Carriage of MRSA Among Dairy Farm Workers in the High Plains

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Introduction & Objective

- Dairy Farmers experience a heavy burden of bioaerosol-related ailments.
- Bioaerosols are known to contain inflammagens (i.e., endotoxins), as well as a diverse bacterial community that is associated with upper respiratory inflammation and pulmonary decrement.

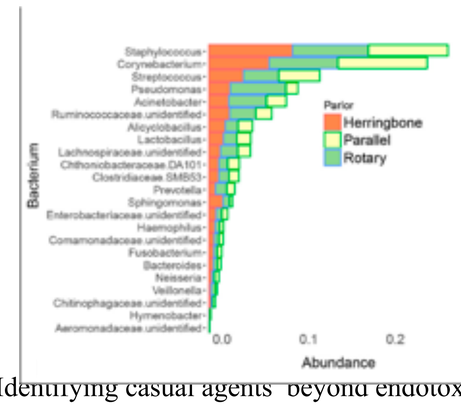
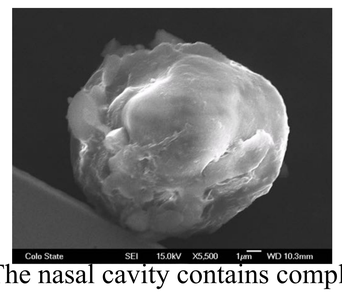
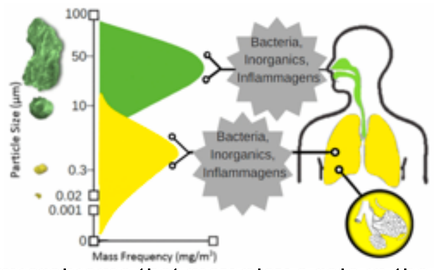


Figure 1. Relative bacterial abundance was comparable across different parlor types with *Staphylococcus*, *Streptococcus*, and *Pseudomonas* as the predominant genera

- Identifying casual agents beyond endotoxin still warrants further research.
- Industrialization and modernization of the dairy industry has led to dramatic changes to production, work organization and tasks. Consequently, exposure patterns have been altered.
- Recently, we demonstrated the particle size range of dairy bioaerosols is predominantly present between 10-100 µm in aerodynamic diameter. These particle sizes are known to deposit in the upper respiratory system.





Chemical & biological composition of inhalable dust inside several Colorado dairy parlors assessed:
Figure 2: SEM image of dust sample. **Figure 3:** Bioaerosol particle size distribution

- The nasal cavity contains complex microbiome that may play a role in the inflammatory response to bioaerosols.
- Recent studies show dairy famers contain over 2X the bacterial diversity and abundance compared to non-farmers.
- It is believed that this diversity is protective against the colonization of *Staphylococcus aureus* (MRSA). In contrast, persistent carriage of methicillin- resistant *Staphylococcus aureus* (MRSA), specifically livestock associated MRSA (LA-MRSA) has been seen among swine production workers.

The objective of this research is to characterize the presence and carriage of *Staphylococcus spp.* with a focus on MRSA and livestock associated MRSA in the nose of dairy workers. The presence of MRSA among dairy workers will be evaluated in the context of the nasal microbiome and its diversity. MRSA and bacterial communities in the nasal passageway will be identified using culture-based methods, PCR, and 16S rRNA sequencing. We will conduct exposure assessments at four dairies in the High Plains region across two different time points. Sampling campaigns are still underway.

Methods

Nasal Lavage :

- Nasal lavages were administered to workers before and after their shift over five consecutive days (8-hour shifts). Dairies were located in the high plains and intermountain region of Colorado.
- Dairy workers volunteered to have air monitoring, pulmonary function testing and naval lavages performed before and after their shift.
- Nasal lavage was performed by administering 5 mL of saline into each nostril (total 10 mL). Volunteers were made to hold their breath for 10 seconds and to not swallow any of the liquid. After the saline was administered, volunteers were instructed to lean forward and allow the saline to drain from their nose into a sterile sample cup.
- After the collection of the nasal lavage, investigators measured the return with a serological pipette for an accurate quantification. The collected lavage sample was then transferred into a sterile 15 mL falcon tube.
- Protease Cocktail inhibitor was added in the field to the lavage for the purpose of preserving cellular information for a study investigating inflammatory response. Samples were stored on ice until they returned to Colorado State University and stored at 6°C in the laboratory.
- A 1 mL aliquot was taken from the lavage sample 12 hours post collection for MRSA carriage testing. Samples were mixed in a 1:1 ration with 40% glycol stock for preservation.

To date:

- Five dairies have been sampled in the high plains of Northern Colorado and northern Texas
- Volunteer sample size = 31
- 119 nasal lavage pre-shift samples
- 119 nasal lavage post-shift samples
- Average nasal lavage sample = 6.6 mLs

Figure 3:. Nasal lavage administration.

