Characterizing *S. aureus* From Mastitis on Maine Dairies

Elisabeth Roadcap[1,2], Brenda Kennedy-Wade[1], Gloria Adjapong[1], Anne Lichtenwalner[1]

[1]Veterinary Diagnostic Laboratory, Cooperative Extension, University of Maine (UMVDL); [2]Microbiology and Molecular Genetics, University of Vermont

eroadcap@uvm.edu

Background

Staphylococcus aureus is an important intramammary pathogen which causes mastitis in dairy livestock. Infections with *S. aureus* (SA) decrease quantity and quality of milk, result in economic loss, and can pose a risk to public health. SA can produce heat-stable toxins which cause food poisoning when consumed in either pasteurized or raw milk products. Some strains of SA are resistant to a range of antibiotics, most notably methicillin (MRSA), which is troubling particularly in nosocomial settings.[1]

Hypothesis

S. aureus mastitis cases in Maine endanger public health.
- Phenotypic and genetic antibiotic resistance
- Increasing prevalence across Maine

Methods

- Database: Epidemiological Analysis:
  - July 2017 to June 2022
  - Evaluated all UMVDL mastitis cases
  - Identified SA-positive cases
- New SA Isolates Evaluated:

Gram Stain → Coagulase Test → Vogel - Johnson agar → Phenotypic Confirmation of SA

Gram-positive cocci (a) and black colonies on yellowed Vogel-Johnson agar (b) suggestive of SA. Kirby-Bauer disk diffusion on Mueller-Hinton plate (c).

- Kirby-Bauer disk diffusion with 12 antibiotics
- Susceptibility determined (CLSI SA values)
- *S. aureus* ATCC 25923 as positive control
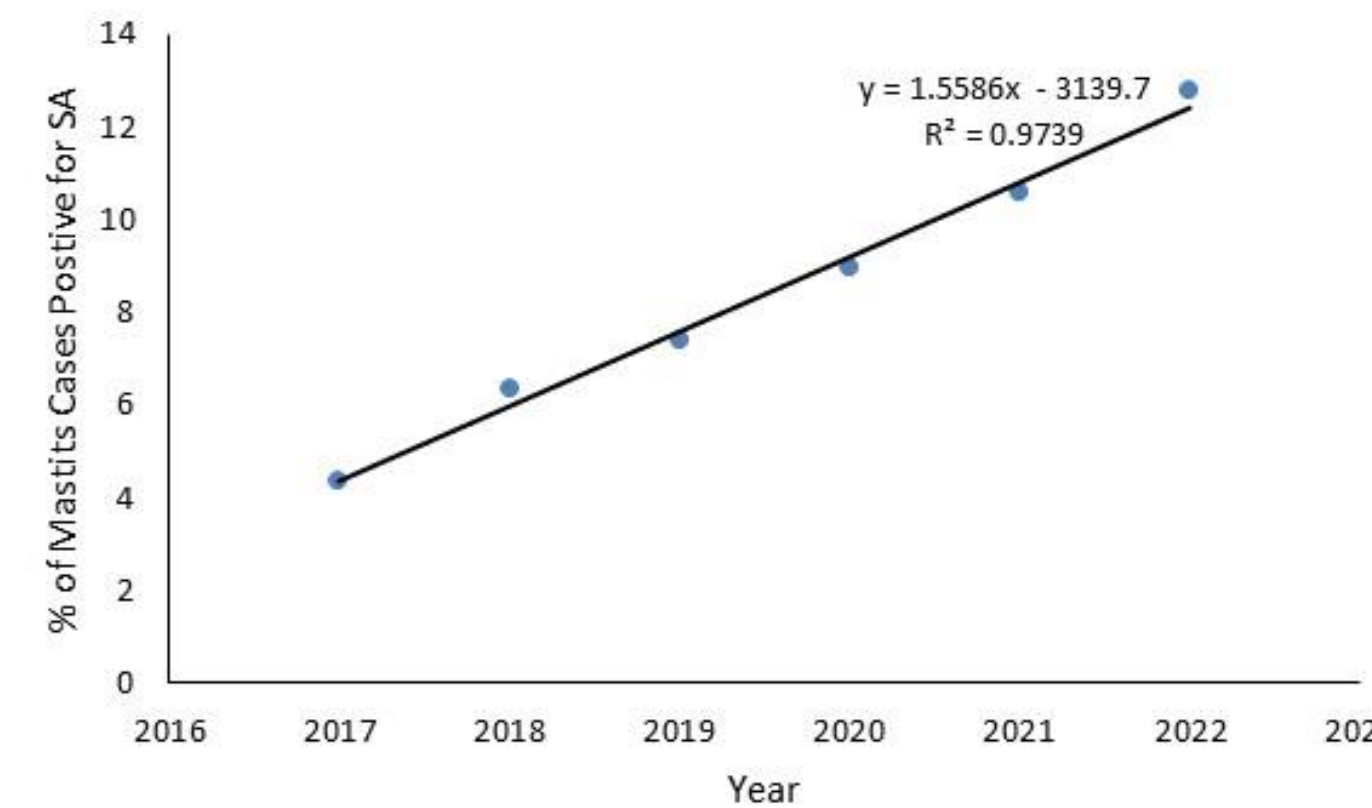
Results

