***Intro***

* Evidence for variation in ST generally for NASM
  + should have stuff from grant narrative, SCC DIM paper
  + Souza 2016
    - **CHRONIC IMI isolate… like mine …** S. chromogenesisolated from a chronic IMIhad greater ability to adhere to bovine mammary epithelial cellscompared to a strain isolated from the teat apex
* Species level, see if gene number associated with SCC – from naushad
  + We also computed the difference in gene associations among NAS species and forisolates from low, medium, and high SCC and CM. Differences in associations forindividual NAS species and isolates from various inflammatory responses suggestcomplex interplay among virulence genes in causing disease. Unraveling these inter-actions will be important to elucidate distinctive pathogenic mechanisms of individualNAS species and assessing species-specific effects on udder health
* AMR in NASM
  + Presence of this AMG confers resistance to benzylpenicillin by the production of beta-lactamases which hydrolytically destroy β-lactam antibiotics.
* Avall: 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
  + 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)

**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)