***Discussion***

*Diversity of strain type as determined by RAPD and MLST*

In all 9 herds, there was at least one RAPD type of *S. chromogenes* identified to be causing an IMI in quarters belonging to different cows. RAPD typing has been used to compare strain types of different isolates of the same species during outbreaks of bacterial infections among animals, in order to see if the transmission pattern was consistent with a pathogen acting in a contagious manner (or originating from a common point source), or the increased number of infections were caused by multiple strains which were not derived from a common source.In combination with sequencing the 16S rRNA gene for representative isolates, RAPD was used to understand the diversity of strain types causing a multistate outbreak of *Corynebacterium tuberculosis* in multiple species (Foley et al. 2004), and also for investigation of a *Campylobacter jejuni* between different flocks on a single broiler farm (in combination with sequencing the 23S rRNA gene; Payne et al., 1999). RAPD alone was used by Zadoks et al. (2003) to identify transmission dynamics of the mastitis isolate *Streptococcus uberis* within a single herd, which revealed that each cow was infected with a unique strain, confirming that the observed mastitis outbreak was not due to contagious transmission, but instead was resulted from infections from environmental sources. Although the objective of the current study was not to identify the transmission dynamics of *S. chromogenes* within a given dairy herd, finding the same RAPD type in more than one cow in the same herd suggests that cow-to-cow spread is occurring within a herd (or spread is occurring via a common point source). These findings are consistent with Wuytack et al. (2020b) and (Reydams et al. 2023), who also used RAPD typing for *S. chromogenes* isolates. Studies using amplified fragment length polymorphisms (AFLP) (Taponen et al. 2007) and pulsed-field gel electrophoresis (PFGE) (Gillespie et al. 2009; Mork et al. 2012) have also demonstrated the same *S. chromogenes* strains have been found in multiple animals from the same farm, suggesting some strains may act in a contagious manner.

In the current study, 10 different strain types determined by MLST were identified for the 30 *S. chromogenes* isolates. As the MLST scheme for *S. chromogenes* was described fairly recently (Huebner et al. 2021), the number of studies describing strain-typing results using this scheme to date is limited (Petzer et al. 2022; Persson Waller et al. 2023). In the phylogenetic analysis, study isolates belonging to ST174, ST175, and ST176 were identified as being closely related to ST1 isolates from PubMLST, and were identified as single locus variants of ST1 by the MLST 2.0 tool. ST1 was the most commonly found strain type in the current study (11/30 isolates, 36.7%). This agrees closely with the work of Huebner et al. (2021), who determined MLST for 120 *S. chromogenes* isolates from Belgium, Vermont (US), and Washington state (US). They found 39/120 (32.5%) of isolates strain-typed belonged to a nodal cluster centered around ST1. For the 48 isolates in Huebner et al. (2021) from Vermont, 36 (75%) belonged to nodal cluster 1. ST1 was also commonly found in 118 *S. chromogenes* isolates from bovine subclinical IMI in Sweden, in a study from Persson Waller et al. (2023), although ST6 and a related novel ST (ST109) were more predominant. For Huebner et al. (2021), ST1 was the only strain type found in all three geographical locations. ST6 was the second most commonly found ST in the current study (9/30 isolates, 30%), and the third most common (15/120, 12.5%) in Huebner et al. (2021). Persson Waller et al. (2023) identified 47 different strain types among 105 isolates from Sweden. Huebner et al. (2021) found a similar degree of diversity, with 47 ST identified from 120 isolates from three geographical locations. After ST1, ST15 was the second most commonly identified by Hubener et al. (2021), with 17/120 (14.2%) of isolates belonging to this strain type. ST15 was primarily identified in isolates from Vermont and Washington state (16/17 isolates), which Huebner et al. (2021) highlight as an example of how the distribution of different strain types varies geographically. Interestingly, 2 of 30 isolates belonged to ST15 in the current study, while only 1 ST15 was found in Sweden (Persson Waller et al., 2023).

