***Stuff to do before publishing***

Add in limitation about RAPD typing

Info about sequence quality etc. from SeqCoast?

Orthologue double check

Confirm etc and coa

Update notes

***Intro***

* Who is staph chromogenes?
* Evidence for variation in ST generally for NASM
  + should have stuff from grant narrative, SCC DIM paper
  + Naushad
    - Cluster with 2 grps of chromogenes
    - Interestingly, in the T-SNE plot (Figure 2),S. chromogenes is the only species split into 2 populations with respect to virulence genes, with a minority of the strains clustering with other members of the clade B (**FUTURE DIRECTION; WOULD BE INTERESTING TO IDENTIFY WHICH ST THESE AONES ARE BY MLST, then consider where they are phylogenetically compared to other trees made with MLST)**, while the majority of the S. chromogenes strains have a distinct profile. An important caveat is that more S. chromogenes isolates were included in this study than other species, but it is tempting to speculate that the larger population of S. chromogenes might represent a pathotype that has adapted to the udder.
  + Haveri et al 2005 Bacterial Genotype Affects the Manifestation andPersistence of BovineStaphylococcus aureusIntramammary Infection
    - Persistence = cows wererevisited 2 and 4 weeks posttreatment for follow-up samplingand clinical examination
    - Finland
    - IMI graded as sublicnical, mild clinical, or sever clinical
    - Two-hundred seventeenStaphylococcus aureusisolates from 116 dairy cows with intramammary infectionswere analyzed by pulsed-field gel electrophoresis to study the association between symptom severity, persis-tence of infection, and bacterial genotype. Among five main genotypes infecting 90% of the cows, one wasassociated with severe clinical symptoms but reduced persistence
  + Haveri 2007
    - To screen putative virulence genes in Staphylococcus aureus causing persistent and nonpersistent bovine intramammary infections (IMI) with different clinical characteristics. To examine, whether a possible relationship exists between genetic profile and infection persistence, clinical signs of infection, clonal type determined by pulsed-field gel electrophoresis (PFGE), and antimicrobial resistance
    - Our results suggest that certain genetic elements are over-representative in S. aureus isolates especially from persistent bovine mastitis. This phenomenon seems to be in connection with clonal type and is often concomitant with penicillin resistance
    - **Isolates from persistent mastitis typically harboured blaZ (P < 0Æ01**
    - **More common pulsotypes had genes encoding PTSAg VF**
    - **a common clonal type carrying a set of genes appeared to predominate in isolates from persistent bovine mastitis**
* Avall: **Definition of a virulence factor:** Virulence factors are seen as properties (i.e., gene products) that enable a microorganism to establish itself on or within a host of a particular species and enhance its potential to cause disease (Virulence Factor Database). Thus, any property of the microorganism which enhances its’ potential to survive within a host can be seen as a virulence factors.
* Various variation within one NAS species to show there is variation occurring at the intraspecies level
  + SCC
  + Persistency
  + Virulence
* Variation within chromogenes specifically
* Link between genotype and phenotype for any NAS?
* Link between genotype and phenotype for chromogenes specifically
* Various variation within one NAS species to show there is variation occurring at the intraspecies level … Intraspecies variation in pathogenicity (ability to cause disease) (Besides the marked differences between different species in the group of bacteria known as non-aureus staphylococci, significant variation also exists within strains of the same species)
  + SCC within a species
    - Fry
    - Condas, L.A.Z., et al., *Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds.* J Dairy Sci, 2017. **100**(7): p. 5592-5612.
    - supre
  + Persistency within a species
    - Mork, T., et al., *Persistence of staphylococcal species and genotypes in the bovine udder.* Vet Microbiol, 2012. **159**(1-2): p. 171-80.
    - Valckenier, D., et al., *Longitudinal study on the effects of intramammary infection with non-aureus staphylococci on udder health and milk production in dairy heifers.* J Dairy Sci, 2021. **104**(1): p. 899-914
  + Difference in interaction with host immune cells within a species
    - Hyvonen, P., et al., *Effect of bovine lactoferrin on the internalization of coagulase-negative staphylococci into bovine mammary epithelial cells under in-vitro conditions.* Journal of Dairy Research, 2009. **76**(2): p. 144-151.
    - Avall-Jaaskelainen, S., et al., *Bovine-associated CNS species resist phagocytosis differently.* Bmc Veterinary Research, 2013. **9**.
  + Effect on milk production
    - Thorberg, B.M., et al., *Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci.* Journal of Dairy Science, 2009. **92**(10): p. 4962-4970.
* Variation within chromogenes specifically (For *S. chromogenes* specifically, the link has been made between particular strain types and phenotypic differences in pathogenicity)
  + **SCC**
    - **Our study:** Quarters with an IMI due to S. chromogenes had an especially wide span of observed quarter SCC in the current study, ranging from 2,000 (the lower limit of detection) to 6,100,000 cells/mL.
    - Wuytack et al. (2020) found S. chromogenes to be the most prevalent NASM species causing IMI in quarters identified both as healthy (≤ 50,000 cells/mL) and infected, but with no observable clinical signs (> 50,000 cells/mL), as well as one of the three most common species in quarters exhibiting clinical signs of mastitis
    - Similarly, Condas et al. (2017b) found that in NASM-positive quarters, S. chromogenes was isolated with similar frequency from quarters classified as low-SCC (< 200,000 cells/mL), high SCC (> 200,000 cells/mL), and those with clinical mastitis.
  + Link between 2 phenotypic traits
    - Persistency and SCC
      * variability in the effect of S. chromogenes on quarter SCC was also noted in Valckenier et al. (2021), where quarters classified as having a transient IMI due to S. chromogenes had a mean SCC of 69,000 cells/mL, while those classified as having a persistent S. chromogenes IMI had a SCC of 351,000 cells/mL
  + interaction with host immune cells and inflammatory response
    - Breyne, K., et al., *Technical note: a pilot study using a mouse mastitis model to study differences between bovine associated coagulase-negative staphylococci.* J Dairy Sci, 2015. **98**(2): p. 1090-100.
    - Piccart, K., et al., *Local host response following an intramammary challenge with Staphylococcus fleurettii and different strains of Staphylococcus chromogenes in dairy heifers.* Veterinary Research, 2016. **47**
    - Souza, F.N., et al., *Interaction between bovine-associated coagulase-negative staphylococci species and strains and bovine mammary epithelial cells reflects differences in ecology and epidemiological behavior.* Journal of Dairy Science, 2016. **99**(4): p. 2867-2874.
      * **CHRONIC IMI isolate… like mine …** S. chromogenes isolated from a chronic IMI had greater ability to adhere to bovine mammary epithelial cells compared to a strain isolated from the teat apex
    - Hyvonen, P., et al., *Effect of bovine lactoferrin on the internalization of coagulase-negative staphylococci into bovine mammary epithelial cells under in-vitro conditions.* Journal of Dairy Research, 2009. **76**(2): p. 144-151.
      * (internalization varied among staphylococcal strains); the effect of bovine lactoferrin (bLf) on the internalization were studied in vitro in a bovine mammary epithelial (BME) cell model; 22 strains CNS of 5 species
  + Habitat niche
    - Wuytack, A., et al., *Fecal non-aureus Staphylococci are a potential cause of bovine intramammary infection.* Vet Res, 2020. **51**(1): p. 32.
    - Jenkins, S.N., et al., *Molecular epidemiology of coagulase-negative Staphylococcus species isolated at different lactation stages from dairy cattle in the United States.* Peerj, 2019. **7**.
* Link between genotype and phenotype for any staph?
  + Haveri et al 2005 Bacterial Genotype Affects the Manifestation andPersistence of BovineStaphylococcus aureusIntramammary Infection
    - Persistence = cows wererevisited 2 and 4 weeks posttreatment for follow-up samplingand clinical examination
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  + Haveri 2007
    - To screen putative virulence genes in Staphylococcus aureus causing persistent and nonpersistent bovine intramammary infections (IMI) with different clinical characteristics. To examine, whether a possible relationship exists between genetic profile and infection persistence, clinical signs of infection, clonal type determined by pulsed-field gel electrophoresis (PFGE), and antimicrobial resistance
    - Our results suggest that certain genetic elements are over-representative in S. aureus isolates especially from persistent bovine mastitis. This phenomenon seems to be in connection with clonal type and is often concomitant with penicillin resistance
    - **Isolates from persistent mastitis typically harboured blaZ (P < 0Æ01**
    - **More common pulsotypes had genes encoding PTSAg VF**
    - **a common clonal type carrying a set of genes appeared to predominate in isolates from persistent bovine mastitis**
* Link between genotype and phenotype for chromogenes specifically
  + The next logical step in understanding the diversity within *S. chromogenes* is to identify the **genetic basis for difference in strain pathogenicity**.
  + Naushad
    - Cluster with 2 grps of chromogenes
    - Interestingly, in the T-SNE plot (Figure 2),S. chromogenes is the only species split into 2 populations with respect to virulence genes, with a minority of the strains clustering with other members of the clade B (**FUTURE DIRECTION; WOULD BE INTERESTING TO IDENTIFY WHICH ST THESE AONES ARE BY MLST, then consider where they are phylogenetically compared to other trees made with MLST)**, while the majority of the S. chromogenes strains have a distinct profile. An important caveat is that more S. chromogenes isolates were included in this study than other species, but it is tempting to speculate that the larger population of S. chromogenes might represent a pathotype that has adapted to the udder.
  + Wuytack et. al (distribution) found genes encoding various virulence factors associated with staphylococci in 44% of NAS isolates originating from cases of more severe mastitis, while only 19% of isolates associated with infections causing a less-severe inflammatory response. These virulence factors were genes associated with biofilm formation to enhance colonization and evasion of host immune response, enzymes associated with other virulence proteins, and capsule formation protecting the bacteria from being killed by host cells
  + As *S. chromogenes* was the most common type of non-*aureus* staphylococcicausing intramammary infections in our longitudinal study, I have a large population of isolates to choose from and an associated measure of host immune response (quarter somatic cell count) to further explore the genetic basis for differential virulence of the most common pathogen causing IMI in dairy cows, *S. chromogenes* S. chromogenes was the most frequently identified SaM associated with subclinical IMI on 10 organic dairy herds in Vermont.

