***Intro***

* Evidence for variation in ST generally for NASM
  + should have stuff from grant narrative, SCC DIM paper
* AMR in NASM
  + Presence of this AMG confers resistance to benzylpenicillin by the production of beta-lactamases which hydrolytically destroy β-lactam antibiotics.

***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”
* Virulence
  + Descriptive information about what genes were found, compare to mine
    - Naushad
      * Naushad et al. (2019) investigated the profile of 191 virulence factors in NAS, based on whole-genome sequencing, and found distinctive patterns of associations for low qSCC (defined as <150,000 cells/mL in that study) and clinical mastitis isolates.
    - From de buck, about … Naushad 2019
      * Interestingly, in the T-SNE plot (Figure 2),S. chromogenesisthe only species split into 2 populations with respect to virulencegenes, with a minority of the strains clustering with othermembers of the clade B, while the majority of theS. chromogenesstrains have a distinct profile. An important caveat is that moreS. chromogenesisolates were included in this study than otherspecies, but it is tempting to speculate that the larger populationofS. chromogenesmight represent a pathotype that has adapted tothe udder.
      * No clear difference was present between the twoS.chromogenespopulations with respect to severity of mastitis(Figure 2B).
    - Wuytack 2020
      * Bap
        + 15/59 isolates of NAS had bap, none were chrom
        + One ST176 and both St25 missing bap
      * Cap5H
        + Only 2 isolates/59, both chrom in clinical, had cap5H
        + Only 2 of my isolates had capH, both St25 in high
    - PW
      * Found 57 unique pVF among their 105 isolates; their chromogenes on avg contained 30 (SD 5.4, range 25-45)
      * Immune evasion: all my isolates had between 4-5 (persistent)
      * All mine had 6 phenol soluble modulin genes, PW only see PSMbeta4
      * Don’t see their have coa
      * Bap was fairly rare, most mine have it
  + ***How did results support initial hypothesis? (VIR)*** (association between phenotypic traits and possessing VIR genes)
    - Did NOT find that # vir genes was a predictor of being in high SCC cat
    - Naushad (check old AND new)
      * Looked at some clinical traits and association with virulence traits
    - 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
* 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)].
  + 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

**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)