Both Persson Waller et al. (2023) and Huebner et al (2021) saw a pattern where ST6 and ST1 as central nodes of ST clusters, with single- and double-locus variants surrounding these two ST. Both authors suggest this indicates a clonal expansion is occurring for *S. chromogenes* isolates belonging to these 2 ST. Results of the current study would support this, as the 3 ST in ST cluster 1 (ST174, ST175, ST176) were all newly-identified single-locus variants of ST1. Describing the diversity of strain type using MLST is an incredibly active area of research. Four of the 10 ST in the current study had previously not been described, while 43% of isolates belonging to 33 new ST were identified by Persson Waller et al. (2023). These results highlight the importance of contributing to publicly-available databases in order to improve our ability to better understand the diversity of *S. chromogenes.*

*Associations between ST and SCC category*

Our initial hypothesis that ST may be a significant predictor of SCC phenotype (HIGH vs. LOW category) was not supported. Persson Waller et al. (2023) also explored associations between genotypes and phenotypic qualities, such as persistency (over a 1 month period) and association with a CMT score at sampling. Although they found no association between ST or ST cluster and persistency, isolates belonging to their cluster VII were significantly more likely to be associated with a high CMT score, indicating a larger inflammatory reaction was occurring in the gland. Isolates belonging to ST6 (the most prevalent ST in cluster VII, and only ST also found in the current study) tended to be more likely to have a high CMT score vs. other STs in the cluster. However, this difference did not achieve the cut-off used for statistical significance.

*Antimicrobial resistance genes and associations between ARG and SCC category*

Overall, both phenotypic resistance and resistance genes are relatively rare in *S. chromogenes* when compared to other non-aureus staphylococci (NAS), with the important exception of the *blaZ* gene (Sampimon 2009; Persson Waller et al. 2011). Our findings support this principle, as the only ARG identified in the 30 *S. chromogenes* isolates was *blaZ. blaZ* encodes β-lactamase enzymes which hydrolytically destroy β-lactam antibiotics, and is the primary determinant of phenotypic resistance to benzylpenicillin in staphylococci (Pinho 2008).In the current study, 10/30 (33.3%) of isolates were *blaZ-*positive, which is higher than the 10% reported for *S. chromogenes* isolates in a Canadian study (Condas et al. 2017) and the 22% reported in Persson Waller et al. (2023), but much less than the 87% of *S. chromogenes* in a Flemish study (Sampimon 2009). Resistance to β-lactam antibiotics is the predominant type of AMR present in staphylococci, and the reported proportion of NAS isolates exhibiting β-lactamase resistance can be fairly high depending on geographical location (51.6% in Argentina, Raspanti et al. 2016; 63% in South Africa, Phophi et al. 2019; 23% in Belgium and Norway, Fergestad et al. 2021; 14% in Korea, Kim et al. 2019). β-lactam antibiotics are among the few choices for treating mastitis in the US, with first- and third-generation cephalosporins being the most commonly-used mastitis treatments (USDA 2016; de Campos et al. 2021). In addition to *blaZ*, Persson Waller et al. (2023) found strpS194, which confers resistance to streptomycin, in 7% of their *S. chromogenes* isolates. This ARG was not identified in isolates from the current study. Nobrega et al. (2018) identified various other ARG in *S. chromogenes* isolates, including genes associated with aminoglycoside resistance [ant(3’’), ant(4’), ant(6)], resistance to amphenicols (fexA), and resistance to tetracyclines (tetK, tetL). However, the estimated prevalence of these genes in the population of *S. chromogenes* tested in their study was low (2-3%).

Carriage of the *blaZ* gene was not found to be a significant predictor of whether an isolate would be associated with a persistently high SCC IMI. Not much is known about the association of ARG carriage by *S. chromogenes* and clinical characteristics of IMI, but previous work has identified a link between phenotypic resistance in *S. aureus* and clinical outcome. Both Sol et al. (2000) and Taponen et al. (2003) found that penicillin-resistant strains of *S. aureus* (those which produced β-lactamase) had a lower bacteriological cure rate *in vivo,* despite use of an appropriate intramammary antibiotic that the isolate was susceptible to *in vitro*. The *S. aureus* isolates in Sol et al. (2000) were associated with clinical mastitis, whereas the isolates in Taponen et al. (2003) were from cases of subclinical mastitis. Further, Sol et al. (2000) report that IMI due to penicillin-resistant *S. aureus* were associated with a more persistently elevated SCC, indicating the IMI was associated with a higher degree of inflammation. The authors of both studies conclude that either 1) the penicillin-resistant strains of *S. aureus* were more virulent than susceptible strains, due to a possible relationship between production of β-lactamase and other virulence factors, or 2) that any antibiotic used to treat mastitis caused by penicillin-resistant strains works less efficiently, due to unidentified pharmacokinetic or pharmacodynamic factors. A more recent example of a possible association between ARG and clinical characteristics of an infection due to NAS staphylococci is described in Wuytack et al. (2020a). When comparing NAS isolates associated with IMI which had an SCC of ≤50,000 cells/mL to isolates from cases of clinical mastitis, Wuytack et al. (2020a) identified *mecA* (a methicillin-resistance gene) in 21/43 (49%) of the NAS isolates originating from clinical mastitis and only in one isolate 1/16 (6%) in IMI from a quarter with an SCC of ≤50,000 cells/mL. Based on these findings, the authors suggest that *mecA* in NAS isolates from bovine IMI may be linked to virulence genes or pathogenicity islands, supposedly both present on a mobile genetic element (*SCCmec,* staphylococcal cassette chromosome *mec).* Of the 22 NAS isolates identified as *mec*-positive in Wuytack et al. (2020a), none were *S. chromogenes.* Further research into exploring associations between ARG and clinical characteristics of IMI including a larger number of *S. chromogenes* are certainly warranted, in order to better understand if particular undesirable traits (e.g., penicillin resistance and a greater inflammatory response) may be genetically linked in this ubiquitous mastitis pathogen.