$y = 1.5586x - 3139.7$
$R^2 = 0.9739$

Figure 1. 467 samples (433 animals) were SA-positive out of 6,118 total mastitis samples at UMVDL over six years. Percent of SA cases increased linearly over time.
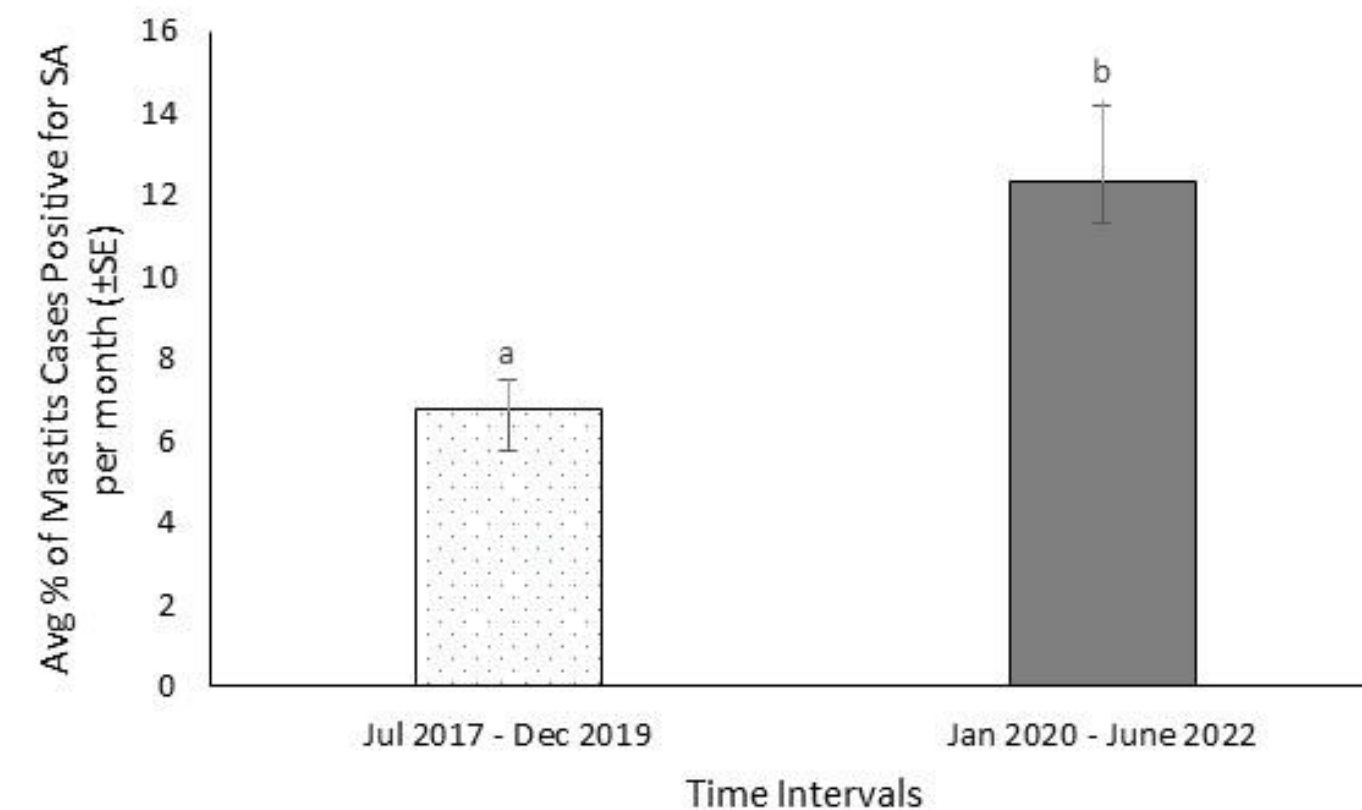

