ST8-t008-SCC\text{mec} IV methicillin-resistant *Staphylococcus aureus* (MRSA) in retail fresh cheese

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**Abstract:** This study reports the finding of 3 ST8-t008-SCC\text{mec} IVa (2B) MRSA strains in fresh cheese purchased within a single market in Costa Rica. In line with the finding of the resistance genes \text{mecA}, \text{blaZ}, \text{mph(C)}, and \text{msr}(A) in their genomes, these bacteria showed phenotypic resistance to multiple β-lactams and erythromycin. Besides, they carry genes for acquired resistance to aminoglycosides (\text{aph}(3′)-\text{III}) and fosfomycin (\text{fosD}), mutations in genes associated with fluoroquinolone resistance (\text{gyr}A-\text{85} and \text{par}C-\text{80}), and genes for a myriad of virulence factors, including adhesins, hemolysins, and exotoxins. Our strains share multiple genomic features with MRSA from the USA300 lineage, which is a highly distributed and highly virulent strain implicated in community infections. As a result, consuming these or similar products could lead to multidrug (MDR) infections in susceptible individuals. These results highlight safety deficiencies in cheese production practices and emphasize the risk of foodborne transmission of hard-to-treat ST8 MRSA strains.

**INTRODUCTION**

*Staphylococcus aureus*, a ubiquitous bacterium prevalent on human and animal skin and mucous membranes, includes virulent strains that can cause a broad spectrum of infections. Over time, this species has become a substantial global health concern for its implication in healthcare and community infections.

The rise of methicillin-resistant *S. aureus* (MRSA) has added complexity to *S. aureus* infection management. These unique strains are distinguished by the expression of \text{mecA} or \text{mecC} genes encoding altered penicillin-binding proteins with reduced β-lactam antibiotic affinity, which are included in a variety of Staphylococcal Cassette Chromosome \text{mec} elements (SCC\text{mec}) (da Silva et al., 2021; Naranjo-Lucena & Slowey, 2023).

Community transmission of *S. aureus* is predominantly through direct human interaction, animals, and animal-derived commodities (Brahma et al., 2022). This pathogen plays a role in contagious mastitis in dairy cattle and can be detected at multiple stages of the dairy production pipeline because dairy products provide favorable conditions for *S. aureus* growth and toxin synthesis (Gajewska et al., 2023).

Cheese is a globally consumed and popular food product. However, it has been implicated in foodborne illness outbreaks of various pathogens, including *S. aureus* (Rangel-Ortega et al., 2023). Its presence in cheese is especially problematic due to its ability to produce heat-stable enterotoxins, which can lead to gastrointestinal symptoms in consumers.

This study aimed to investigate the presence and traits of MRSA in fresh cheese that was presumably prepared with pasteurized milk and marketed at Costa Rican retail stores. Through phenotypic testing and genomic analyses, we assessed their antibiotic resistance profiles and identified genes and genetic elements contributing to their resistance and virulence.

**MATERIALS AND METHODS**

**Isolates**

Isolates H1R2–1T, H1R3–4T, and H3R3–2T were obtained from fresh cheese samples collected on 2 separate occasions from 2 retailers in a single market in Costa Rica. These bacteria were cultivated by inoculating sample homogenates onto Baird-Parker agar plates and grew at population densities ranging from 10¹ to 10⁶ cfu/g. Using the Vitek2 system (Bio-Mérieux), we confirmed that these isolates show phenotypic resistance to oxacillin (MIC > 4 µg ml⁻¹) and other antibiotics (see below).

**RAPD profiling**

We implemented the RAPD-PCR procedure described by Reinoso et al. (2004) to evaluate the diversity of the isolates. These PCR reactions were prepared using a commercial mastermix (ThermoScientific™ PCR Master Mix 2X), genomic DNA extracted with a commercial kit (NucleoSpin Tissue, Macherey-Nagel) from overnight cultures in trypticase soy broth, and oligonucleotides OLP6 (5′ACCGCCTGCT3′), OLP11 (5′ACGATGAGCC3′), and OLP13 (5′ACGGCCCTGCT3′). The resulting amplicons were separated by electrophoresis on 1.5% agarose gels, which were prepared with 1X TBE buffer.

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The list of standard abbreviations for JDS Communications is available at adsa.org/jdsc-abbreviations-24.
Whole-genome sequencing

The genomic DNA preparations presented above were also used for sequencing by synthesis at MicrobesNG (UK). In this regard, libraries were prepared using the Nextera XT Library Prep Kit (Illumina), and the sequencing was performed on a HiSeq platform (Illumina, 2x250 bp). Adapter and low-quality sequences were removed from the data sets using Trimmomatic 0.30, employing a sliding window quality cutoff of Q15. The resulting reads were then utilized for de novo assembly with SPAdes v3.7. Annotation of the assembled genomes was conducted using Prokka v1.13. The assembled genomes showed at least 30X coverage and N50 values ranging from 299 to 377 and 299–390 kb, respectively. Raw sequencing data can be downloaded from the following link: https://microbesng.com/portal/projects/8E75A3F4-F7B2-374B-8679-1EEEB9E3D896/

Bioinformatic analyses and comparative genomics

The multilocus and SCCmec types of the isolates were determined with the MLST 2.0 and SCCmecFinder tools, respectively. Their spa types, instead, were assigned using the Ridom SpaServer. Parsnp was used to call pairwise SNPs using default settings. To identify the predicted resistome of the isolates, we used resfinder and the Resistance Gene Identifier (RGI) pipeline. In addition, we employed ABRicate in conjunction with the VFDB database to identify sequences encoding virulence factors.