*Virulence genes and associations between VF and SCC category*

The overall number of unique VF identified in the current study (62) from 30 *S. chromogenes* isolates was similar to the findings of Perrson Waller et al. (2023), who identified 57 unique genes among the 105 *S. chromogenes* isolates from Sweden. The average number of VF per isolate reported from Persson Waller et al. (2023) was 30 (SD: 5.4, range: 25-45), which is somewhat lower than the median (44) and range (43-51) reported for isolates in the current study. The same database of VF was used in the current study and Persson Waller et al. (2023), facilitating a direct comparison of these values. In a smaller-scale study of 8 *S. chromogenes* from Finland using a different database for identifying VF, a range of 37-49 VF were identified (Åvall-Jääskeläinen et al. 2018). Although species-specific summary statistics are not provided for the 83 *S. chromogenes* separately, a large Canadian study investigating the profile of 191 VF in 441 isolates belonging to 25 different species of NAS found that NAS isolates contained on average 30 VF genes (Naushad et al. 2019). Naushad et. al (2019) report that the phylogenetic grouping of NAS species with the highest virulence potential (defined by total number of VF) was clade B, which contains *S. chromogenes, S. agnetis, and S. hyicus*. A proportionately higher number of exotoxin genes, host evasion genes, and capsular genes contributed to clade B’s high virulence potential.

One of the better-studied virulence genes of NAS is *bap*, encoding a surface protein which is a pathogenic factor of biofilm formation. *bap* was not detected in any of the 83 *S. chromogenes* isolates in Naushad et al. (2019), or in any of the 25 isolates of *S. chromogenes* included in a Belgian study of clinical and low-SCC IMI (Wuytack et al. 2020a). It was also rare in Åvall-Jääskeläinen et al. (2018), only found in 1/8 *S. chromogenes* isolates, and was somewhat sporadically found in Persson Waller et al. (2023) in 13/105 isolates. In light of these findings, it was surprising that *bap* was in 28 of the 30 isolates (93.3%) in the current study. It has been suggested that biofilms increase the ability of NAS to persist in the udder (Piessens et al. 2012; Tremblay et al. 2013). As all 30 isolates in the current study are from persistent IMI, finding *bap* in such a high proportion would support the notion that biofilms play a role in the ability of *S. chromogenes* to cause chronic infections. Another staphylococcal virulence factor playing a role in evasion of the host immune response is genes encoding for a polysaccharide capsule which resists phagocytic cell uptake. In Naushad et al. (2019), *S. chromogenes* isolates were seen to have 12 different capsular genes in low frequencies (7-11%). Only 1 of 8 *S. chromogenes* in Åvall-Jääskeläinen et al. (2018) had any capsular genes, and only 2/25 isolates in Wuytack et al. (2020a) was positive for cap5H with PCR. All 30 isolates in the current study contained at least 3 different *cap* genes, with 28 all having *capN, capO, capP.* Two isolates in the current study were missing *capN,* but possessed both *capJ* and *capH.* There is conflicting evidence on the associations between capsule genes and overall virulence of staphylococci. Some evidence exists that staphylococci lacking a capsule are able to better persist in the mammary gland, while encapsulated strains elicit more inflammation and are thereby eliminated faster (Tuchscherr et al. 2005), but other research suggesting that the antiphagocytic properties of the capsule allows staphylococci to persist in infected hosts (Thakker et al. 1998). Citing work showing that lack of a capsule has shown to be advantageous for *S. aureus* during chronic infections, Naushad et al. (2019) argue that the low prevalence of capsule genes in their *S. chromogenes* may explain the how the pathogen has become widespread in the population of Canadian dairy animals. Finding such a large proportion of isolates carrying multiple capsular genes in the current study of *S. chromogenes* isolates from persistent IMI would more support that the ability to form a capsule may enhance the ability of the organism to evade the host’s immune response.

