

Genomic Characterization of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* ST7 Isolates from a Case of Human Bacteremia in China

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Abstract: The detection of novel livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) is important in both clinics and livestock. In this study, we report a MRSA-infected patient who was associated with livestock as a butcher, from whom we collected two MRSA strains FJ0318 and FJ0322. To further understand the correlation between these MRSA isolates and livestock, whole-genome sequencing and comparative genomic analyses were performed for these two isolates. Phylogenetic analysis revealed that these two strains were homologous. Multilocus sequence typing showed that these two strains belong to ST7, which is a common lineage in retail meat and meat products in China. The genetic islands in FJ0318 and FJ0322 were different from those in other common clones, such as ST59, ST8, and ST5. A mosaic plasmid with a sequence identical to that of the plasmid pE2 from livestock was found in strain FJ0318. Additionally, a novel prophage island was identified on the chromosome. Furthermore, the sequence of the island was similar to that of phage SP6 identified in livestock. ST7 may originate from livestock and be transmitted to communities, causing invasive infections.

Keywords: MRSA, ST7, whole genome analysis, livestock

Simple Summary

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multi-drug resistant pathogen that poses a pertinent danger to livestock and humans. Here, we report two MRSA strains isolated from a butcher and genetically characterized. We suggest that these strains belong to a lineage that likely originates from livestock and can be transmitted to humans, causing potential disease outbreaks.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important multidrug-resistant pathogens in both clinics and livestock.¹ MRSA can cause a variety of infections, such as bacteremia, endocarditis, osteoarticular infections, toxic shock syndrome, and complicated skin and soft tissue infections.² Infections caused by MRSA are no longer limited to hospitals and the community, since livestock-associated MRSA (LA-MRSA) emerged in the mid-2000s.³ LA-MRSA has continued to raise concerns in the past few decades.

Since the first report of LA-MRSA infection in a family who worked on a swine farm, the potential for animals to act as a reservoir for *S. aureus* zoonotic infections has been exemplified.⁴ Those persons associated with livestock, such as

farmers, veterinarians, slaughterhouse personnel, and transporters are at high risk for exposure to LA-MRSA due to their direct contact with MRSA-colonized livestock.⁵ LA-MRSA lineages can be characterized by their geographic location. Infections in livestock caused by LA-MRSA CC398 are common in Europe.⁶ In Asia, ST9 MRSA is an epidemic in livestock.⁷ However, other lineages recognized as LA-MRSA have not yet been examined in depth.

In this study, we report the case of a butcher who had a severe MRSA infection. The MRSA strains isolated from this patient were confirmed as ST7 MRSA. Whole-genome sequencing and comparative genome analyses were performed to better understand this lineage.

Materials and Methods

Bacterial Strains

Strains FJ0318 and FJ0322 were isolated from bronchoalveolar lavage fluid and pus samples from one patient with skin and soft tissue infections and sepsis at Fujian Provincial People's Hospital. The strains were identified as *S. aureus* using VITEK2. The genome sequences for the comparison of ST1 (MW2), ST5 (N315), ST8 (LAC), ST59 (SA 268), and ST250 (COL) were obtained from the NCBI database.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the strains was performed using disk diffusion according to the manufacturer's instructions while cefoxitin, gentamicin, erythromycin, clindamycin, ciprofloxacin, quinupristin-dalfopristin, tetracycline, and linezolid were tested. Susceptibility testing for vancomycin was performed using the Etest strip. The results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI).⁸ *Staphylococcus aureus* ATCC 25923 was used as a quality control.

Whole Genome Sequencing and Comparative Genome Analysis

The genomes of the strains mentioned above were sequenced using the HiSeq X-Ten platform (Illumina, San Diego, CA, USA) with 2×150 bp paired-end reads. Long-read genome sequencing was performed using a MinION sequencer (Oxford Nanopore Technologies, Alameda, CA, USA). Hybrid assembly was performed using Unicycler (0.4.8) with Illumina and Nanopore reads.⁹ Homology analysis was performed using breseq (0.34.1).¹⁰ The assembled contigs were annotated using the RAST server (<https://rast.nmpdr.org/>).¹¹

Plasmid and Prophage Analysis

A map of the plasmid sequence comparison was generated using BRIG.¹² Recombination was identified using Mauve.¹³ The genetic environment of the prophage was compared with that of other phages using Web-based BLASTN, and a comparison map was generated using Easyfig 2.1.¹⁴

Nucleotide Sequence Accession Number

The genomes of FJ0318 and FJ0322 were deposited in the NCBI database under the accession numbers CP096272-CP096273 and CP096274, respectively. The accession numbers for the genomes of the strains SA268, LAC, MW2, N315 and COL are CP006630, CP055225, BA000033, BA000018 and CP000046 respectively.

