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Genome Note

Complete genome of a methicillin-resistant *Staphylococcus vitulinus* from Danish ground beef meat carrying a *mecA2* resistance gene and a novel *ccr* allotype

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ABSTRACT

Objectives: To report the complete genome sequence of a methicillin-resistant *Staphylococcus vitulinus* from ground beef to allow comparison with other available *S. vitulinus* genomes and to investigate its SCC*mec* element.

Methods: Meat samples from grocery stores in Denmark were examined for the presence of staphylococcal species by plating on selective plates. One colony isolated from beef was identified as *S. vitulinus* by MALDI-TOF and genome sequenced using a combination of Illumina and Oxford Nanopore technologies. Phylogenetic and *in silico* resistome analyses were performed for all available *S. vitulinus* genomes.

Results: The closed genome of *S. vitulinus* Tienloo1 isolate had a chromosome size of 2,628,028 bp and contained a single novel 2,380 bp plasmid based on a hybrid assembly. It carried *mecA* as the only resistance marker. The isolate was found not to carry any immune evasion cluster genes, which have been putatively associated to human origin. Comparison with all publicly available *S. vitulinus* draft genomes showed a diverse population and revealed that only the Danish beef isolate contained a *mec* gene in addition to a *ccr* gene complex. Additionally, the single *ccrC* gene within the isolate was novel and distant from the *mecA2* gene.

Conclusion: This isolate, Tienloo1, from a ground beef meat sample represents the first complete genome of *S. vitulinus* found to carry a *mecA2* gene and a novel *ccr* allotype in its SCC*mec* element that is distinct from all publicly available draft *S. vitulinus* genomes.

Retail meat is often contaminated by bacteria, including various *Staphylococcus* species, of which some may be disease-causing in humans [1–3]. The resistance profiles, and in particular the beta-lactam resistance of these species, are of special interest in relation to human disease and public health surveillance. Beta-lactam resistance is well described in staphylococci, as well as in *Staphylococcus vitulinus* [4].

In an ongoing study on the presence of staphylococcal species in Danish retail meat, samples of beef, chicken and pork meat were purchased at various grocery stores in Copenhagen, Denmark. Within 1 day after purchasing and prior to expiration dates, 50 g of each meat sample was homogenised for 2 min in 100 mL buffered peptone water (BPW), using the Seward Stomacher 400 Laboratory Blender (Seward Limited, West Sussex, UK). Subsequently, 1 mL of the sample was enriched in 9 mL tryptic soy broth (TSB) at 37 °C for 24 h before plating 500 μL on SA-Select plates (Bio-Rad, Hercules, CA, USA). After culturing overnight, species identification was performed by MALDI-TOF. One colony isolated from beef was identified as *S. vitulinus*. For short-read sequencing, DNA was purified with the Qiagen Blood and Tissue DNA kit followed by paired-end sequencing using the Illumina NextSeq sequencing

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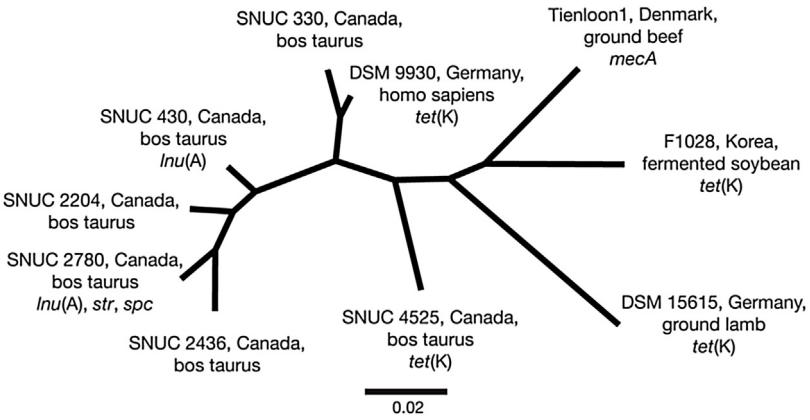

Fig. 1. Phylogenetic tree of all 10 available *Staphylococcus vitulinus* genome sequences. The analysis was based on 39,734 SNPs detected in a ~2.17 Mb core genome across the collection. Information on isolate ID, country of origin, sample material and detected resistance genes are presented for all isolates. Scalebar indicates substitutions per site.