Staphyolococcal complement inhibitor (encoded by the gene *scn*) is another virulence factor conferring the ability of the bacterium to evade the host’s immune system. *scn* encodes a protein which inhibits the complement system, reducing phagocytosis of the bacterium following opsonization. Although staphylococcal complement inhibitor had been thought to be highly specific to isolates of human origin, Naushad et al. (2019) detected in a high proportion (88%) of *S. chromogenes* isolates in their study. In agreement with these fidnings, all 30 isolates of *S. chromogenes* in their current study were positive for *scn.* Adenosine synthase A (*adsA*) is an immune evasion factor identified in *S. aureus,* which is responsible for increasing the amount of extracellular adenosine, a potent immuno-suppressive signaling molecule. *scn* allows staphylococci to survive after being engulfed by neutrophils, allowing it to evade the bactericidal activity of host leukocytes. *scn* was found in a high proportion (99%) of isolates from Naushad et al. (2019), and all 30 isolates in the current study.

Another widely-found VF in *S. chromogenes* is β-hemolysin, a phospholipase C toxin secreted by *S. aureus*. β-hemolysin was the most frequent and predominant gene detected in *S. chromogenes* isolates and other species of clade B in Naushad et al. (2019), was found in all 8 isolates in (Åvall-Jääskeläinen et al. 2018), and in all 30 of the isolates in the current study. In contrast, the gene encoding for exfoliative toxin type C (*etc,* a toxin that cause the loss of cell‐cell adhesion in the superficial epidermis in the host) was found identified in any of the *S. chromogenes* isolates in Naushad et al. (2019) or (Åvall-Jääskeläinen et al. 2018), but was present in all 30 of the isolates in the current study. Exfoliative toxins in NAS have been identified in *S. agnetis* and *S. chromogenes* from broiler chickens (as reviewed in Szafraniec et al., 2020), but are not widely reported from isolates of bovine IMI. Another category of toxin gene commonly identified in NAS is the β-type phenol-soluble modulins (PSMs), which have been shown in *S. aureus* to cause lysis of red and white blood cells, contributing to biofilm development and stimulation of inflammatory responses in the host. In Naushad et al. (2019), all *S. chromogenes* isolates possessed a single gene associated with PSMs (*PSMβ4*)*,* which was also widely found in isolates from Persson Waller et al. (2023). All isolates in the current study had the entire suite of PSM-associated genes described in the comprehensive NAS database (*PSMβ1- PSMβ6*), although more research is needed to understand if having a larger number of genetic determinants associated with PSMs plays an important role in the pathogenesis of *S. chromogenes.*

Two *S. chromogenes* isolates in the current study was positive for *coa,* the gene encoding for staphylocoagulase. Staphylocoagulase binds to prothrombin in the host to form a complex which ultimately forms a fibrin clot which shields the bacteria from the host's defenses and causes localized clotting. *coa* has previously been identified in *S. agnetis* and *S. hyicus,* which are considered coagulase variable (Vanderhaeghen et al. 2015). Except for *S. aureus, S. hyicus,* and *S. agnetis*, coagulase positive staphylococci are rarely isolated from bovine IMI, which is why the coagulase test has been so widely used to classify staphylococci from mastitis into coagulase-positive (primarily *S. aureus*) and coagulase-negative (largely all other species of staphylococci) (Vanderhaeghen et al. 2015). None of the 441 NAS isolates in Naushad et al. (2019) were identified as *coa-*positive, while 4/4 *S. agnetis* but 0/8 *S. chromogenes* were *coa-*positive in Åvall-Jääskeläinen et al. (2018). Carriage of the *coa* gene by *S. chromogenes* from bovine IMI has not yet been widely reported. Another protein exhibiting coagulating ability, the von Willebrand factor-binding protein, is widely carried by NAS bovine IMI isolates. All 30 isolates in the current study were positive for *vWbp,* as were 94% of *S. chromogenes* isolates in Naushad et al. (2019).