Ethics

This study was approved by the local ethics committee of Sir Run Run Shaw Hospital with a waiver of informed consent (Approval No. 20190821-9). The patient provided consent for publication of the clinical details, and written informed consent was obtained.

Results

Case Report

A 33-year-old man, previously a healthy butcher, severed the hypothenar of the right palm with a knife during pork-processing on February 12, 2021. The wound was approximately 2.5 cm long and was in direct contact with raw pork. Two

days later, skin lesions appeared on his back. He was admitted to a local hospital with a fever and shortness of breath on February 27. His white blood cell count was $17.19 \times 10^9/L$, with a neutrophil ratio of 90.6%, and C-reactive protein was 87.1 mg/L. Computed tomography (CT) revealed pneumonia with pleural effusion, and a blood culture grew MRSA (isolate not stocked). His condition worsened, and he was referred to a tertiary hospital on March 11. Strain FJ0318 was isolated from bronchoalveolar lavage fluid on February 18 and strain FJ0322 was isolated from the pus of skin lesions on February 22. The patient was treated with vancomycin for 4 days and then treated with linezolid for 14 days due to acute kidney injury. Finally, the patient was discharged, and a lung CT scan was performed on July 7, 2021 (Figure 1).

Susceptibility Profiles

Both FJ0318 and FJ0322 were resistant to ceftazidime (Table 1). These two strains were susceptible to gentamicin, erythromycin, clindamycin, ciprofloxacin, quinupristin-dalfopristin, linezolid and vancomycin. However, FJ0322 was susceptible to tetracycline, whereas FJ0318 was resistant to tetracycline.

Genome Information and Molecular Subtyping

The complete genome of strains FJ0318 and FJ0322 were analyzed. Both FJ0318 and FJ0322 had a 2,809,462 bp chromosome with just three single nucleotide polymorphisms (SNVs), two in the intergenic and one in the transposase ISSau3, indicating that these two strains were the same. However, FJ0318 also contained a 32,359 bp plasmid.

Strains FJ0318 and FJ0322 were classified as ST7, CC7 and spa-type t1509, respectively. SCCmec type IV was located on chromosomes FJ0318 and FJ0322. Meanwhile, these two strains both carried agr I.

FJ0318 Plasmid Structure

The plasmid pTET-ST7 carried by strain FJ0318 was 32,359 bp with 38 open reading frames. Three types of resistance genes, tetracycline resistant gene *tetK*, penicillin resistant gene *blaZ* and aminoglycosides resistant gene *ANT(4')-Ia*, and one cadmium resistance operon conferred heavy metal resistance to the plasmid. Six genes associated with replication and two genes associated with transfer, *IS431* and *Tn552*, were found in the plasmid. The other two genes *mobE* were associated with mobilization.

