***Abstract***

*Staphylococcus chromogenes* is the dominant species associated with mammary gland infections in dairy animals one of the most persistent intramammary pathogens*.* The objectives of the current study were to: 1) identify if distinct strain types (ST) of *S. chromogenes* were associated with intramammary infections (IMI) where quarter SCC is consistently elevated (HIGH SCC IMI) vs. consistently low (LOW SCC IMI), 2) identify if *S. chromogenes* from HIGH SCC IMI are more likely to carry antimicrobial resistance genes (ARG) vs. LOW SCC IMI, and 3) identify if *S. chromogenes* from HIGH SCC IMI possess more genes encoding previously-described staphylococcal virulence factors (VF) vs. LOW SCC IMI. Isolates originate from a longitudinal observational study of 10 organic dairy farms in Vermont (US), where aerobic culture of quarter-milk samples to identify IMI was conducted in parallel with determination of quarter SCC. Two groups were selected from persistent *S. chromogenes* IMI (as confirmed by RAPD PCR): 1) IMI associated with high SCC, where all quarter-day observations had an associated SCC of ≥200,000 cells/mL; and 2) IMI associated with low SCC, where all quarter-day observations had an associated SCC of <200,000 cells/mL. Representative isolates from 15 LOW SCC IMI and 15 isolates from HIGH SCC IMI were submitted for whole genome sequencing and strain-typed according to a 7-locus MLST scheme*.* ARGs and VF were identified from assembled genomes. Separate mixed-effects logistic regression models were made using ST, ARG carriage, and VF number as the predictor, SCC category as the outcome, and herd as a random effect. Ten different ST were identified, including 4 novel ST. Seven ST were identified in each SCC category, with 3 unique to each. In a mixed-effects logistic regression, ST was not a significant predictor of SCC category. The only ARG identified was *blaZ,* encoding for resistance to penicillin (33.3% of isolates; 6/15 in the HIGH category and 4/15 in the LOW category). *blaZ* was not a significant predictor of SCC category in a mixed-effects logistic regression model. *blaZ* was consistently present in all isolates for 4/5 ST with multiple isolates. Sixty-two unique VF were detected (median: 44 per isolate; range: 43-21). Thirty-nine VF were identified which were present in all isolates, including genes associated with iron uptake and metabolism, production of phenol soluble modulins, hemolysins, and an exfoliative toxin. Presence of VF associated with adherence, host immune evasion, type VII secretion system, and production of exoenzymes and exotoxins was variable. In the HIGH category, 677 VF total were identified vs. 670 in the LOW category. In a mixed-effects logistic regression, number of VF identified was not a significant predictor of SCC category. Genes encoding for exfoliative toxin type C (*etc*) and staphylocoagulase (*coa*) were identified in isolates in the current study, neither of which have been widely reported for *S. chromogenes* isolates of bovine origin. *blaZ* carriage, number and type of VF appears to be a function of ST for *S. chromogenes*, but more research is needed to confirm these findings.

***Introduction***

* 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
  + 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**
* 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.

The objectives of the current study were to: 1) identify if distinct strain types of *S. chromogenes* are associated with IMI where quarter SCC is consistently elevated (HIGH SCC phenotype) vs. consistently low (LOW SCC phenotype), 2) identify if *S. chromogenes* from HIGH SCC phenotype are more likely to carry genes encoding for antimicrobial resistance (as determined by whole-genome sequencing) vs. LOW SCC phenotype, and 3) identify if *S. chromogenes* from HIGH SCC phenotype possess a higher number of genes encoding previously-described staphylococcal virulence factors vs. LOW SCC phenotype.