Figure 2. The average percent of mastitis samples positive for SA per month increased significantly from the first 30 months of the evaluated period to the second 30 months. ($P = 0.008$).
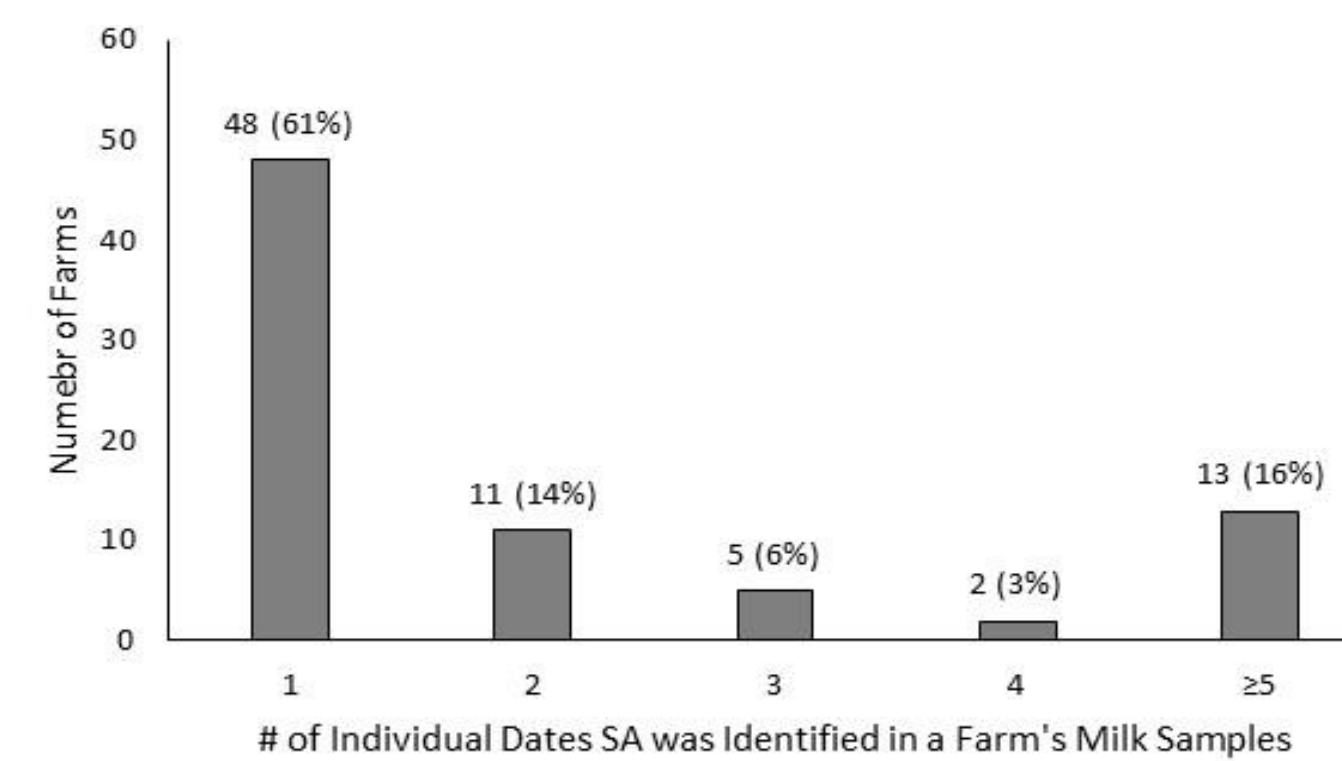