In the current study, our hypothesis that the number of virulence genes for each isolate would be a significant predictor of whether or not it was associated with a HIGH or LOW SCC IMI was not supported. A number of other studies exploring virulence potential in NAS of bovine origin came to similar conclusions. In their study of virulence factors found in 4 different staphylococcal species (4 isolates each of *S. aureus* and *S. agnetis,* 8 isolates each of *S. chromogenes,* and *S. simulans*), Åvall-Jääskeläinen et al. (2018) found no association by visual inspection between the type of mastitis (clinical vs. subclinical) and specific virulence genes, virulence gene profiles, or the cumulative number of virulence genes. As statistical power to analyze these relationships by species was limited by number of isolates, logistic regression analyses of pooled data for all NAS isolates was carried out; however, they did not observe any clear difference in the virulence gene profiles or cumulative number of different virulence genes between the isolates from clinical and subclinical mastitis (Åvall-Jääskeläinen et al. 2018). They reported that most of the isolates had unique virulence gene profiles, and when two isolates of the same species shared an identical profile, 1 of the isolates was clinical while the other was subclinical (Åvall-Jääskeläinen et al. 2018). When comparing isolates from clinical and subclinical mastitis caused by *S. aureus,* Haveri et al. (2007) found no difference in the cumulative number of VF between the two groups. In a Canadian study of 255 NAS IMI isolates, no association between biofilm formation and SCC associated with the IMI was observed (Tremblay et al. 2013). Similarly, no association was found between the phenotypic ability of a NAS isolate to form biofilm and the persistence of IMI when isolates from 63 persistent and 55 transient were compared (Simojoki et al. 2012). In the same study, researchers found no association between the ability of 114 NAS isolates to form biofilms with the severity of the associated mastitis as measured by measured with milk N-acetyl-b-D-glucosaminidase activity (an enzyme which reflects tissue damage and is an indicator of inflammation in the udder; Simojoki et al. 2012).

In contrast, other work has found some associations between the clinical characteristics of an IMI and VF in the NAS isolates causing the infections. In a linear regression with all virulence factors considered together, Naushad et al. (2019) did not find that an increase in the overall number of VF for a NAS isolate was associated with an increase in the logSCC of the associated IMI. However, when stratified by type of virulence, the presence of each additional toxin gene for a NAS isolate was associated with a 0.024 increase in logSCC of the associated IMI for toxin and host immune evasion genes specifically (Naushad et al. 2019). Similarly, in a logistic regression with ordinal categories for IMI severity (low SCC, medium SCC, high SCC, and clinical mastitis), an overall increase in the number of VFs was not associated with increased severity of an IMI (Naushad et al. 2019). In a regression analysis with VF stratified by functional category, the presence of each additional VF gene associated with host immune evasion increased the odds of having a more severe immune response by 0.074 (Naushad et al. 2019). Naushad et al. (2019) applied various clustering approaches in order to determine whether particular VF distributions had any association with SCC category or clinical mastitis associated with the isolate, but no clustering of isolates representing low SCC, medium SCC, or high SCC or CM was identified. For *S. chromogenes* specifically, Persson Waller et al. (2023) also identified some associations between clinical characteristics of an IMI and VF. They found that higher number of exoenzyme genes were present in isolates associated with milk samples that had a with low CMT vs. a high CMT (Persson Waller et al. 2023). Additionally, isolates from low CMT quarters had higher number of evasion genes than those with high CMT, and the *geh* gene (encoding a lipase) was associated with increased odds of having a low CMT (Persson Waller et al. 2023). As these findings contrasted with those of Naushad et al. (2019), Persson Waller et al. (2023) were unable to identify why they may have observed this association. In the current study, all 30 isolates from both HIGH and LOW SCC categories were positive for *geh,* which conflicts with their finding it consistently in isolates from IMI with less inflammation occurring. Persson Waller et al. (2023) also found that *capJ* and *capH* were significantly associated with IMI that came from quarters with a lower CMT. This would be consistent with the finding that unencapsulated staphylococci may be better able to persist in the udder, as they may cause less of an inflammatory response (Thakker et al. 1998). However, the only 2 isolates carrying *capJ* and *capH* in the current study were associated with a persistently high SCC IMI.