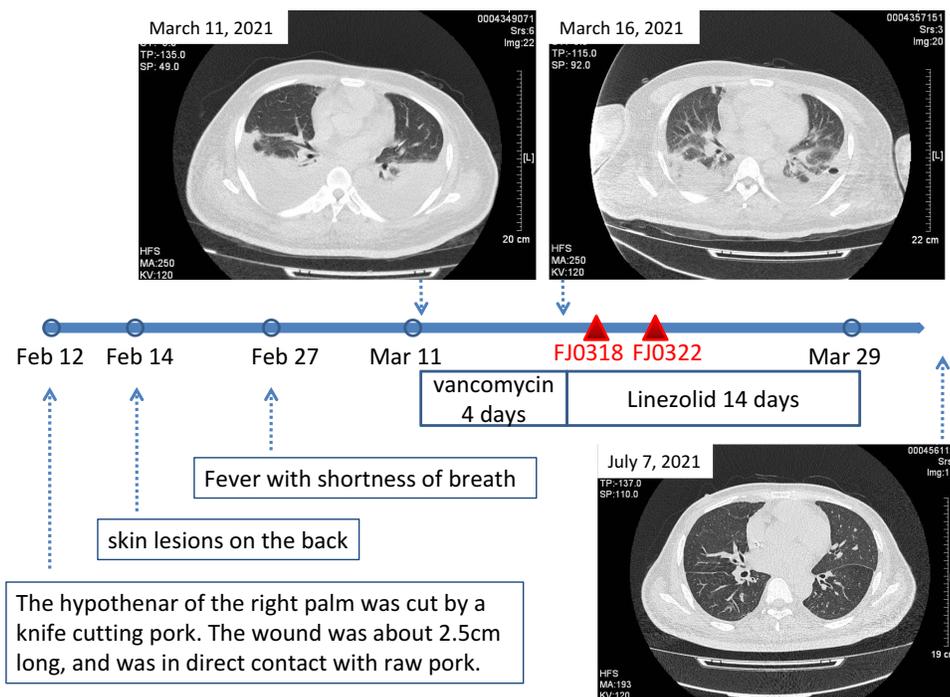


Figure 1 Timeline of the disease. Lung CT scan at different time points were listed. Isolation of strain FJ0318 and FJ0322 were marked with triangle.

Table 1 Antimicrobial Susceptibility Testing of Strains

Antimicrobial Agent	FJ0318		FJ0322	
	Diameter/MIC	Result	Diameter/MIC	Result
Cefoxitin	12	R	14	R
Gentamicin	24	S	24	S
Erythromycin	30	S	28	S
Clindamycin	24	S	22	S
Ciprofloxacin	26	S	24	S
Quinupristin-dalfopristin	28	S	26	S
Tetracycline	8	R	28	S
Linezolid	34	S	32	S
Vancomycin	0.75*	S	0.75*	S

Notes: The unit of number is millimeter or Minimum Inhibitory Concentration (MIC), R/S: resistance/ susceptibility, *Susceptibility testing for vancomycin was performed using the Etest strip.

BRIG and web-based BLASTN were used to characterize the structure and origin of plasmid pTET-ST7 (Figure 2). The plasmid was identical to pE2 (CP080006) isolated from livestock, implying that this plasmid may be transmitted between humans and animals.

