* *https://www.jdscommun.org/pb-assets/Health%20Advance/journals/jdsc/JDSC-Long-IFORA-Mar2023-1678716091670.pdf*
* *5 pages composed (approximately 5,000 words or 30,000 keystrokes*
* *Supplemental data files not accepted*
* *No more than 3 references needed to support a specific concept*
* *Maximum of 3 tables or figures; maximum size is one half page for any table or figure. If total space occupied by figure and tables exceeds 1.5 pages, word count needs to be reduced accordingly (~500 words per half page)*
* *Tables should be “self-explanatory and understandable without excessive reference to the text.” Must be prepared using table feature in Word (boooooo)*
* *Prepare figures at final size for publication. Figures should be sized to fit one column (8.9 cm wide), 2 columns (14 cm wide), or full-page width (19 cm wide).*
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* *The authors must state explicitly that institutional animal care and use (IACUC) or equivalent approval was obtained before commencement of the study*

***Staphylococcus aureus* isolates from humans and cattle on Vermont dairy farms making farmstead cheese belong to different clonal complexes**

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**Abstract**

*Staphylococcus aureus* is a multihost pathogen that causes significant human and livestock

**Keywords:** Mastitis, Antimicrobial resistance, Multi Locus sequence typing, beta-lactam, zoonotic transmission

1. **Introduction**

*Staphylococcus aureus* is a common bacterium found in the anterior nares of 20–60% of healthy humans

profile and with potential host transmission events occurring on the enrolled dairy farms.

1. **Materials and methods**

**2.1 Study design, setting and participants**

In this pilot study, 19 Vermont dairy farms that produce farmstead cheese or milk for artisan cheese

**2.2 Sample Collection**

The samples included human nasal and hand swabs, quarter milk (CQM) from lactating cows, and

were transported on ice to the laboratory and stored at -20 ºC up to 90 days prior to processing.

**2.3 Bacterial culture**

The samples collected from humans, dogs and BTM were grown on non-selective Tryptic Soy. All plates were incubated at 37°C for 24 h, except for TSAWB plates, which were incubated for 48 h.

**2.4 Presumptive Isolation and Identification of *Staphylococcus aureus***

Presumptive identification criteria for staphylococci included round colonies 2-3 mm in diameter; opaque greyish white, white, pale yellow, or golden yellow colonies generally hemolytic on TSAWB; clear to white or yellow colonies that ferment mannitol on MSA; and mauve to pink colonies on CHRSA and CHRMRSA. After incubation, the hemolytic pattern on TSAWB was observed, followed by Gram staining, catalase, and coagulase tests on each presumptive isolate. Presumptive *S. aureus* were gram-positive, catalase and coagulase tests positive, cocci with complete or partial hemolysis. The presumptive isolates were stored frozen in sterile Tryptic Soy Broth with 15% glycerol until further processing.

**2.5 DNA extraction and Multiplex PCR2.6 Multilocus Sequence Typing (MLST)**

For MLST analysis, genomic DNA from all *nuc* positive isolates was subjected to PCR using or alignments with mismatches were re-sequenced. Consensus sequences were queried against the *S. aureus* MLST database (<https://pubmlst.org/>) to determine allele and sequence type matches. Novel alleles or allelic profiles were submitted to the MLST database curator for new allele and ST number assignment. All identified isolates were submitted to the database.

**2.7 Antimicrobial susceptibility testing (AST)**

Antimicrobial sensitivity testing was performed using agar disc diffusion (DD) and broth microdilution assays, following CLSI

**2.8 Data management and Statistical analysis**

In this study, we defined isolates as bacterial colonies selected from primary culture plates and sub-cultured on secondary plates showing homogenous morphology. Sequence types(STs) were defined as isolates with a common MLST allelic profile, and clonal complex(CC) was defined as

y interaction or association was considered significant with a p-value of less than 0.05.

1. **Results**

**3.1 Descriptive Analysis**

This study included 41 human participants (1-4) from 19 farms and 589 cows (3 to 204 per herd) from 17 participating herds, BTM samples from all 19 farms, and 13 dogs from 9 farms. A total of 1628 isolates were collected, of which 1260 were gram-positive, catalase-positive cocci (GPCPC). Out of 352 human hand isolates and 365 nasal isolates, 166 were GPCPC and 69 were

**3.2 MLST Profiles**

The 89 *S. aureus* isolates were classified into 14 different MLST sequence types (STs) and 8 clonal

1. **Results**

In this study, the 19 dairy farms represent approximately 28% of the total number of farms engaged in farmstead or artisan cheese production in Vermont during 2015-2018.

**4.1 Prevalence of *S. aureus* in dairy farms**

The prevalence of *S. aureus* positive BTM samples in our study is consistent with previous studies . Because cows with *S. aureus* mastitis shed into milk, BTM samples are considered useful for

.

**4.2 MLST profiles**

. However, no significant association was found between the carriage of *blaZ* by *S. aureus* isolates and the type of farm (organic or conventional). This could be due to the unbalanced sample size, as there was a large discrepancy between the number of conventional and organic farms in the study.

**4.4 Potential Host Transmission Events**

1. **Conclusion**

In conclusion, this study provides insights into the prevalence and clonal diversity of *S. aureus* strains among dairy workers and dairy cows in cheesemaking farms in Vermont. The study found that human carriers had a higher likelihood of carrying penicillin-resistant *S. aureus* strains, possibly because of the increased use of antibiotics in human medicine. Similarly, humans working on organic dairy farms may be the main source of resistant isolates because of the widespread use of β-lactam antibiotics in human medicine. Further research, specifically focusing on organic dairy farms, could help further understand the dynamics of antibiotic-resistant gene transfer. Additionally, the study hints at potential spillover events of *S. aureus* sequence types between hosts. These findings support the importance of the "One Health Initiative" for continued monitoring of *S. aureus* at the human-animal interface.

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**Conflict of Interest**

The authors declare no conflicts of interest.

**Author Contributions**

Ashma: Lab work, data collection & analysis, manuscript writing

Chrsitine: Field sample collection

Ariela: Field sample collection, Lab work

Robert: Sample collection, lab work, data collection & analysis

Amanda: sample collection, lab work

John: Study design, field sample collection, lab work, data collection & analysis, manuscript preparation

**References**