* + - REASONS why we may not find a difference
      * Language from De buck
        + The contribution of virulence genes on disease outcomes or development can also be affected by intrinsic factors (within the udder) or extrinsic factors (in the cow’s environment) that influence gene expression. The latter is likely influenced by factors such as herd management, climatic conditions, and geographic location
        + development of disease and interactions of VFs with the host are complex and determined by interplay of genes rather than just presence of specific virulence genes

example of interaction with host:

Several components of mastitic milk can affect biofilm formation, for example calcium, lactoferrin (which concentration increases in mastitic milk), and iron contents in milk

Lactoferrin inhibits biofilm formation; It can be speculated that biofilm formation of staphylococci in the mastitic udder is prevented by lactoferrin; as summarized **in Simojoki 2012**

* + - * From Naushad
        + Additionally, many other factors (e.g., host environment, nutritional status, presence of other competing microbes, and host genetics) have crucial roles in successful colonization, persistence, and pathogenicity of mammary pathogens. Pathogenesis is complex and often involves an organized and systematic participation of various VFs to establish disease. Often VFs complement each other to promote pathogen colonization and persistence of disease
        + These findings suggest that the development of disease and the interactions of VFs with the host are complex and determined by the interplay of genes rather than just the presence of virulence genes
      * These results indicate that, possibly excluding the most severe peracute *S. aureus* mastitis, similar symptoms can be caused by several different combination of virulence factors rather than by any of them alone. In addition, not only the properties of microbes but also the immune system of the host, the cow, has an important role in the manifestation of the inflammation.

Åvall-Jääskeläinen, S., et al. (2018). "Comparative genome analysis of 24 bovine-associated Staphylococcus isolates with special focus on the putative virulence genes." PeerJ **6**: e4560.

Condas, L. A. Z., et al. (2017). "Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds." J Dairy Sci **100**(7): 5592-5612.

de Campos, J. L., et al. (2021). "Quantification of antimicrobial usage in adult cows and preweaned calves on 40 large Wisconsin dairy farms using dose-based and mass-based metrics." J Dairy Sci **104**(4): 4727-4745.

Fergestad, M. E., et al. (2021). "Antimicrobial resistance and virulence characteristics in 3 collections of staphylococci from bovine milk samples." Journal of Dairy Science **104**(9): 10250-10267.

Foley, J. E., et al. (2004). "Molecular epidemiologic features of Corynebacterium pseudotuberculosis isolated from horses." Am J Vet Res **65**(12): 1734-1737.

Gillespie, B. E., et al. (2009). "Prevalence and persistence of coagulase-negative Staphylococcus species in three dairy research herds." Vet Microbiol **134**(1-2): 65-72.

Haveri, M., et al. (2007). "Virulence genes of bovine Staphylococcus aureus from persistent and nonpersistent intramammary infections with different clinical characteristics." J Appl Microbiol **103**(4): 993-1000.

Huebner, R., et al. (2021). "Characterization of genetic diversity and population structure within Staphylococcus chromogenes by multilocus sequence typing." PLoS One **16**(3): e0243688.

Kim, S. J., et al. (2019). "Antimicrobial resistance and genetic characterization of coagulase-negative staphylococci from bovine mastitis milk samples in Korea." J Dairy Sci **102**(12): 11439-11448.

Mork, T., et al. (2012). "Persistence of staphylococcal species and genotypes in the bovine udder." Vet Microbiol **159**(1-2): 171-180.

Naushad, S., et al. (2019). "Comprehensive Virulence Gene Profiling of Bovine Non-aureus Staphylococci Based on Whole-Genome Sequencing Data." mSystems **4**(2): 10.1128/msystems.00098-00018.

Nobrega, D. B., et al. (2018). "Prevalence and Genetic Basis of Antimicrobial Resistance in Non-aureus Staphylococci Isolated from Canadian Dairy Herds." Front Microbiol **9**: 256.

Payne, R. E., et al. (1999). "Molecular epidemiology of Campylobacter jejuni in broiler flocks using randomly amplified polymorphic DNA-PCR and 23S rRNA-PCR and role of litter in its transmission." Appl Environ Microbiol **65**(1): 260-263.

Persson Waller, K., et al. (2011). "CNS species and antimicrobial resistance in clinical and subclinical bovine mastitis." Veterinary Microbiology **152**(1-2): 112-116.