* Avall: **Definition of a virulence factor:** Virulence factors are seen as properties (i.e., gene products) that enable a microorganism to establish itself on or within a host of a particular species and enhance its potential to cause disease (Virulence Factor Database). Thus, any property of the microorganism which enhances its’ potential to survive within a host can be seen as a virulence factors.

***Discussion***

* ST diversity
  + Compare to what PW found as far as number of ST and diversity
  + Talk about how my isolates clustered with publicly available ones?
  + Papers about geographic diversity of chrom ST (Huebner, Roberts paper?)
  + **Huebner**
    - PW sasys saw “same core ST” in ST6 and ST1 with each of those having a cluster of SLV
    - Majority of isolates belonged to ST1 -- I have ST1 cluster – 174, 175, 176; these were novel allelles for MLST database, which may support findings of Huebner and PW that there is a clonal expansion of ST1 happening
    - 120 isolates, saw 46 ST
    - Huebner saw big difference in ST between Belgium and US
    - Saw ST1 in All three locations (Belgium, VT, Washington state)
    - VT: St1 had 11, ST5 had 7, st15 had 6, St10-11-13 all had 4 each; only 1 st6… others I didn’t see st2, st3, st7, st18-19-20-21-22-23 with 1 or 2 isolates
    - The geographic distribution of strain types indicated a high degree of genetic isolation between locales
  + **PW 2024;**
    - 105 chrom from 105 cows in 77 herds throughout Sweden;
    - 47 ST were identified; 45 or 43% of isolates belonged to 33 new ST (still very active area identifyinf strain divserity of chromogenes)
    - ST6, ST109 were most prevalent, followed by ST1 and ST19, ST102, ST59, ST103, ST127
* RAPD type diversity
  + Can I say that the herd pattern may suggest contagious manner of spread for some? Very thin ice… vs. purely environmental source
    - Zadoks PFGE images
    - Coryne paper: To determine whether bacteria in different RAPD types (classified on the basis of RAPD PCR assay) had consistent genetic changes that allowed them to be grouped by sequence of the 16S rRNA gene, PCR assay was performed for the 16S rRNA gene
  + PW
  + herds. The fact that the same genotypes of S. chromogenes were sometimes found in more than one cow in the same herd indicates that spread within-herd had occurred either between cows (e.g., at milking, or from the same source in the environment to the cows)
* ***How did results support initial hypothesis? (ST predicts SCC category)***
  + Did NOT find that ST was not a predictor of which SCC category would fall into
  + Compare to PW paper
    - Tested their 105 isolates for associations between cluster and phenotypic traits
      * Did not find any particular ST or cluster which was more associated with persistent IMI (BUT this persistent IMI is like, 4 days)
      * One cluster (VII) had a significant association with high CMT score
* AMR
  + Descriptive - blaZ in staph from bovine isolates
    - Frequency of blaZ carriage compared to other studies (lit review)
  + Whatever else nobrega found for chrom
  + ***How did results support initial hypothesis? (AMR)***
    - Did NOT find that AMR (or blaZ presence) was a predictor of being in high SCC cat
    - Different than Belgian paper, where high scc group had more blaZ
    - PW
      * 3 out of 8 isolates in cluster III were linked with persistent infection, of which 2 were collected from the same farm. Interestingly, all these isolates, belonging to ST-102 and ST-103, were also resistant to penicillin as evidenced by detection of the *blaZ* gene and β-lactamase production
      * BUT found that cluster IV all of them also had blaZ but were not persistent “indicating other factors may also be of importance”
* Avall
  + s. Haveri et al. (2005) and Haveri et al. (2007) also found that while the most prevalent pulsotypes were associated with certain types of mastitis (symptoms, persistence, and response to the antimicrobial treatment) (Haveri et al., 2005),
    - go back and read this- might go in ST/SCC category section
* Virulence
  + Descriptive information about what genes were found, compare to mine
    - Range of num of vir genes
      * Avall: Range of vir genes: 37-49 for 8 chrom isolates
      * PW: Found 57 unique pVF among their 105 isolates; their chromogenes on avg contained 30 (SD 5.4, range 25-45)
      * Naushad:
        + Canadian; Naushad et al. (2019) investigated the profile of 191 virulence factors in NAS, for 25 different species, 441 isolates, 83 chromogenes, based on whole-genome sequencing
        + All isolates of each NAS species contained on average 30 or more VF genes, with thehighest virulence potential (defined by total number of VFs), assigned to SAG, SHY, andSCH (clade B), largely due to exotoxins, host evasion and capsular gene
      * Bap
        + Previously,bapwas described as a cattle-specificpathogenic factor of biofilm formation
        + Bap was not found in any chrom in Naushad!
        + Avall: 1/8 had it
        + Most of mine have it, One ST176 and both St25 missing bap
        + PW: 13/105
        + Naushad: none had it
        + Wuytack : 0/25 chrom had bap
      * Coa (staphylocoagulase)
        + Coa wasn’t found in any of Naushad (in any NAS) but ID’d in mine in 1 isolate
        + Avall 2018 found it in s agentis (but not chrom)
        + PW did not report coa
      * β-hemolysin (hlb)
        + Naushad:

was the most frequent and predominant gene detected inS.chromogenesisolates and other species of clade B (7), also for 8 chrom in Avall 2018

* + - * + Yup all mine have it
        + Avall:

also for 8 chrom in Avall 2018

* + - * Exfoliative toxin c
        + Not in any isolates in Naushad
        + Not in any chrom in avall
        + present in 100% of mine
      * Adenosine synthase A
        + Adenosine synthase A is an immuneevasion factor forS. aureusresponsible for increasing theoverall abundance of extracellular adenosine, which may bethe most potent immuno-suppressive signaling molecule. Thisfactor is necessary for staphylococcal survival within neutrophils,allowingS. aureusto escape bactericidal activity of leukocytes andother host immune responses
        + 99% of Naushad chrom have it
        + Yep all mine have it
      * Phenol soluble modulins
        + similarly, most NAS species containedβ-type phenol-soluble modulins (PSMs), which have been considered majordeterminants ofS. aureusvirulence (7). Phenol-soluble modulinshave multiple roles in staphylococcal pathogenesis, causing lysisin red and white blood cells, contributing to biofilm developmentand stimulation of inflammatory responses
        + chrom in Naushad only had psmb4, but 100% of them had it
        + Pw: only report PSMb4, but almost all have it
        + yup all mine had them (beta) psmb1-6
      * Set7 staphylococcal exotoxin 7
        + All 8 Avall had it
        + none of mine did
        + no chrom in Naushad had it
      * Cap genes –
        + Naushad: In our study, 12 capsular genes (capAtocapL), in agreement with the resultsof a recent study (31), were present in very low frequencies (7 to 11%) among SCHisolates, the most common species of NAS in IMI worldwide. The absence of capsulargenes may explain the persistence of SCH in Canadian IMI
        + Avall: only 1 isolate had any cap genes (of 8), had h I j k
        + All 30 of mine had at least 3 cap genes (28 all had capn, capo, capp- in two isolates of st25 they were missing capN but had capJ and capH (these 2 isolates had 4 cap genes)
        + Wuyack: Only 2 isolates/59, both chrom in clinical, had cap5H (were looking ONLY for cap5h, pcr)
      * Scn
        + Staphylococcal complement inhibitor (scnproduct) thought to be highly specific for staphylococcal isolates ofhuman origin;
        + Naushad: but scnwas detected in species of clade B(SAG, SHY, and SCH),
        + which is in contrast with the results of a recent study (Avall) who didnot detectscnin any NAS isolates
        + all mine had it
  + ***How did results support initial hypothesis? (VIR)*** (association between phenotypic traits and possessing VIR genes)
    - I did NOT find that # vir genes was a predictor of being in high SCC cat
    - Other studies that found **NO DIFFERENCE**:
    - **Avall 2018**
      * By visual inspection it was impossible to find any association between the type of mastitis and specific virulence genes, virulence gene profiles, or cumulative number of virulence genes (Table S3). Logistic regression analyses of the pooled NAS data did not either yield statistically significant (p < 0.5) effects of any virulence genes or groups of genes on the type of mastitis
      * No association between virulence gene profiles and the type of mastitis were found. Most of the isolates had unique virulence gene profiles. When two isolates in one species shared an identical profile, as within S. agnetis, S. chromogenes and S. simulans, one of the isolates was clinical while the other was subclinical.
      * virulence gene profile or accumulation of virulence genes did not predict the type of mastitis (SCM or CM) or the severity of inflammation
      * The type of mastitis was not associated with any specific virulence gene profile. It seems that the virulence gene profiles or cumulative number of different virulence genes are not directly associated with the type of mastitis (clinical or subclinical), indicating that host derived factors such as the immune status play a pivotal role in the manifestation of mastitis.
      * In this study, we 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. [Haveri et al. (2005)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5880176/#ref-28) and [Haveri et al. (2007)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5880176/#ref-27) also found that while the most prevalent pulsotypes were associated with certain types of mastitis (symptoms, persistence, and response to the antimicrobial treatment) ([Haveri et al., 2005](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5880176/#ref-28)), such association was not found with any single virulence gene or gene group (e.g., hemolysin genes) or the cumulative number of virulence genes ([Haveri et al., 2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5880176/#ref-27)). 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.
    - **Tremblay 2013**
      * 255 isolates from Canada; no association between biofilm and SCC was observed; CNS generally (didn’t say they analyzed at species level)
      * The reason for such selection is unclear, but it is likely that biofilms increase the ability of CNS to persist in the intramammary environment. This indirectly indicates a relationship between biofilm formation and persistent IMI
    - **Simojoki 2012**
      * No association was found between the phenotypic ability to form biofilm and the persistence of IMI (63 persistent, 55 transient; 3-4 weeks, strain-typed) or severity of mastitis (for 114 isolates; as measured by measured with milk N-acetyl-b-D-glucosaminidase (NAGase) activity; an enzyme which reflects tissue damage and is an indicator of inflammation in the udder)
      * except a tendency for milder inflammation in IMIs caused by isolates with more intense biofilm production in TCP; Isolates with more intense biofilm formation in TCP tended to cause milder inflammation measured with milk NAGase activity (coeff. 0.09, p = 0.07)
      * CNS in Finland
    - **Studies that DID find association between vir gene number and a clinical quality**
    - **Naushad**
      * Relationships between the VFs from five categories were investigated using an association plot – then, did this analysis of patterns for each category of SCC and CM separately and compared – found there were *distinctive patterns of associations for low sCC and clinical isolates –*
        + Associations were also computed for low, medium, and high somatic cell count (SCC) and clinical mastitis (CM) isolates, demonstrating distinctive patterns of associations for low SCC and CM isolates, but no differences between high SCC and CM isolates … here were many distinct positive and negative association patterns in CM isolates (in presence or absence of certain genes)
        + To determine **whether VF distributions had any association with SCC or CM**, various clustering approaches, including complete linkages, Ward clustering, and t-distributed stochastic neighbor embedding, were applied. However, **no clustering of isolates representing low SCC, medium SCC, or high SCC or CM was identified**
      * Although the overall number of VFs was not associated with disease severity, increasing numbers of toxin and host immune evasion genes specifically were associated with more severe disease outcomes. 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
        + **Linear regression: overall, increasing number of VF did not cause increase in logSCC; overall with all vir factors together**

