

## Exploring persistence of non-aureus staphylococci in the mammary gland using a lactating cow model

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Overuse of antibiotics has resulted in the emergence of antibiotic-resistant bacteria, and thus, alternative treatments are required. A promising alternative is the use of bacteriocins – antimicrobial proteins produced by bacteria that can inhibit *S. aureus*. Our hypothesis is that by creating a non-aureus staphylococcus (NAS) probiotic through genetically engineering a bacteriocin gene cluster into its genome, it will be able to inhibit *S. aureus* and prevent mastitis. To achieve this, we needed to find a persistent and non-inflammatory NAS strain that can colonize cow mammary glands by using an experimental mammary infusion model. Future objectives include the genetic modification and characterization of the persist NAS strain to produce bacteriocins. We infused 4 different mixtures of NAS containing a total of 16 different NAS isolates into 3 lactating cows' mammary glands. The milk samples were tested daily to see which strains remained. At the end of the two-week trial, the cows were euthanized, and tissue analysis was performed to see where different NAS colonized. From the 4 different mixtures, the three most persistent NAS isolates were *S. devriesei* 1316, *S. pasteurii* 2657, and *S. warneri* 2993. These three persistent NAS were then infused individually into 3 lactating cow mammary glands to see their persistence and individual effect on somatic cell count (SCC). Euthanasia and tissue analysis was performed in a similar manner to above afterwards. *S. warneri* 2993 was the most persistent strain as it was isolated from milk 28 out of 42 times while the other two isolates grew less than 4 out of 42 times. *S. warneri* 2993 did increase the SCC past the subclinical mastitis threshold at days 2 to 5. However, for the rest of the days, the SCC was stayed below the subclinical mastitis threshold. To conclude, *S. warneri* 2993 was the most persistent strain with the lowest effect on SCC and will be used to introduce protective bacteriocin genes into.

**Take home message:** This was the first lactating cow infection trial done at the University of Calgary showing an intramammary NAS infusion can result in colonization of bovine mammary glands. *S. warneri* 2993 was the most persistent strain with the lowest effect on SCC. This strain will now be modified to be a probiotic producing bacteriocins to prevent mastitis.



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