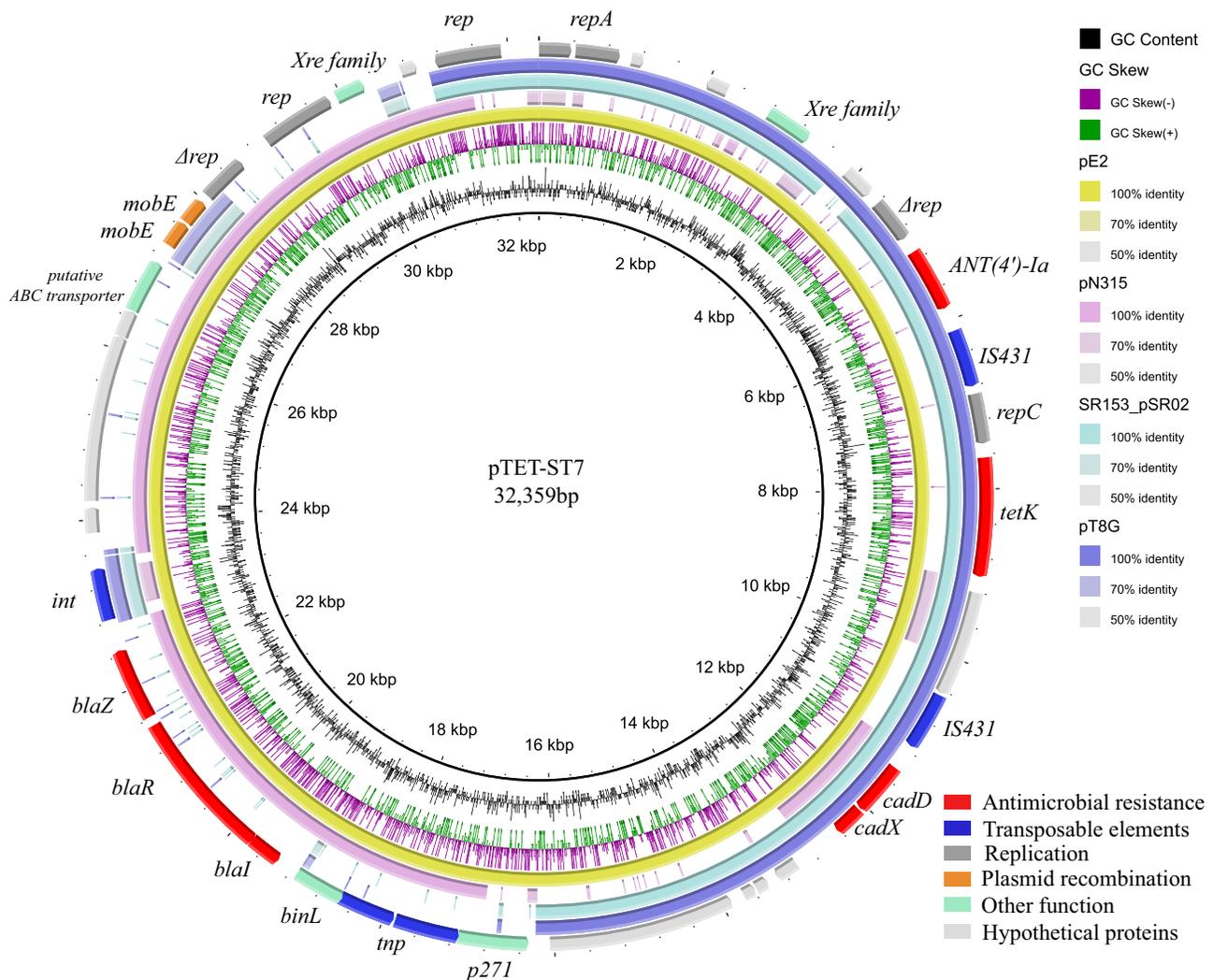


Figure 2 Structure of pTET-ST7, the plasmid in strain FJ0318. The inner circle represents GC content. The outer circle represents ORFs. The resistance genes, replications, transposable elements, and plasmid recombination were shown beside the relevant ORFs. The plasmid sequence was aligned to pE2, pN315, SR153_pSR02 and pT8G. The relevance of circle color and plasmid was in top right corner.

Further comparative genome analysis of pTET-ST7 revealed that it is mosaic, comprising parts of two staphylococcal plasmids from different species (Figure 2). A ~17.5 Kbp region containing *tetK*, *ANT(4')-Ia*, and one cadmium resistance operon was nearly identical to the plasmid pSR02 (CP048645) from the *Staphylococcus aureus* strain SR153 and the plasmid pT8G (KU882684) from the *Staphylococcus lugdunensis* strain. The other ~15 kb region was homologous to pN315 from the *Staphylococcus aureus* ST5 strain N315. The plasmid pTET-ST7 may have originated from rearrangement of the plasmids.

Genomic Islands, Pathogenicity Islands, Prophage, and Virulence Genes

Genomic islands, pathogenicity islands, prophage, and virulence genes of ST7 strain FJ0318 were compared with the epidemic CA-MRSA lineages ST59, ST8, ST1, and the classical HA-MRSA ST5 (Table 2). FJ0318 harbored the genomic islands vSa α , vSa β , and Φ Sa3 (Figure 3), but the PVL-encoding prophage Φ Sa2 was absent. None of the pathogenicity islands existed in FJ0318; however, FJ0318 contained virulence genes, such as staphylokinase gene *sak*, staphylococcal complement inhibitor gene *scn*, *psm* which coding phenol-soluble Modulins, etc. The composition of enterotoxins of FJ0318 was different from other lineages, only enterotoxin *sep* was present in FJ0318.