**stratified by vir factor type, separate analysis for each**

**increase in toxin number increased scc**

* + - * + **Did logistic regression, ordinal:**

**Stratified by type of virulence factor**

**Outcomes were ordinal, low-med-high-clinical**

**Increasing num of vir factors (in that category) was significant predictor of being in next highest category of inflammation**

**Each additional vir gene for host immune evasion made it more likely to be associated with a higher category of host response**

* + - * + Regression analysis to test for associations with individual VF functional categories demonstrated that each additional toxin and **host immune evasion gene** increased the odds of having high SCC or CM, although an overall increase in the number of VFs was not associated with increased SCC or occurrence of CM

**Overall, an increase in the number of putative VFs was not associated with an increase in log SCC**; but then stratified by type of VFs, the presence of each additional toxin gene was associated with a 0.024 increase in log SCC (P0.006). None of the other VF types were associated with changes in log SCC

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 (P0.003) (having one more host immune evasion gene made the isolate 1.07 times more likely to cause a more severe inflammation [i.e., increased SCC and/or CM]). Other types of VFs, however, were not associated with an increased risk of having a more severe immune response

* + - **PW**
      * They found higher number of exoenzyme genes for samples with low CMT vs high
      * Low CMT quarters had higher number of evasion genes than those with high CMT
        + Specifically found that presence of geh sig associated with increased odds of having low CMT
        + All mine had geh
        + Cap j and caph associated significantly with lower CMT; for mine, the only ones WITH these genes were 2 in the HIGH SCC group
    - 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.
* Mine looks like both vir and amr more factor of phylogeny
  + **AMR** - PW paper – association between genetic grouping and AMR carriage (not association between phenotypic trait/high SCC and AMR carriage)
    - Tested their 105 isolates for associations between cluster and AMR traits
      * All isolates of their ST19, St102, ST103 carried blaZ; particular clusters were more likely to carry gene; clusters III and IV vs other clusters
      * that *blaZ* was found in all *S. chromogenes* isolates belonging to ST-19, ST-102, and ST-103, distributed over different farms and counties, which indicate that spread of resistance is at least partly due to spread of certain lineages rather than horizontal transfer of genes encoding resistance between strains or species

