Communication between *Staphylococcus aureus* and Non-aureus Staphylococci from Bovine Intrammary Infections and Teat Apex

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Udder health management in automatic milking systems (AMS) differs from conventional milking systems (CMS). Dairy cows in AMS can be milked up to five times daily without any human contact with the udder and preparation for milking is usually by individual teats. The longer milking duration may be at higher risk of acquiring new intramammary infections (IMI) with non-aureus staphylococci (NAS). Epidemiological characteristics of NAS have been extensively investigated in CMS but knowledge about these characteristics is sparse for AMS herds. Understanding the patterns of habitat-specific NAS distribution could improve the udder health in AMS herds. The role of NAS on the risk to acquire of IMI with *Staphylococcus aureus* (*S. aureus*) is poorly understood. The quorum-sensing accessory gene regulator (*agr*) system of *S. aureus* plays a central role in its pathogenesis and virulence. Some staphylococcal species (e.g., *S. epidermidis*) produce auto-inducing peptide (AIP)-like molecules, which inhibit *S. aureus* agr and toxin production. The objectives of this study were to 1) investigate the patterns of NAS species distribution in milk and teat skin in AMS herds, and 2) examine if the isolated NAS influence the *S. aureus* virulence factors expression.

Among cows with elevated SCC (≥ 200,000 cells/mL) in eight herds with AMS, 30-40 cows were randomly selected for teat skin swabbing and aseptic collection of quarter foremilk samples. Of these, samples from right rear and left front quarters of cows with odd laboratory running numbers were used for further analysis. Samples from teat skin and milk were subjected for bacterial culture and, subsequently, Maldi-Tof for species identification. To investigate the interaction between *S. aureus* and NAS, 81 isolates from milk and teat skin were subjected to qualitative beta-galactosidase reporter plate assay using three reporter strains of *S. aureus*, *hla*, encoding a-hemolysin, *RNAIII*, the key effector molecule of *agr* and *spa*, encoding Protein A.

Out of 284 quarters (142 cows), 80% harbored at least one species of NAS. In total, 15 species from teat skin and only 10 species from milk. The most prevalent NAS species identified from milk were *S. epidermidis*, *S. haemolyticus*, and *S. chromogenes* confirming their major role in causing IMI in AMS herds. Conversely, *S. equorum*, *S. haemolyticus*, and *S. cohnii* were the most common NAS species in teat skin. Out of 81 isolates, CNS supernatants reduced expression of *hla* (72%) and *RNAIII* (68%) but increased expression of *spa* (61%) indicating that CNS species isolated from different habitats in dairy cows interfere with *agr* quorum system of *S. aureus*. Crosstalk between NAS and *S. aureus* showed three patterns (a) downregulation effect such as *S. chromogenes* (milk), (b) no effect such as *S. sciuri* (teat), and (c) variant effect such as *S. epidermidis* (milk and teat).

Staphylococcus epidermidis and *S. chromogenes* are milk-associated while *S. equorum* and *S. cohnii* are teat-associated. NAS species, habitat type, and herd factors affect NAS and *S. aureus* crosstalk patterns. Crosstalk patterns of NAS and *S. aureus* could explain varying protective effect of NAS on *S. aureus* IMI reported in previous studies. Knowledge of how NAS influence *S. aureus* virulence factors expression may ultimately help in controlling *S. aureus* IMI.

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