The Unique Prophage in FJ0318 and FJ0322

Mauve was performed to explore the differences between these two strains and other Methicillin-susceptible *Staphylococcus aureus* (MSSA) ST7 strains. Compared with the MSSA ST7 strain 08–02300 isolated from Germany, whose whole genome sequence was deposited in NCBI (CP015646), FJ0318 and FJ0322 harbored a unique prophage integrated between *rpmf* and *isdB* genes (Figure 4). This novel prophage was designated as prophage-ST7, with 64 open reading frames, most of which were associated with the phage structure. Comparative genome analysis showed that this prophage was similar to (coverage 84%, identity 93%) *Staphylococcus aureus* phage SP6 (JX274647), which was collected from livestock. These results indicated that this phage may have originated from livestock and then invaded *Staphylococcus aureus* and integrated into the genome of the strain as a prophage.

Table 2 Genomic, Pathogenicity Islands and Virulence Genes in FJ0318

Island/Gene	FJ0318 (ST7)	SA268 (ST59)	LAC (ST8)	MW2 (ST1)	N315 (ST5)	COL (ST250)
vSa1	–	+	+	–	–	+
vSa2	–	–	–	–	–	–
vSa3	–	–	–	+	–	–
vSa4	–	–	–	+	+	+
vSa α	+	+	+	+	+	+
vSa β (lukDE)	+	+	+	+	+	+
Φ Sa1	–	–	–	–	–	–
Φ Sa2 (containing PVL)	–	+	+	+	–	–
Φ Sa3	+	–	+	+	+	–
<i>aur</i> , <i>hlgA</i>	+	+	+	+	+	+
<i>hlgB</i> , <i>hlgC</i>	+	+	+	+	+	+
<i>hla</i>	+	+	+	+	+	+
<i>sak</i>	+	–	+	+	+	+
<i>chp</i>	–	+	+	–	+	–
<i>scn</i>	+	+	+	+	+	–
<i>lukDE</i>	+	–	+	+	+	+
<i>tsst</i>	–	–	–	–	+	–
<i>psmα1–4</i>	+	+	+	+	+	–
<i>psmβ1–2</i>	+	+	+	+	+	–
Enterotoxin	<i>sep</i>	<i>seb</i> , <i>sek</i> , <i>seq</i>	<i>sek</i> , <i>seq</i>	<i>sea</i> , <i>sec</i> , <i>sek</i> , <i>sel</i> , <i>seq</i>	<i>sec</i> , <i>seg</i> , <i>sei</i> , <i>sel</i> , <i>sem</i> , <i>sen</i> , <i>seo</i> , <i>sep</i> , <i>seu</i>	<i>seb</i> , <i>sek</i> , <i>seq</i>

Abbreviations: +/-, presence/absence of islands or genes; *aur*, aureolysin; *hlg*, gamma-hemolysin component; *hla*, alpha hemolysin; *sak*, staphylokinase; *chp*, chemotaxis inhibitory protein; *scn*, staphylococcal complement inhibitor; *tsst*, toxic shock syndrome toxin-1; *psm*, phenol-soluble Modulins.