RESULTS

All 3 isolates exhibited distinct RAPD patterns (Figure 1) and were distinguished from each other by 14–190 SNPs in their core genomes.

Although they represent different strains, these bacteria were invariably assigned to the ST8-t008 lineage (Table 1). Furthermore, they were found to carry Iva-2B SCCmec elements (sequence similarity > 90%) (Table 1). All strains lack the arc and opp3 ACME clusters, PVL genes, and seq and sek genes from the pathogenicity island PaI5. Furthermore, only H1R2–1T and H1R3–4T possess a merARB operon, which confers resistance to mercury (Table 1).

Our strains exhibited phenotypic resistance to a range of β-lactam antibiotics (penicillin, oxacillin, ampicillin, amoxicillin, and cephalosporin) and erythromycin. By contrast, they were found to be susceptible to clindamycin, gentamicin, ciprofloxacin, linezolid, nitrofurantoin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin (Table 2). These findings were, for the most part, supported by our in silico analyses, which revealed hits with > 98% identity and 100% coverage to mecA and blaZ for β-lactam

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<thead>
<tr>
<th>Isolate</th>
<th>ST spa type</th>
<th>SCCmec</th>
<th>copB/mco</th>
<th>ACME</th>
<th>PVL genes</th>
<th>PVL (op3 clusters)</th>
<th>PVL</th>
<th>18S</th>
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<th>VfDB</th>
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<tr>
<td>H1R2–1T</td>
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<td>H1R3–4T</td>
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<td>H3R3–2T</td>
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*ND: not detected.
*Such as those found in p18805-p03.
*Serine residue.

Figure 1. RAPD patterns obtained for the 3 MRSA isolates analyzed
resistance and mph(C) and msr(A) for macrolide resistance. Additionally, all 3 strains carried the aph(3’)-III gene (100% identity and coverage), conferring resistance to aminoglycosides, and fosD (79% identity and 100% coverage) associated with fosfomycin resistance. H1R2–1T and H1R3–4T were further distinguished by the presence of the tetracycline-resistance gene tet(K) (100% identity and coverage), while H1R2–1T also carried erm(C) gene (100% identity and coverage). In addition to these acquired antibiotic resistance genes, the 3 strains displayed mutations in the gyrA-85 and parC-80 genes that lead to fluoroquinolone resistance and hits to genes of the resistance plasmid p1885-p03 (Table 1), indicating the potential involvement of plasmids in the dissemination of antibiotic resistance among these MRSA strains.

Several virulence factors were computationally detected in all 3 strains. These included genes encoding major autolysin (atl), clumping factor precursors (cflAB), extracellular adherence protein (eap), extracellular matrix-binding protein homolog (ebh), fibrinogen-binding protein (efb), intercellular adhesion locus (icaA/BCD), and hemolysin genes (hla, hld, hlgABC). Furthermore, toxin genes such as exfoliative toxin A (eta), leukocidin (lukDE), and a variety of exotoxins (set30, set31, set34, set35, set36, set39, set40) were also identified in these genomes.

**DISCUSSION**

Our study confirms that methicillin-resistant *S. aureus* (MRSA) can be transmitted in the community through raw cheese consumption, posing a potential consumer risk (Titouche et al., 2019). However, it should be noted that the magnitude of this risk is low, as indicated by our infrequent recovery of MRSA isolates despite extensive sampling efforts (3/211 isolates, data not shown) and recent literature (Schnitt & Tenhagen 2020, Gajewska et al., 2023).

Although our phenotypic susceptibility testing did not include aminoglycosides and fosfomycin, our genomic analysis revealed the potential for multidrug resistance in the 3 strains. This worrisome trait, combined with the presence of genes encoding various virulence factors, further emphasizes the significance of our findings in terms of public health.

All 3 strains were classified as ST8-t008 and carried SCCmec IVa(2B) elements. This genomic profile was somewhat expected, as strains from clonal complex CC8, including ST8, have been previously detected in animal products (Herrera et al., 2016; Titouche et al., 2019). Additionally, MRSA strains carrying SCCmec IV have been shown to spread among livestock (Schnitt & Tenhagen 2020).

In North and South America, strains belonging to the USA300 pulsotype are prevalent and notorious for their high virulence and enhanced environmental survival and transmission properties (Glaser et al., 2016). Our strains are t008 and ST8, display acquired plasmid-mediated resistance to clindamycin (*ermA* or *ermC*), tetracycline (*tetK* or *tetM*), and mupirocin (*mupA*), as well as chromosomal mutations conferring resistance to fluoroquinolones, possibly indicating their association with this pandemic lineage (Nimmo 2012).

Variants of *S. aureus* USA300 and related strains can be differentiated based on the presence or absence of β-hemolysin converting phages, enterotoxin genes, and ACME and PVL genes. Our study detected such genetic variations in the 3 strains, highlighting the
genomic diversity of MRSA and confirming that lateral gene transfer is a significant driver of their evolution (Glaser et al., 2016).

This study reveals potentially highlights deficiencies in the manufacturing process or end product management. Accordingly, we advise retailers and the dairy industry to continuously review and strengthen their hygiene and disinfection measures.

We also advocate for surveillance programs focusing on raw materials, personnel, and finished products. A subsequent study analyzing clinical MRSA isolates from hospitals within the same geographical zones is also desirable, aiming to recognize potential niche overlaps. This could further reinforce the role of food in the proliferation of community-acquired MRSA.

In conclusion, the identification of MDR, virulence factors, and genomic features of strains from the *S. aureus* USA300 lineage in our strains confirm their clinical relevance, underlining the importance of addressing food safety measures and implementing surveillance programs to mitigate the potential spread of CA-MRSA through food sources.

**References**


**Notes**

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