Persson Waller, K., et al. (2023). "Genotypic characterization of Staphylococcus chromogenes and Staphylococcus simulans from Swedish cases of bovine subclinical mastitis." J Dairy Sci **106**(11): 7991-8004.

Petzer, I. M., et al. (2022). "Species identification and cow risks of non-aureus staphylococci from South African dairy herds." Onderstepoort J Vet Res **89**(1): e1-e10.

Phophi, L., et al. (2019). "Antimicrobial resistance patterns and biofilm formation of coagulase-negative Staphylococcus species isolated from subclinical mastitis cow milk samples submitted to the Onderstepoort Milk Laboratory." BMC Vet Res **15**(1): 420.

Piessens, V., et al. (2012). "Characterization of coagulase-negative staphylococcus species from cows' milk and environment based on bap, icaA, and mecA genes and phenotypic susceptibility to antimicrobials and teat dips." J Dairy Sci **95**(12): 7027-7038.

Pinho, M. G. (2008). "Mechanisms of beta-lactam and glycopeptide resistance in Staphylococcus aureus." Staphylococcus molecular genetics: 207-226.

Raspanti, C. G., et al. (2016). "Prevalence and antibiotic susceptibility of coagulase-negative Staphylococcus species from bovine subclinical mastitis in dairy herds in the central region of Argentina." Rev Argent Microbiol **48**(1): 50-56.

Reydams, H., et al. (2023). "Comparison of non-aureus staphylococcal and mammaliicoccal species found in both composite milk and bulk-tank milk samples of dairy cows collected in tandem." Journal of Dairy Science **106**(11): 7974-7990.

Sampimon, O. (2009). Coagulase-negative staphylococci mastitis in Dutch dairy herds., Utrecht University.

Simojoki, H., et al. (2012). "Is the biofilm formation and slime producing ability of coagulase-negative staphylococci associated with the persistence and severity of intramammary infection?" Veterinary Microbiology **158**(3): 344-352.

Sol, J., et al. (2000). "Factors associated with cure after therapy of clinical mastitis caused by Staphylococcus aureus." J Dairy Sci **83**(2): 278-284.

Szafraniec, G. M., et al. (2020). "A Review of Current Knowledge on Staphylococcus agnetis in Poultry." Animals (Basel) **10**(8).

Taponen, S., et al. (2003). "Efficacy of Targeted 5-day Combined Parenteral and Intramammary Treatment of Clinical Mastitis Caused by Penicillin-Susceptible or Penicillin-Resistant Staphylococcus aureus." Acta Veterinaria Scandinavica **44**(1): 53.

Taponen, S., et al. (2007). "Bovine Intramammary Infections Caused by Coagulase-Negative Staphylococci May Persist Throughout Lactation According to Amplified Fragment Length Polymorphism-Based Analysis." Journal of Dairy Science **90**(7): 3301-3307.

Thakker, M., et al. (1998). "Staphylococcus aureus serotype 5 capsular polysaccharide is antiphagocytic and enhances bacterial virulence in a murine bacteremia model." Infect Immun **66**(11): 5183-5189.

Tremblay, Y. D. N., et al. (2013). "Characterization of the ability of coagulase-negative staphylococci isolated from the milk of Canadian farms to form biofilms." Journal of Dairy Science **96**(1): 234-246.

Tuchscherr, L. P., et al. (2005). "Capsule-negative Staphylococcus aureus induces chronic experimental mastitis in mice." Infect Immun **73**(12): 7932-7937.

USDA. (2016). "Dairy 2014: Milk Quality, Milking Procedures and Mastitis in the United States, 2014. Accessed July 12, 2024. <https://www.aphis.usda.gov/sites/default/files/dairy14_dr_mastitis.pdf>."

Vanderhaeghen, W., et al. (2015). "Identification, typing, ecology and epidemiology of coagulase negative staphylococci associated with ruminants." Vet J **203**(1): 44-51.

Wuytack, A., et al. (2020a). "Distribution of non-aureus staphylococci from quarter milk, teat apices, and rectal feces of dairy cows, and their virulence potential." J Dairy Sci **103**(11): 10658-10675.

Wuytack, A., et al. (2020b). "Fecal non-aureus Staphylococci are a potential cause of bovine intramammary infection." Vet Res **51**(1): 32.

Zadoks, R. N., et al. (2003). "Clinical, epidemiological and molecular characteristics of Streptococcus uberis infections in dairy herds." Epidemiol Infect **130**(2): 335-349.