*Prose from my literature review*

* Even within a given species, AMR carriage has been linked to certain strain types. For *S. aureus*, carriage of methicillin resistance has been associated with particular clonal complexes both in human medicine (Smith et al., 2021; Garrine et al., 2023) and certain clusters of *spa* ­type for bovine clinical mastitis isolates (Freu et al., 2022). The linkage between strain type and AMR is not as well studied for NASM, but Persson Waller et al. (2023) found that *blaZ* was significantly more common among *S. chromogenes* strains belonging to 2 specific clusters of strain types vs. strains belonging to other clusters.
  + Future directions: where is blaZ carried?
    - blaZ – might make sense from my findings that that ST seemed to determine carriage IF it was chromosomally carried
      * + future direction: where is blaZ carried in these chromogenes isolates
        + In 26 out of 34 Finnish isolates (76.5%) and in 25 out of 44 Swedish isolates (56.8%) the *blaZ* gene was localized on a plasmid. Six different protein signatures were found.
        + why penicillin-resistance is clearly more common in Finland than in the neighbouring Nordic countries with similar conditions for milk production. One explanation could be the more common plasmid location of the *blaZ* gene and plasmid-mediated spread of penicillin-resistance.
        + Genetic basis of penicillin resistance of S. aureus isolated in bovine mastitis; Arzu Funda Bagcigil,1,2 Suvi Taponen,corresponding author1 Joanna Koort,3 Björn Bengtsson,4 Anna-Liisa Myllyniemi,5 and Satu Pyörälä1
      * ***from bagcigil paper:***
        + Resistance to benzylpenicillin is mainly caused by the *blaZ* gene encoding production of beta-lactamases, which hydrolytically destroy beta-lactams [[13](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B13)]. The *blaZ* gene can be located chromosomally or on plasmids [[14](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B14)]. This type of penicillin resistance in *S. aureus* may thus emerge via two mechanisms: spread of resistant clones or through horizontal dissemination of mobile elements containing the *bla*Z gene [[15](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B15),[16](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B16)]. Location of the resistance determinants on transferable elements generally promotes efficient spread [[16](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B16)]. In Denmark the *blaZ* gene of penicillin resistant *S. aureus* isolates has been predominately located chromosomally [[17](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B17)]…. ***Possession of the blaZ gene was partly linked to pulsotype, which may indicate a clonal spread of resistance.***
        + Certain genotypes of mastitis causing *S. aureus* can become dominant in the dairy herds [[25](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B25)]. In the three most common pulsotypes here *bla*Z-negative isolates were over-represented, indicating that penicillin-resistance was partly related to pulsotype. An association between certain pulsotypes and penicillin susceptibility has also been shown previously [[32](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B32),[33](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B33)]. Penicillin-resistance may be linked to other virulence factors of *S. aureus*, which may facilitate the spread of resistant clones [[33](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B33)]. Intramammary infection remained significantly more often chronic if it was caused by *bla*Z positive (61.0% remained persistent) than *blaZ* negative (25.0%) strains [[34](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575348/#B34)].
* Mine looks like both vir and amr more factor of phylogeny
  + **VIR** – PW paper (association between genetic groupings and virulence gene carriage)
    - Numbers of pFV did not differ significantly between 5 most prevalent ST, BUT pVF lower for isolates in III, IV, and VII vs all other isolates
    - Were 2 singleton ST which were quite distant from others which had higher number of VF (ST59 44.3, ST127 42.7)
    - St59 had higher number of adherence genes vs other ST
    - Cluster IV had significantly more exoenzyme genes vs other clusters
    - Atl seemed to follow pattern by cluster; present in V, VI, VII but absent in II III, and IV
      * Atl adherence is present in all 30 of mine
  + Naushad
    - To investigate whether any unique pattern of the presence and absence of VF could predict disease outcome, a decision tree was generated. Although this revealed many unique patterns of VF distributions, none of these patterns were clearly associated with the level of host immune responses (low SCC, medium SCC, high SCC, and CM) … most of the clustering in these dendrograms was according to species. … isolates from the same species, regardless of their isolation stage (low, medium, or high SCC or CM) were grouped together. Similarly, based on distribution patterns (generated using t-SNE algorithms), most NAS isolates clustered according to their respective species
* Limitations
  + Limited number of isolates due to financial constraints; would be nicer to have more power to compare carriage of VF or AMR between ST (given that there *was* differences in ST, with larger numbers or more diversity)
  + Gene identification is not = to gene expression
  + Using SA genes: Identification of VFs basedon genetic similarity could be problematic for two reasons. First, we assumed a highlevel of sequence conservation signified conserved function. Identification and predic-tion of functions of NAS VFs were extrapolated from well-characterized analogues inSAU or from NAS of human origin. However, SAU VF genes may have niche-adaptedfunctions, impacting their roles in virulence.
  + As previously mentione… the presence/absence of virulence-associated genes maynot directly correlate with pathogenesis and severity of disease, since in addition toexpression of these genes, host environment and host genetics also have major rolesin disease development and progression
  + Avall: Therefore, the presence of a single virulence factor rarely determines the microorganisms’ capacity to act as a pathogen. In addition, also the expression level of the virulence factors may have an influence on the outcome of the disease (Le Maréchal et al., 2011) The line between virulence factors and eg. proteins involved in normal metabolism is not as clear as thought earlier.
* General notes from Naushad
  + **Cap5 vs. cap8 issue; capgenes were considered present if hits were detected foreither isoformcap5orcap8**
* General notes from de buck (citations all fucked up)
  + Interestingly, in the T-SNE plot (Figure 2),S. chromogenes is the only species split into 2 populations with respect to virulence genes, with a minority of the strains clustering with other members of the clade B (**FUTURE DIRECTION; WOULD BE INTERESTING TO IDENTIFY WHICH ST THESE AONES ARE BY MLST, then consider where they are phylogenetically compared to other trees made with MLST)**, while the majority of the S. chromogenes strains have a distinct profile. An important caveat is that more S. chromogenes isolates were included in this study than other species, but it is tempting to speculate that the larger population of S. chromogenes might represent a pathotype that has adapted to the udder.
  + No clear difference was present between the two S. chromogenes populations with respect to severity of mastitis(Figure 2B).
  + **Need further gene expression studies to resolve which are associated** with disease severity bc he merepresence of genes does not guarantee their expression =ref 120 in naushad)
  + In someS. chromogenesisolates capsular genes from thelarger VF-based cluster are missing (7), which seems to beone factor that causes the population split in this species -- InS. aureus,expression of these genes results in formation of apolysaccharide capsule that helps resist phagocytic cell uptake,thus playing a role in evasion of the host immune response
  + **Future directions – compare persistent to NOT persistent infections and see what differences there are in biofilms … all mine are persistent!** NT strains are non-reactivewith antibodies to CP types 1, 2, 5, or 8 (87), and these isolatesfrom chronically infected hosts were shown to have conservedtheir acapsulated phenotype over successive passages on artificialmedia without reverting back to encapsulation (87). Isolates fromcows with SCM revealed that the proportion of non-typeable(NT)S. aureusstrains was 86% (88). These findings reveal thatability to persist in chronic infections is strongly associated withNT strains (i.e., acapsulated pathogens). With a majority ofS.chromogenesisolates lacking capsule genes, it may be of furtherinterest to study the relationship between acapsulation andthepersistence ofS. chromogenesin IMI.
  + **Future directions -- Perhaps elucidating their role inpathogenesis ofS. chromogenesin future studies may explainits dominance in bovine mastitis and persistence in the udder – from de buck.** We know what role these virulence factors play for s aureus and are assuming they are the same… maybe they are and maybe they aren’t?
  + A correlation was observed between the average SCC of milksamples from which specific NAS species were isolated and thenumber of exoenzyme, host evasion and iron uptake genes thesespecies carried (7,9). These virulence genes might hold the key towhy certain NAS species provoke somewhat more inflammationthan others. Absence of these virulence genes may result in NASspecies becoming more host adapted or even commensal. Thisis somewhat illustrated byS. chromogenes, which is considereda host-adapted NAS, and has moderate numbers of exoenzyme,host evasion and iron uptake genes. Furthermore, interestingassociations were found between virulence genes identifiedin NAS, with striking differences in the strength of theseassociations between isolates that caused low SCC and CMisolates (7)
  + Virulence potential function of phylogeny at the species and clade level, as determined in Naushad et al 2019 (species clustered together distinctly -- t-Distributed Stochastic Neighbor Embedding(T-SNE), a method to visualize high-dimensional datasets,demonstrated that all species studied can be defined as separateand homogenous bacteria (7) because of clear clustering byspecies)

**------ notes from adding section about biofilms 9.26.2024**

Biofilm formation has been proposed as an important virulence factor by which staphylococci causing intramammary infections evade the cow's immune response, leading to persistent intramammary infections (NAS: Simojoki et al., 2012 Tremblay et al., 2013; *S. aureus:* Keefe, 2012). Biofilms are a structured surface-associated community of bacterial cells, and are characterized by an extracellular polysaccharide matrix (Tolker-Nielsen, 2015). Biofilms protect bacteria from the host immune defense by impairing phagocytosis and antimicrobial peptides (Costerton et al., 1999), as well as potentially protect bacteria from antimicrobial therapy (Fey and Olson, 2010).

