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Reverse zoonotic transmission of community-associated MRSA ST1-IV to a dairy cow

Sir,

Here we report the isolation and molecular characterisation of a community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) clone ST1-IV in an Australian dairy cow with subclinical mastitis. In January 2015, a dairy herd manager requested a veterinary evaluation to determine the risk factors contributing to an elevated bulk milk cell count (BMCC) and a high rate of clinical mastitis on a farm in Victoria. The herd consisted of ca. 480 Friesian and Friesian-cross cattle, managed by seven stock handlers. The herd had an extended period with a BMCC of >300 000 cells/mL.

As part of the investigation, composite milk samples were collected from 17 cows with a history of elevated BMCC. Milk from seven of the cows was contaminated with enteric and/or skin bacteria. *Streptococcus uberis* was isolated from four animals, *S. aureus* from four animals, *Corynebacterium bovis* from one animal and *Streptococcus dysgalactiae* from one animal. *Staphylococcus aureus* and *S. uberis* were identified from a sample collected from a 4-year-old Friesian cow that had calved 2 months previously without a history of clinical mastitis. She had a history of an elevated cell count in her previous lactation and in January 2015. The *S. aureus* isolate was identified as MRSA by routine susceptibility testing according to Clinical and Laboratory Standards Institute (CLSI) recommendations [1]. Cefoxitin was used as a surrogate antimicrobial for identifying methicillin resistance. The MRSA-positive
cow was aseptically resampled and MRSA was cultured from this second milk sample.

Antimicrobial susceptibility testing was performed by disk diffusion according to CLSI recommendations [1] for the following antimicrobial agents: cefoxitin; chloramphenicol; ciprofloxacin; clindamycin; erythromycin; gentamicin; rifampicin; tetracycline; and trimethoprim.

Whole-genome sequencing (WGS) was performed on the isolate using Illumina MiSeq chemistry as previously described [2]. The Nullarbor pipeline was used to assemble raw reads, to identify virulence and resistance genes, and to identify single nucleotide polymorphisms (SNPs) in core genes for the generation of a maximum parsimony phylogenetic tree. Read mapping was based on the ST398 *S. aureus* reference genome (GenBank accession AM990992).

To evaluate the molecular context of the isolate, comparative genomic analysis was performed using a collection of 21 previously sequenced human isolates of ST1-IV and a single ST1-I, all carrying *blaZ* and *mecA* genes, from sepsis cases obtained from the Australian Group on Antimicrobial Resistance (AGAR).

WGS confirmed the isolate was a ST1, *spa* type t127 *S. aureus* with staphylococcal cassette chromosome mec (SCCmec) type IV. The isolate (SA606) was phenotypically resistant only to penicillin and cefoxitin and carried the *blaZ*, *mecA* and *norA* antimicrobial resistance genes.
Although hlb, hlgA, hlgB, hlgC, lukE, lukF and seh virulence genes were detected, the isolate did not harbour the Panton–Valentine leukocidin genes lukF-PV or lukS-PV or genes associated with the human immune evasion cluster (IEC).

Comparative genome analysis grouped the identified dairy cow isolate in a cluster of ST1-IV isolated from humans, sharing 47 SNPs (Fig. 1). The closest human ST1 isolate related to the MRSA ST1 isolate from the dairy cow was obtained from a human sepsis case in 2015 (S23603-2015) with eight common SNPs. Within this cluster, the majority of isolates belong to the same spa type (t127) and SCCmec type IV. The other cluster of ST1 isolates was grouped sharing 57 common SNPs and was found to belong to the SCCmec class type I. There were no major differences in the carriage both of antimicrobial resistance and virulence genes between MRSA ST1 from the cow from this study and the human isolates (Fig. 1).

Here we report the first case of MRSA isolated from cattle in Australia. ST1-IV, also known as WA-MRSA-1, is one of the most frequently isolated CA-MRSA clones circulating within the Australian community [3]. The ST1-IV isolated from the cow was found to be closely related to WA-MRSA-1, suggesting human-to-animal transmission has occurred (reverse zoonosis or anthropozoonosis). Given the high prevalence of ST1-IV in the Australian community, it is highly plausible that transmission occurred via animal handlers who were colonised with the MRSA ST1-IV clone. Unfortunately, no samples from the farm manager or farmhands could be obtained to confirm this hypothesis.
Although this is the first reported case of ST1-IV in a dairy cow in Australia, ST1-IV spa t127 has previously been reported from a cow with mastitis by a group of Italian researchers who also concluded, based on epidemiological data, that human-to-animal transmission had occurred [4]. Amplification and spread of livestock-associated MRSA (LA-MRSA) clones such as ST398 and mecC-carrying clones reported in Europe and their subsequent impact on public health highlights the importance of thorough genetic characterisation of clinical mastitis isolates found to be methicillin-resistant [5]. In addition, the findings reported here highlight the importance of antimicrobial resistance surveillance in cases both of clinical and subclinical dairy cow mastitis.

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**References**


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**Fig. 1.** Phylogenetic relationships between methicillin-resistant *Staphylococcus aureus* (MRSA) ST1 isolates from humans and a dairy cow. A maximum parsimony tree generated using single nucleotide polymorphisms (SNPs) of MRSA ST1 isolates is shown. MRSA isolated from the dairy cow in association with subclinical mastitis is highlighted in red. The numbers on the branches represent SNPs. All isolates are *spa* type t127, except S32103-2015 (t114), S41214-2015 (t559) and S41218-2015 (t948).