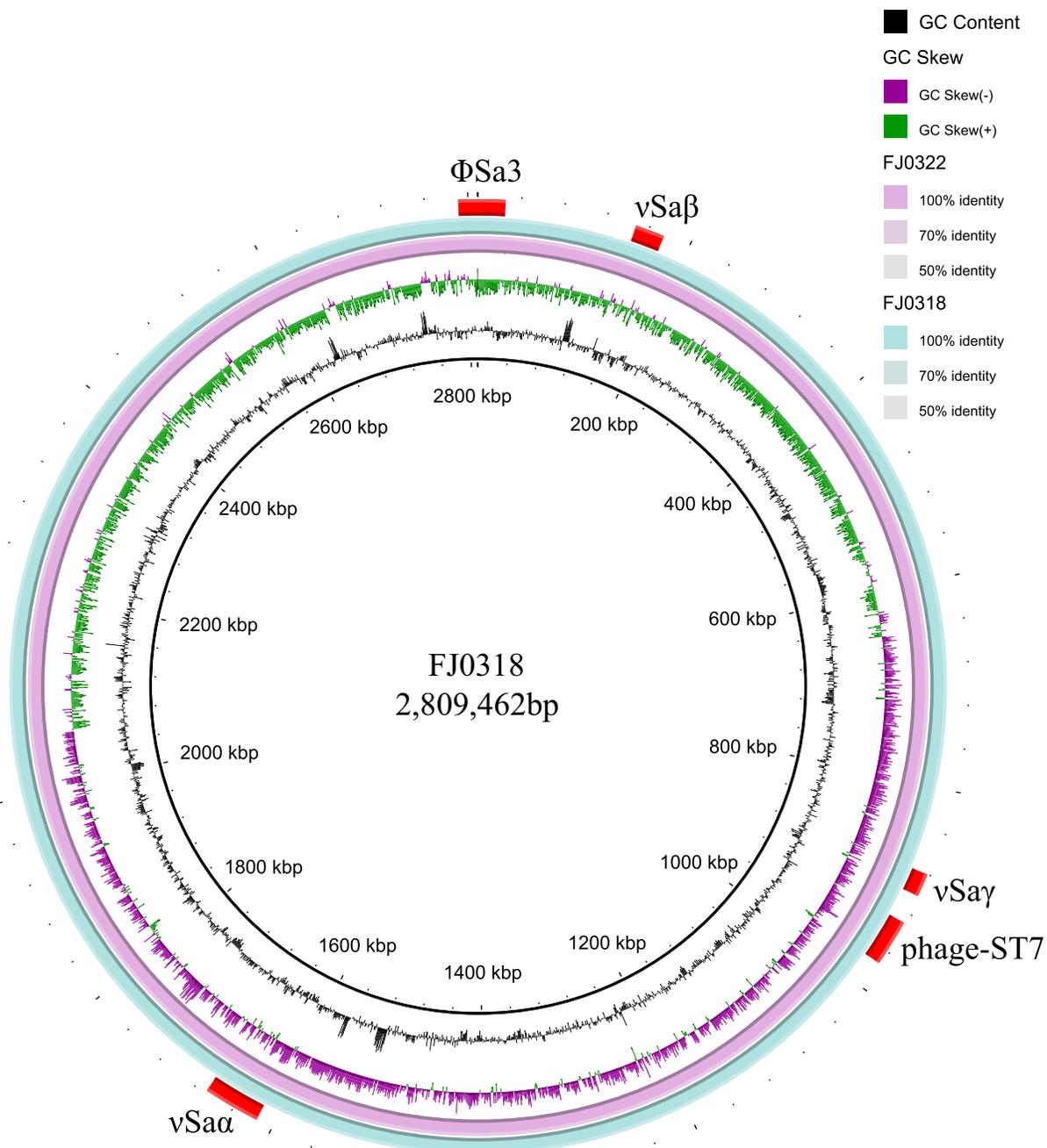


Figure 3 Chromosome of FJ0318 and FJ0322. The inner circle represents GC content and GC skew. The outer circle represents genomic islands and prophage. The relevance of circle color and strain was in top right corner.

Discussion

An increasing number of researchers have recently focused on LA-MRSA transmission between livestock and humans. Some studies have revealed that livestock workers are at a significantly higher risk of LA-MRSA colonization and subsequent infections.¹⁵ This may be attributed to direct contact between livestock workers and colonized animals. In addition to direct animal-human transmission, LA-MRSA could also be transmitted via contaminated meat,¹⁶ since the presence of MRSA in meat products has been demonstrated.^{17,18} In this case study, we reported a severe MRSA infection in a butcher who worked in the market handling raw pork. Due to the specific association with livestock, we presumed that it was an LA-MRSA infection.