Although biofilm formation is influenced by multiple factors, it is strongly related to the production the surface protein *bap,* or biofilm-associated protein (Otto, 2013). *bap* was first described in bovine mastitis strains from Spanish dairy herds (Cucarella et al., 2001), and has been shown to confer a strong biofilm-forming phenotype (Lasa and Penadés, 2006).

Some work has suggested that *S. aureus* positive for *bap* were more able to persist inside the mammary gland, and that *bap*-mediated biofilms demonstrated higher resistance to antimicrobials (Cucarella et al. 2002 and 2004). However, other work has found no significant difference in antimicrobial susceptibility between biofilm and non-biofilm-producing *Staph. aureus* mastitis isolates (Fidelis et al., 2024).

Zuniga et al. (2015) found that staphylococcal isolates positive for a group of adhesin genes (*eno, fnbA,* and *fib*) and *bap* originated from quarters with higher somatic cell counts when compared to isolates from quarters which were negative for these genes, as well as culture-negative quarters. They suggest that the presence of these genes may be related to a higher intensity of the inflammatory process (Zuniga et al., 2015).

In NAS, it has been suggested that biofilms increase the ability of NAS to persist in the mammary environment (Tremblay et al., 2013; Piessens et al, 2012; Zuniga et al., 2015). However, other work suggests that biofilm formation by NAS seems not to be substantial factors for the persistence of infection or a stronger inflammatory reaction (Simojoki et al., 2012). As the relationship between pathogenicity and biofilm formation is not clear for NAS causing mastitis, additional in vivo testing is needed (De Buck et al., 2021). As reviewed by Pederson et al. (2021), in vitro observations of biofilm formation are not easily comparable with what happens in vivo within the bovine mammary gland.

it has been suggested that biofilms increase the ability ofNAS to persist in the mammary environment (79,80). Tremblay, piessens

(Pedersen et al., 2021)

**Pederson review:**

However, in vitro observations of biofilm formation are not easily comparable with the in vivo situation inside the udder. The biofilm acts as a physical barrier, impeding direct contact between antimicrobials and microorganisms, a condition not mimicked during our in vitro testing.

(Zuniga et al., 2015)

**Zuniga:** The higher frequency of occurrence (P < 0.005) of the bap gene in CNS compared with CPS suggests that in these species biofilm formation is an important mechanism for the persistence of the infection. The medians of the SCCs in the samples where eno, fnbA, fib and bap genes were detected were higher compared with Staphylococcus without the assessed genes (P < 0.05) and negative samples (P < 0.01), which indicated that the presence of these MSCRAMM may be related to a higher intensity of the inflammatory process.

**fidelis:** our study did not find a significant difference in antimicrobial susceptibility between biofilm and non-biofilm-producing *Staph. aureus* isolates.

**Francisco:** Even though bap and icaA are widespread among staphylococci, we found no significant correlation between genotype (the presence of these genes) and phenotype (biofilm production). This lack of correlation has been reported before for CoNS causing mastitis in dairy animals (Coimbra-e-Souza et al., 2019), and indicates that other factors must be involved in biofilm formation by non-aureus staphylococcal species

**Simojoki:** We conclude that in CNS species commonly isolated in mastitis, biofilm and slime formation seem not to be substantial factors for the persistence of infection or a stronger inflammatory reaction.

**Tremblay:** a solid statistical model cannot be established to test whether an association exists between biofilm formation and persistent IMI. However, our statistical analysis did reveal a positive relationship between DIM and biofilm strength. This suggests that strong biofilm formers are selected for during lactation. The reason for such selection is unclear, but it is likely that biofilms increase the ability of CNS to persist in the intramammary environment. This indirectly indicates a relationship between biofilm formation and persistent IMI.

**De buck:** Additionalin vivotesting is needed to better characterize theassociations between pathogenicity and biofilm production inS. chromogenes.

it has been suggested that biofilms increase the ability ofNAS to persist in the mammary environment (79,80). Tremblay, piessens

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**Snell et al. (2015) Snell GGM, Monecke S, Ehricht R, Piccinini R. Molecular characteristics of bap-positive *Staphylococcus aureus* strains from dairy cow mastitis. *Journal of Dairy Research.*2015;82:312–316.**

The biofilm-associated protein (Bap) ofStaphylococcusaureusis a high-molecular weight cell-wall-anchoredprotein of 2276 amino acids and was first described inbovine mastitis strains from Spanish dairy herds (Cucarellaet al.2001). Bap was shown to be involved in intercellularadhesion and accumulation in multilayer cell clusters, andalso in primary attachment to abiotic surfaces. These func-tional characteristics confer a strong biofilm-forming pheno-type to strains carrying thebapgene (Lasa & Penadés,2006

, in the experimental infection,the samebap-positive strain was more able to persistinside the mammary gland (Cucarella et al.2002). Also,bap-mediated biofilm demonstrated higher resistance toantimicrobials (Cucarella et al.2004

(Keefe, 2012)

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Although the presence of genes associated with forming biofilms in both S. aureus and NAS has been previously described, the exact pathogenesis of biofilm formation by NAS in vivo is still not yet fully understood. Genotype and phenotype have a poor correlation.

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**Fidelis:**

Biofilm, which is characterized by an extracellular polysaccharide matrix, serves as a protective layer for microorganisms, allowing their proliferation within and subsequent release into the environment [[11](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11053524/#B11-vetsci-11-00170)] While primarily composed of polysaccharides, proteins, nucleic acids, and lipids, the structure and composition of biofilms exhibit significant variability [[12](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11053524/#B12-vetsci-11-00170)].  our study did not find a significant difference in antimicrobial susceptibility between biofilm and non-biofilm-producing *Staph. aureus* isolates.

The biofilm acts as a physical barrier, impeding direct contact between antimicrobials and microorganisms, a condition not mimicked during our in vitro testing. The lack of association between biofilm production and observed antimicrobial resistance might be explained by the absence of environmental conditions during our in vitro antimicrobial susceptibility tests.

**Pederson review:**

However, in vitro observations of biofilm formation are not easily comparable with the in vivo situation inside the udder. Only two papers investigate the location and distribution of bacterial biofilms inside udders of dairy cows with mastitis. Based on the current knowledge, the role of biofilm in bovine mastitis is still unclear and more in vivo investigations are needed to uncover the actual role of biofilm formation in the pathogenesis of bovine mastitis.

Hence to find the actual role of biofilm in bovine mastitis, the approach needs to change from *in vitro* to *in vivo* investigations of biofilms in infected udders.