Methods continued

Procedure:

• In the field, nasal lavage samples were kept between 2º - 8ºC until they could be placed in a laboratory refrigerator to await further processing (within 12 hours). Long-term preservation was achieved with a 40% glycerol stock solution in a 1:1 ratio to create a 20% solution.
• Samples were then placed for long term preservation in a -80ºC freezer.
• Preserved samples were thawed slowly prior to analyses.
• Tryptic Soy Agar (TSA), Chromagar MRSA, Chromagar Staph aureus, and Mannitol Salt Agar (MSA) were inoculated with each nasal lavage sample (pre and post).
• Inoculated media was incubated for 24 hours at 37.5ºC . A positive and negative control was also included for each media. Positive controls were :
Staphylococcus aureus ATCC 25923 (MSSA)
Staphylococcus aureus ATCC 700699 (MRSA)
• Post 24-48 hour incubation, samples were monitored for growth.
• Colonies from selective media were collected and propagated in Brain Heart Infusion Broth (BHI) for 24-48 hours at 37.5ºC.
• BHI samples were plated onto TSA plates to grow isolates of original media in preparation for Antibiotic Sensitivity Testing (AST) via the Kirby Disc Diffusion method. Plates were incubated at 37.5ºC for 24-48 hours.
• After appropriate incubation and growth, isolated colonies were selected to transfer into 2 mL of Saline, and vortexed to match a 0.5 McFarland standard.
• Within 15 minutes of creating desired saline mixture, TSA plates were lawn streaked and allowed to dry for 5 minutes prior to placing antibiotic disks.
• Inoculated TSA plates were divided into four quadrants prior to antibiotic sensitivity testing (AST).
• Tetracycline, Vancomycin, Cefoxitin, and a blank disk were placed on each TSA plate. A blank disk was included as a control. Plates were incubated for 24 hours prior to interpretation.
• Organisms will be confirmed using Matrix Assisted Laser Desorption/Ionization – Time of Flight (MALDI-TOF).
• DNA extraction will be performed using Qiagen's DNeasy Blood and Tissue kit for use in multiplex PCR.
•Multiplex PCR will be performed targeting/amplifying mecA, scn (immune evasion cluster) and the PVL genes to determine LA-MRSA and CA-MRSA.

Table 1. Analysis Method for determining presence of MSSA, CA-MRSA & LA-MRSA

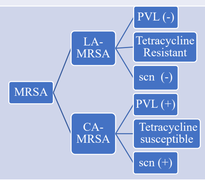
Methods	Materials	Outcome
Isolation	- Chromagar Staph - Chromagar MRSA II - Tryptic Soy Agar	- Selective media isolate for MSSA & MRSA - General propagation
Antibiotic Sensitivity Testing	Disk diffusion : - Tetracycline - Vancomycin - Cefoxitin	- Resistance indicates LA-MRSA - Resistance provides evidence of further public health concern - mecA gene = oxacillin resistance
MALDI	Rapid MRSA confirmation	- Confirms MRSA by the presence of mecA or mecC
Identification of LA-MRSA and CA-MRSA	PCR : - PVL gene presence - Tetracycline susceptibility - Scn immune evasion cluster (IEC) gene presence	

Table 1. Methods used for determining the presence and designation of MSSA and MRSA

Results

To date, a total of 31 dairy workers have participated in our study. Exposure data were available for 119 work shifts based on number of consecutive days of monitoring. As such, 238 nasal lavage samples (pre and post-shift) were collected.

- A total of 80 samples tested positive for MSSA (34%), 23 samples tested positive for MRSA (9.7%).
- To date, 3 samples out of 23 that were MRSA positive have undergone AST. Results for vancomycin susceptibility indicated 3 out of 3 were susceptible to vancomycin (resistance indicates potential public health concerns).
- Additionally, results for tetracycline indicated resistance in two samples (n=2), and susceptibility observed in one sample (n=1).
- AST results for cefoxitin indicated one sample was resistant (n=1; mecA +), and two samples were susceptible to cefoxitin and therefore mecA – (n=2). *

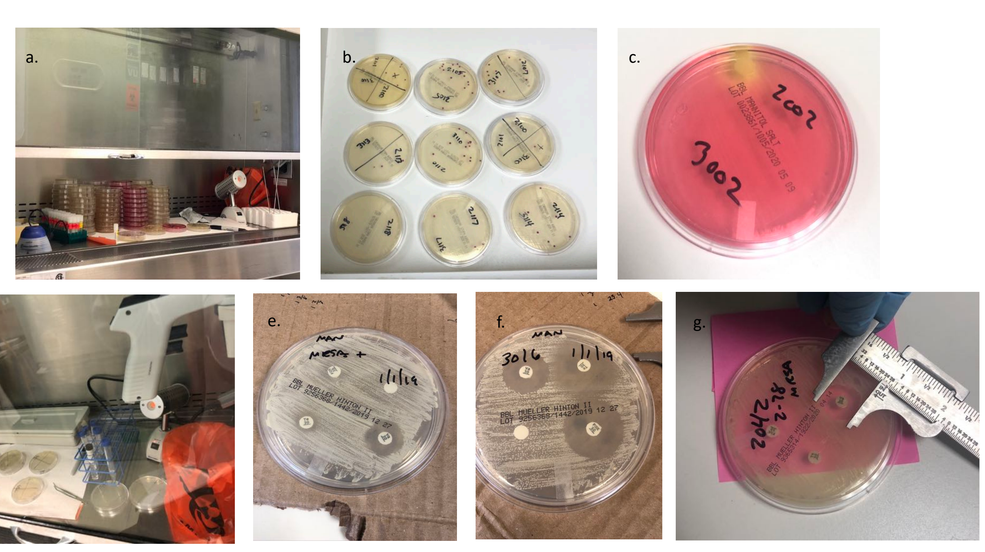


Figure 4: from left to right (a) BSCII prepared for media inoculation. (b) Samples on selective media Chromagar MRSA II and Chromagar *Staph aureus*. (c) Positive growth on MSA indicating MRSA. (d) BSCII prepared for AST. (e) AST for MRSA Control ATCC 700699. (f) AST for a MRSA + post nasal lavage sample . (g) AST for a MRSA + pre nasal lavage sample.

* To date 3 samples have undergone AST

Future Recommendations

- Perform AST on remaining 20 MRSA + samples
- Confirm isolates as LA-MRSA using PCR and MALDI - TOF

Acknowledgements : This research was funded by the National Institute for Occupational Safety and Health UO1 grant #1U01OH010840