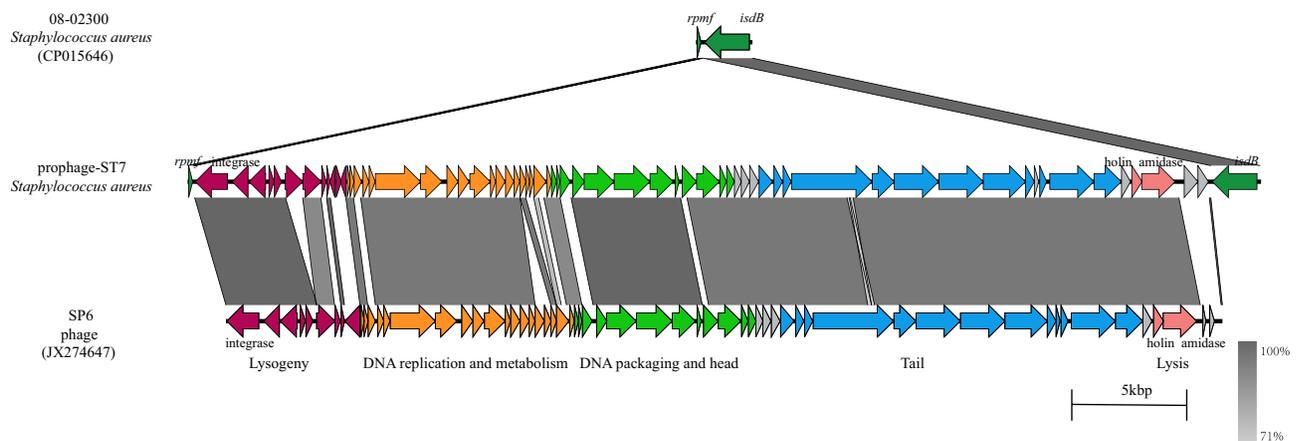


Figure 4 Structure and comparative analysis of prophage page-ST7. Compared with MSSA ST7 strain 08-02300 (CP015646) and *Staphylococcus aureus* phage SP6 (JX274647).

Some reports have revealed that ST9 MRSA was the major LA-MRSA clone in China, especially in swine farms.^{19,20} Molecular characteristics of *S. aureus* observed from food surveillance has shown that ST7 should not be ignored in retail meat because it is common to both meat and meat production as Methicillin-susceptible *S. aureus* clone.^{21–23} The strains FJ0318 and FJ0322 collected in this case were confirmed to be ST7, according with the epidemiology of *S. aureus* in meat. Furthermore, FJ0318 and FJ0322 were identified as MRSA based on their phenotypes and genotypes, which are at higher risk. A detailed description of ST7 was not available from previous studies. In our study, whole genome sequencing was performed to better understand ST7, which is a potential livestock-associated clone.

ST9, the most dominant LA-MRSA clone in China, is multidrug-resistant and carries many resistance genes.^{19,20,24} In this study, two ST7 strains were susceptible to other antimicrobials and only carried the *mecA* gene on the chromosome. Meanwhile, other researchers reported that ST7 clone was susceptible,²¹ in accordance with our results. This may be because this clone is emerging in livestock and has not adapted to the host environment with a high antibiotic selective pressure. Meanwhile, the pathogenicity islands and prophage in ST7 were less abundant than those in other classical CA-MRSA and HA-MRSA clones, implying that ST7 had a simpler genetic background. However, the significant virulence potential of ST7 was displayed in this case, which may be due to virulence determinants in the core genome. Identification of the unique prophage-ST7, whose sequence was similar to that of phage from livestock, revealed that the genome of ST7 may undergo rearrangement in livestock. Furthermore, this clone may have originated in livestock.

Mosaic plasmids have been reported in recent years.^{25,26} Some mosaic plasmids were derived from homologous recombination,²⁷ and some plasmids were created by rare genomic rearrangements that were apparently mediated by non-homologous recombination events.²⁸ In this study, the mosaic plasmid pTET-ST7 may be derived from the rearrangement between different staphylococcal species, according to the comparative analysis. However, homologous fragments have not yet been identified; hence, the mechanism of recombination remains unclear. Nevertheless, the mosaic plasmid pTET-ST7 possesses multi-resistance to both antibacterial agents and heavy metals, which supports the existence of plasmids in extreme environments, such as agriculture and animal husbandry.^{29,30} The plasmid pTET-ST7 may also be transmitted in livestock, as that was where the homologous plasmid was collected. Interestingly, in this case, strain FJ0322 lost this plasmid during infection, illustrating the transfer ability of the plasmid pTET-ST7.

Conclusions

In conclusion, we report a MRSA infection associated with livestock and present the whole genome of ST7, a potential LA-MRSA clone. Genome analysis will help us to better understand this LA-MRSA lineage. Better monitoring is needed for the emergence of potential LA-MRSA clones and the transmission of LA-MRSA between livestock and humans in communities.

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Disclosure

The authors declare no conflict of interest.

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