**Fidelis CE, Orsi AM, Freu G, Gonçalves JL, Santos MVD. Biofilm Formation and Antimicrobial Resistance of *Staphylococcus aureus* and *Streptococcus uberis* Isolates from Bovine Mastitis. Vet Sci. 2024 Apr 10;11(4):170. doi: 10.3390/vetsci11040170. PMID: 38668437; PMCID: PMC11053524.**

*Staphylococcus* (*Staph.*) *aureus* and *Streptococcus* (*Strep.*) *uberis* are key causes of intra-mammary infection in dairy cows, and their ability to form biofilms is recognized as a significant virulence factor influencing mastitis pathogenesis and the response to antimicrobial treatment. This study aimed to evaluate (a) the biofilm producer ability and antimicrobial resistance of *Staph. aureus* (*n* = 197) and *Strep. uberis* (*n* = 119) isolated from cows with clinical and subclinical mastitis, and (b) the association between biofilm formation and antimicrobial resistance. Both *Staph. aureus* and *Strep. uberis* exhibited high biofilm formation ability. However, no correlation was found between the form of mastitis presentation (clinical or subclinical) and the biofilm-forming capacity. Moreover, a significant proportion of *Staph. aureus* and *Strep. uberis* isolates demonstrated resistance to penicillin, ampicillin, and tetracycline. Interestingly, we observed no association between biofilm formation ability and antimicrobial resistance.

**Franciso 2021:**

As noted here, most Staphylococcus involved with subclinical mastitis can form biofilms. While biofilm formation is known to be a multifactorial feature, it is strongly related to the production of two of the most studied factors: (i) the surface protein Bap (biofilm-associated protein) and (ii) the products of the ica operon, which coordinate the synthesis and export of the most important adhesive molecule of staphylococcal biofilms, the (exo)polysaccharide intercellular adhesion (PIA), also called poly-N-acetylglucosamine or PNAG (Otto 2013; Salina et al., 2020). Even though bap and icaA are widespread among staphylococci, we found no significant correlation between genotype (the presence of these genes) and phenotype (biofilm production). This lack of correlation has been reported before for CoNS causing mastitis in dairy animals (Coimbra-e-Souza et al., 2019), and indicates that other factors must be involved in biofilm formation by non-aureus staphylococcal species

***Simojoki:***

Biofilm formation is suspected to be one means by which mastitis-causing staphylococci avoid the immune response of the cow and cause persistent IMI. (Simojoki et al., 2012)

Biofilms protect bacteria from the host immune defence. They impair phagocytosis and the function of antimicrobial peptides ([Costerton et al., 1999](https://www.sciencedirect.com/science/article/pii/S0378113512001411?fr=RR-2&ref=pdf_download&rr=8b00554dcda19059" \l "bib0020)). Biofilms also protect bacteria from antimicrobial therapy ([Fey and Olson, 2010](https://www.sciencedirect.com/science/article/pii/S0378113512001411?fr=RR-2&ref=pdf_download&rr=8b00554dcda19059" \l "bib0030)).

The aim of the present study was to investigate the biofilm and slime formation of CNS isolated in mastitis, the presence of nine adhesion genes, the genes *bap*, *icaA* and *icaD*, and the association between these characteristics and the persistence of IMI or severity of inflammation.

Numerous orthologues for genes encoding different microbial surface components recognizing adhesive matrix molecules (MSCRAMM)

*bap* (encoding biofilm associated protein) ([Baba et al., 2008](https://www.sciencedirect.com/science/article/pii/S0378113512001411?fr=RR-2&ref=pdf_download&rr=8b00554dcda19059" \l "bib0010), [Tormo et al., 2005](https://www.sciencedirect.com/science/article/pii/S0378113512001411?fr=RR-2&ref=pdf_download&rr=8b00554dcda19059" \l "bib0155))

In previous studies, biofilm production has been connected with nosocomial infections in humans, especially persistent infections with devices ([Fey and Olson, 2010](https://www.sciencedirect.com/science/article/pii/S0378113512001411?fr=RR-2&ref=pdf_download&rr=8b00554dcda19059#bib0030)). Based on our results, biofilm production is not associated with the persistence of bovine IMI caused by CNS

We conclude that in CNS species commonly isolated in mastitis, biofilm and slime formation seem not to be substantial factors for the persistence of infection or a stronger inflammatory reaction.

***Avall:***

Only a few studies concentrated on NAS, and these studies have mainly focused on detection of genes associated with biofilm formation

Piessens et al. (2012) Piessens V, De Vliegher S, Verbist B, Braem G, Van Nuffel A, De Vuyst L, Heyndrickx M, Van Coillie E. Characterization of coagulase-negative staphylococcus species from cows’ milk and environment based on *bap*, *ica*A, and *mec*A genes and phenotypic susceptibility to antimicrobials and teat dips. *Journal of Dairy Science.*2012;95:7027–7038. doi: 10.3168/jds.2012-5400.

**Tremblay et al. (2013) Tremblay YDN, Lamarche D, Chever P, Haine D, Messier S, Jacques M. Characterization of the ability of coagulase-negative staphylococci isolated from the milk of Canadian farms to form biofilms. *Journal of Dairy Science.*2013;96:234–246. doi: 10.3168/jds.2012-5795.**

**(Tremblay et al., 2013)**

Biofilm formation is critical for the virulence of several pathogens and has also been proposed as an important virulence factor for the mastitis pathogens *Staph. aureus*, *E. coli*, and *Strep. uberis* ([Melchior, 2011](https://www.journalofdairyscience.org/article/S0022-0302(12)00819-3/fulltext)). In the case of CNS (not mastitis), it has been established that biofilms play an important role in the virulence of *Staph. epidermidis*, especially for infections associated with indwelling medical devices ([Otto, 2008](https://www.journalofdairyscience.org/article/S0022-0302(12)00819-3/fulltext)). With the exception of *Staph. epidermidis*, information on the biofilm formation of other CNS species has been very limited and, in the case mastitis-associated CNS, is limited to 2 studies ([Tormo et al., 2005](https://www.journalofdairyscience.org/article/S0022-0302(12)00819-3/fulltext); [Simojoki et al., 2012](https://www.journalofdairyscience.org/article/S0022-0302(12)00819-3/fulltext)). The objective of our study was to characterize the ability of CNS associated with mastitis to form biofilms.

