratory work and wrote the manuscript, Elizabeth M Lay collected samples and performed laboratory work, Gavin K Paterson performed some of the laboratory work and contributed to the manuscript, Ewan M Harrison performed some of the analysis and contributed to the manuscript, Sharon J Peacock edited the manuscript, Julian Parkhill edited the manuscript, Ruth N Zadoks edited the manuscript, Mark A Holmes designed the study, supervised the laboratory work, undertook some of the analysis and edited the manuscript.

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