platform and their 300-cycle V2 kit (Illumina, San Diego, CA, USA). Adaptor sequences were removed with Trimmomatic v0.36 [5] using the following settings: ILLUMINACLIP:~/NexteraPE-PE.fa:2:30:10 LEADING:20 TRAILING:20. For long-read sequencing, DNA was purified from a colony scrape of half a 1 μL sterile inoculation loop resuspended in 200 μL PBS using an Agencourt Genfind V2 kit (Beckman Coulter, Brea, CA, USA) scaled to 300 μL magnetic beads and a DynaMag-2 Magnet (ThermoFisher, Waltham, MA, USA). The purified DNA was applied to an R9.4.1 flow cell in a MinION Mk1B using a Ligation Sequencing Kit 1D, SQK-LSK109 with Native Barcoding Kit 1D, SQK-NBD103 (Oxford Nanopore Technologies, Oxford, UK), following the standard protocol starting with 1 μg DNA, omitting the optional 'DNA fragmentation' and choosing the 'Long Fragment Buffer'. Raw reads were base-called with ONT's Albacore v2.3.4 software. Sequencing adaptors were removed with Porechop v0.2.3 [6] and reads quality filtered to at least q8 with NanoFilt v2.2.0 [7] prior to hybrid assembly using Unicycler v0.4.7 [8]. The complete genome in a single contig was annotated using NCBI's annotation pipeline and analysed for antimicrobial resistance genes using ABRicate with the ResFinder database. Phylogenetic analysis of *S. vitulinus* with all available draft genomes from RefSeq (https://www.ncbi.nlm.nih.gov/refseq/, accessed on 23 September 2019, $n = 9$) was performed using SNPs detected with NASP [9] after removal of duplicated regions using NUCmer in the Tienloo1 reference chromosome and exclusion of positions with less than 10-fold coverage and <90% unambiguous variant calls. Phylogenetic reconstruction on the resulting SNP alignment was performed using the maximum likelihood as implemented in IQ-TREE [10].

The *S. vitulinus* Tienloo1 isolate had a chromosome size of 2,628,028 bp with a GC-content of 32.9% and contained a single novel 2,380 bp plasmid, which had a sequencing depth ~15 times higher than the chromosome. ResFinder (v3.1) analysis did not reveal any resistance genes other than *mecA* using a threshold of 90% and 80% minimum hit length. Hence, no resistance marker was found on the small high copy number plasmid. The isolate did not encode genes for the Panton-Valentine leukocidin (*lukS*/*F*-PV) or genes (*scn*, *sak* or *chp*) related to the immune evasion cluster (IEC) and human origin in *Staphylococcus aureus*. The complete and annotated (NCBI Prokaryotic Genome Annotation Pipeline (PGAP)) chromosome and plasmid are available under GenBank accession IDs CP051882 and CP051881, respectively.

Staphylococcus vitulinus has previously been described in horse samples from Denmark [11], and in chicken and bovine samples from the US [12,13]; this corroborates the livestock-related origin of the Danish isolate. Further investigations into the staphylococcal cassette chromosome *mec* (SCC*mec*) revealed no readily identifiable direct repeats in the vicinity of a detected *mecA2* gene, nor any identifiable *ccr* gene-complex genes, similar to what has previously been described [14]. Interestingly, a phenol-soluble modulin (PSM) encoding gene was present upstream of *mecA*. The *psm-mec* gene encodes a cytolytic amphipathic peptide toxin that has been shown to influence methicillin resistance, biofilm formation, cell spreading and the expression of other virulence factors [15]. Analyses including all nine available *S. vitulinus* draft genomes revealed a core genome of 82.6% (~2.17 Mb) with a divergent population with ~5,800–18,700 SNPs separating all isolates (see Fig. 1). Investigations into *mecA* and SCC*mec* content in the publicly available *S. vitulinus* draft genomes showed that two-thirds of the RefSeq isolates contained one to three *ccr* genes that generally differed in allotype between isolates. However, the genome of the Tienloo1 isolate presented here is the only genome that contained a *mec* gene in addition to any *ccr* gene complex. Analysis of *mecA* itself in staphylococci has shown that the *mecA2* allele likely emerged in *S. vitulinus* more than 50 years ago, but also that *S. vitulinus*, despite encoding *mecA*, displays varying levels of phenotypic resistance to beta-lactams [16]. Our isolate also harboured a single novel *ccrC* gene (<76% sequence similarity to *ccrC1* and <73% to *ccrC2*) that was found >90 kb from the *mecA2* gene. Given the distance between the *mecA* gene and the novel *ccr* gene, the latter was perhaps placed the outside the SCC*mec* element itself. However, whereas the region contains no indication of insertion sequences (IS), prophage structural elements or plasmid replicons, the genetic content between the two genes includes restriction-modification systems, efflux pumps and a toxin-antitoxin system that combined could be the result of horizontal gene transfer. Interestingly, two other isolates, SNUC 330 and F1028, from Canada and Korea contained a similar novel *ccrC* allotype. *In silico* analysis revealed a scarce repertoire of resistance genes in most isolates (Fig. 1).

In conclusion, analyses of the now 10 available *S. vitulinus* genomes showed a diverse population with few resistance markers. However, multiple acquisitions of the SCC element were observed. Additionally, the IEC gene cluster associated to human adaptation was absent from the genomes, thereby highlighting the species' non-human reservoir.

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Conflict of interest

None declared.

Ethical approval

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