The majority (85.1%) of the isolates tested were biofilm positive, and a minority (14.9%) were biofilm negative. Using a similar assay, [Simojoki et al. (2012)](https://www.journalofdairyscience.org/article/S0022-0302(12)00819-3/fulltext) observed the opposite: most mastitis-associated CNS were biofilm negative (68.7%) and a minority of isolates were biofilm positive (31.3%). The distribution of CNS species in both studies was similar, with the exception that *Staph. xylosus* was not reported as a separate group by [Simojoki et al. (2012)](https://www.journalofdairyscience.org/article/S0022-0302(12)00819-3/fulltext). In our study, we observed that the ability to form biofilms varied among CNS species and that *Staph. haemolyticus* and *Staph. xylosus* were strong biofilm formers and *Staph. simulans* and *Staph. epidermidis* were weak biofilm formers.

a solid statistical model cannot be established to test whether an association exists between biofilm formation and persistent IMI. However, our statistical analysis did reveal a positive relationship between DIM and biofilm strength. This suggests that strong biofilm formers are selected for during lactation. The reason for such selection is unclear, but it is likely that biofilms increase the ability of CNS to persist in the intramammary environment. This indirectly indicates a relationship between biofilm formation and persistent IMI.

Previously, [Simojoki et al. (2012)](https://www.journalofdairyscience.org/article/S0022-0302(12)00819-3/fulltext) did not observe an association between biofilm formation and CNS IMI persistence and severity. We did not observe a relationship between biofilms and SCC;

**De buck:** Additionalin vivotesting is needed to better characterize theassociations between pathogenicity and biofilm production inS. chromogenes.

it has been suggested that biofilms increase the ability ofNAS to persist in the mammary environment (79,80). Tremblay, piessens

**Toledo-Silva, B., de Souza, F.N., Mertens, K. *et al.* Bovine-associated non-*aureus* staphylococci suppress *Staphylococcus aureus* biofilm dispersal in vitro yet not through *agr* regulation. *Vet Res* 52, 114 (2021). https://doi.org/10.1186/s13567-021-00985-z**

Biofilms are defined as a structured surface-associated community of bacterial cells (sessile) encompassed by an extracellular matrix [[24](https://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-021-00985-z#ref-CR24)]. Biofilm development is related to environmental signals and communication systems [[25](https://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-021-00985-z#ref-CR25)]. In staphylococci it relates to specific gene expression such as the intracellular adhesion locus (*ica*ABCD) [[26](https://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-021-00985-z#ref-CR26)], the biofilm-associated protein *bap* [[27](https://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-021-00985-z#ref-CR27)], and the accumulation-associated protein *aap* [[28](https://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-021-00985-z#ref-CR28)].

Piessens et al. [[48](https://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-021-00985-z#ref-CR48)] also observed that only nine out of the 30 *bap*- and/or *icaA*-positive NAS isolates produced biofilm in the phenotypic assay

Additionally, it was reported [[48](https://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-021-00985-z#ref-CR48)] that so-called environmental NAS species (only causing IMI sporadically), such as *S. epidermidis*, *S. sciuri*, and *S. xylosus*, are a more significant reservoir of biofilm-related genes than so-called contagious NAS species (primarily causing IMI).

**Snell et al. (2015) Snell GGM, Monecke S, Ehricht R, Piccinini R. Molecular characteristics of bap-positive *Staphylococcus aureus* strains from dairy cow mastitis. *Journal of Dairy Research.*2015;82:312–316.**

The biofilm-associated protein (Bap) ofStaphylococcusaureusis a high-molecular weight cell-wall-anchoredprotein of 2276 amino acids and was first described inbovine mastitis strains from Spanish dairy herds (Cucarellaet al.2001). Bap was shown to be involved in intercellularadhesion and accumulation in multilayer cell clusters, andalso in primary attachment to abiotic surfaces. These func-tional characteristics confer a strong biofilm-forming pheno-type to strains carrying thebapgene (Lasa & Penadés,2006

, in the experimental infection,the samebap-positive strain was more able to persistinside the mammary gland (Cucarella et al.2002). Also,bap-mediated biofilm demonstrated higher resistance toantimicrobials (Cucarella et al.2004

**Zuniga et al. (2015) Zuniga E, Melville PA, Saidenberg ABS, Laes MA, Gonsales FF, Salaberry SRS, Gregori F, Brandão PE, Dos Santos FGB, Lincopan NE, Benites NR. Occurrence of genes coding for MSCRAMM and biofilm-associated protein Bap in *Staphylococcus* spp. isolated from bovine subclinical mastitis and relationship with somatic cell counts. *Microbial Pathogenesis.*2015;89:1–6. doi: 10.1016/j.micpath.2015.08.014.**

This study aimed to elucidate aspects of the epidemiology of bovine [subclinical mastitis](https://www.sciencedirect.com/topics/veterinary-science-and-veterinary-medicine/subclinical-mastitis) through the assessment of genes encoding MSCRAMM (*microbial surface components recognizing adhesive matrix molecules* – a group of adhesins) and protein Bap (implicated in biofilm formation), in coagulase-positive (CPS) and coagulase-negative (CNS) *Staphylococcus* isolated from subclinical mastitis. The higher frequency of occurrence (P < 0.005) of the *bap* gene in CNS compared with CPS suggests that in these species biofilm formation is an important mechanism for the persistence of the infection.

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Tremblay, Y. D. N., D. Lamarche, P. Chever, D. Haine, S. Messier, and M. Jacques. 2013. Characterization of the ability of coagulase-negative staphylococci isolated from the milk of Canadian farms to form biofilms. J. Dairy Sci. 96(1):234-246.

Zuniga, E., P. A. Melville, A. B. Saidenberg, M. A. Laes, F. F. Gonsales, S. R. Salaberry, F. Gregori, P. E. Brandão, F. G. dos Santos, N. E. Lincopan, and N. R. Benites. 2015. Occurrence of genes coding for MSCRAMM and biofilm-associated protein Bap in Staphylococcus spp. isolated from bovine subclinical mastitis and relationship with somatic cell counts. Microb Pathog 89:1-6.

**General notes**

***AMR***

* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6604941/
  + Intrinsic resistance may be defined as a trait that is shared universally within a bacterial species, is independent of previous antibiotic exposure, and not related to horizontal gene transfer
  + Natural resistance may be intrinsic (always expressed in the species), or induced (the genes are naturally occurring in the bacteria, but are only expressed to resistance levels after exposure to an antibiotic
  + Acquisition of genetic material that confers resistance is possible through all of the main routes by which bacteria acquire any genetic material: transformation, transposition, and conjugation (all termed horizontal gene transfer—HGT); plus, the bacteria may experience mutations to its own chromosomal DNA. The acquisition may be temporary or permanent. Plasmid-mediated transmission of resistance genes is the most common route for acquisition of outside genetic material;
  + many mutations that confer antimicrobial resistance do so at a cost to the organism. For example, in the acquisition of resistance to methicillin in *Staphylococcus aureus*, the growth rate of the bacteria is significantly decreased (Reygaert WC. Methicillin-resistant *Staphylococcus aureus* (MRSA): molecular aspects of antimicrobial resistance and virulence. *Clin Lab Sci.*2009;22:115–119)

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