Figure 3. Chronicity of SA on dairy farms as demonstrated by the number of individual dates SA was detected in milk samples sent to the UMVDL between July 2017 and June 2022.
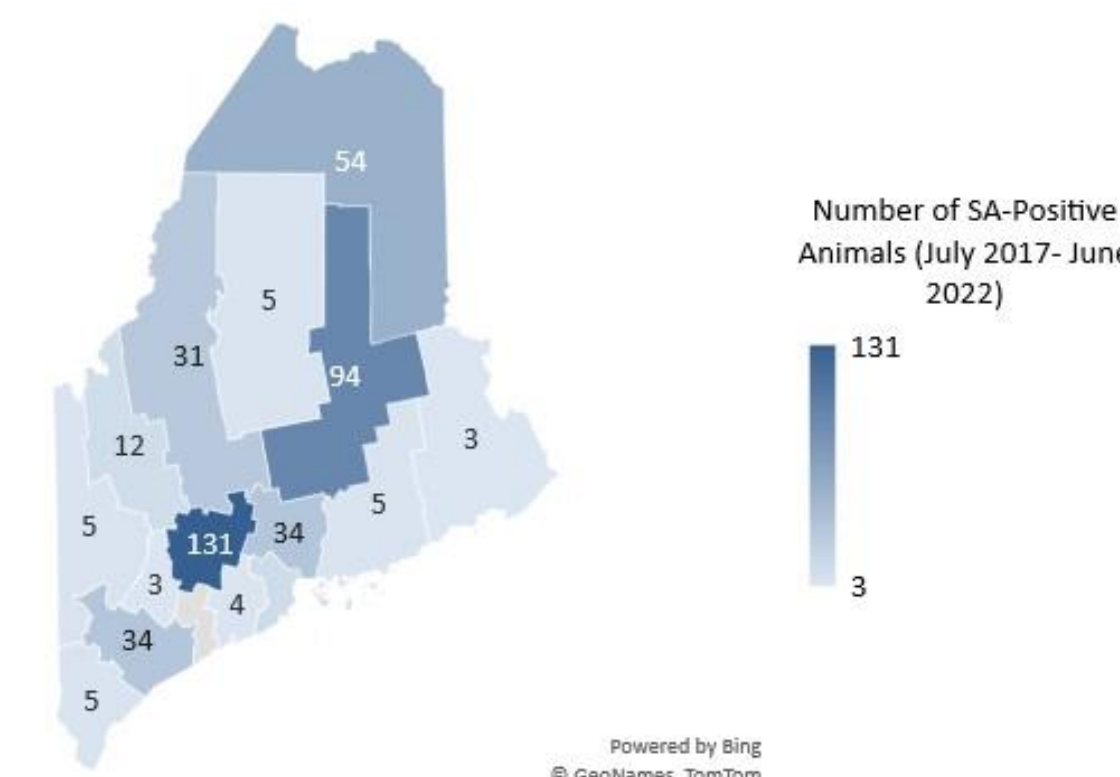

Figure 4. Aggregate number of SA-positive animals by county between July 2017 and June 2022. Some animals tested SA-positive on multiple dates.

Phenotypic Antibiotic Susceptibility

- 91 SA isolates had been tested for susceptibility to oxacillin by farmer request between July 2017 and June 2022.
  - 27.5% (25 isolates) were oxacillin resistant
- None of the 29 recent isolates were phenotypically resistant to any of the 12 antibiotics tested.

Conclusions

- SA mastitis is increasing in Maine.
- SA is a chronic issue on some farms, while others seem to manage SA infections effectively.
- Oxacillin resistance, a proxy for methicillin resistance, was found on some Maine dairies in the last five years.
- Antibiotic susceptibility in current SA isolates is encouraging because these antibiotics are critically or highly important to human medicine[2]

Future Directions

1. Analyze DNA of the most recent isolates for antibiotic resistance and toxin genes
2. Conduct a comparative study between the on-farm techniques at farms with the highest incidence and lowest incidence of SA mastitis

References

[1]Campos, et al. 2022. "Diversity and Pathogenesis of Staphylococcus Aureus from Bovine Mastitis: Current Understanding and Future Perspectives." *BMC Veterinary Research* 18 (1): 115. https://doi.org/10.1186/s12917-022-03197-5
[2]"WHO Critically Important Antimicrobials for Human Medicine 6th Revision," 2018. World Health Organization.

Acknowledgements