***Chapter 6: Summary, future directions, general conclusions and overall significance***

***6.1 Summary***

The overall objective of this dissertation research was to better understand the epidemiology of the most relevant pathogens causing intramammary infections (IMI) in organic dairy cows in Vermont. In particular, this work generated foundational knowledge about staphylococcal species causing mastitis on organic dairies. Bacteria belonging to the genus *Staphylococcus* are the predominant pathogens causing IMI in dairy animals, both on organic farms in Vermont and across the globe.

Chapter 1 of this dissertation described the findings of an observational study of organic dairies which was undertaken to identify differences in udder health outcomes between herds using different housing systems. Previous research had shown that tiestall barns were the most frequently used housing type for lactating dairy cattle on organic farms in Vermont, followed by freestall barns. For most of the udder health metrics and the two udder hygiene measures studied, herds using a bedded pack system either performed slightly better or were equivalent in comparison to the more commonly used facility types.

The diversity of bacterial species causing IMI on 10 organic farms in Vermont were then identified in a longitudinal study, for all types of pathogen in Chapter 5 and specifically staphylococci and mammaliicocci in Chapter 3. An even number of organic herds using bedded packs and tiestall barns were enrolled in this study. Species belonging to the genus *Staphylococcus* were primarily responsible for causing mastitis in these herds. Overall, the majority of IMI were caused by all non-*aureus* staphylococci and mammaliicocci (NASM) combined. At the species level, *Staph. chromogenes* was the leading cause of IMI for both facility types, followed by *Strep. uberis*, *Staph. aureus*, and *Staph. haemolyticus*. Quarter-level prevalence of IMI by pathogen was similar between bedded pack and tiestall farms, with slight variability found for the most prevalent species. For the bedded pack farms, the most commonly identified pathogens were *Staph. chromogenes,* followed by *Strep. uberis*, *Staph. haemolyticus,* and then *Staph. aureus.* For the tiestall farms, the most commonly identified pathogens were *Staph. chromogenes,* followed *Staph. aureus*, *Strep. uberis*, and then *Corynebacterium* spp*.*

Chapter 3 focused specifically on IMI caused by staphylococci and mammaliicocci in the longitudinal study. Within this group of species, *Staph. chromogenes* was the most frequently found, followed by *Staph. aureus*. The diversity of staphylococcal species observed and the species-level effect on inflammatory reaction (as measured by somatic cell count, or SCC) was similar to that previously reported for conventional herds. Compared to healthy quarters, SCC was higher in quarters infected for 9 of 10 staphylococcal species identified. Although the increase in SCC was modest for most staphylococcal species, their widespread nature can still result in sizeable increases in bulk tank SCC. A large amount of variability was observed in SCC associated with *Staph. chromogenes* IMI, with some causing an elevation comparable to that of major mastitis pathogens and others similar to healthy quarters.

The majority of *Staph. chromogenes* IMI observed in the longitudinal study were persistent. Chapter 4 describes the findings of a study to better understand the epidemiology of this subgroup of *Staph. chromogenes* causing persistent IMI. Two categories selected from persistent *Staph. chromogenes* IMI: those associated with a consistently high SCC and those with a consistently low SCC. Representative bacterial isolates from these IMI were submitted for whole genome sequencing and strain-typed. Particular strain types (ST) of *Staph. chromogenes* were not found to be associated with either SCC category. Ten different ST were identified, 4 of which were newly-described. The only antimicrobial resistance gene (ARG) identified was *blaZ*, encoding for resistance to penicillin in a third of the study isolates. Neither overall number of virulence factors nor *blaZ* carriage was found to be a significant predictor of SCC category. *blaZ* carriage, number and type of virulence factor instead appeared to be a function of strain type (Table 6.1).

***6.2*** ***Directions for future research***

*6.2.1 Further work exploring associations between management factors and udder health for bedded pack farms*

The biggest limitation encountered in the cross-sectional study described in Chapter 2 was the relatively small number of farms included for each of the 3 facility types of interest. This limited statistical power when comparing udder health and hygiene metrics between tiestalls, freestalls, and bedded pack farms. Further complicating analysis between farms was how similar milk quality and udder health were among all 21 enrolled herds; most herds were below benchmark cutoffs for the number of cows developing new IMI each month, cows with chronic IMI month to month, proportion of cows with an elevated SCC, and all bulk tank SCC were fairly low (54,000-250,000 cells/mL). A related limitation is that well-established mastitis control practices were widely adapted by all participating herds, so we were unable to analyze associations between certain practices and bulk tank milk quality, udder health, and hygiene. The potential still exists for future studies with a larger number of farms enrolled to further characterize milk quality and udder health on bedded pack systems in the Northeastern US climate. By enrolling farms from a larger geographic area (vs. only Vermont), future studies may be able to include a larger number of bedded packs, with more variation in milk quality and mastitis rates. This could increase the statistical power needed to identify particular management factors which are beneficial or detrimental for these farms specifically. Although we were limited by statistical power for our cross-sectional study, our data could be used to inform new hypotheses and power calculations for future study design.

*6.2.2 Further work exploring virulence potential for Staph. chromogenes isolates from the current study*

In Chapter 4, the total number of virulence factors identified for each *Staph. chromogenes* isolate was not a significant predictor of whether that isolate would belong to the persistently high or persistently low SCC IMI category. In a linear regression with all virulence factors considered together, Naushad et al. (2019) did not find that an increase in the overall number of virulence factors for a NASM isolate was associated with an increase in SCC of the associated IMI. However, when stratified by type of virulence category, the presence of each additional toxin gene for a NASM isolate was associated with a 0.024 increase in logSCC of the associated IMI (Naushad et al., 2019). Similarly, in a logistic regression with ordinal categories for IMI severity (low SCC, medium SCC, high SCC, and clinical mastitis), an overall increase in the number of virulence factors was not associated with increased severity of an IMI (Naushad et al., 2019). In a regression analysis with virulence factors stratified by type of virulence category, the presence of each additional gene associated with host immune evasion increased the odds of having a more severe immune response by 0.074 (Naushad et al., 2019). For the 30 *Staph. chromogenes* isolates included in Chapter 4, it would be worth repeating the analysis of possible associations between number of virulence factors and SCC category, but stratified by the different groupings of virulence gene functional category. Naushad et al. (2019) define five separate functional groupings for virulence genes (adherence, exoenzymes, host immune evasion, iron metabolism, and toxins), and analysis of number of virulence genes by functional group may be associated with SCC category even though overall number was not. Furthermore, Naushad et al. (2019) applied various clustering approaches in order to determine whether particular virulence distributions had any association with SCC category or occurrence of clinical mastitis, as well as relationships between the virulence factors from five categories stratified by IMI severity (low SCC, medium SCC, high SCC, and clinical mastitis). Although this methodology was beyond the scope of the current dissertation, cluster analyses of the study isolates may reveal interesting associations between different virulence factors, or patterns of virulence factors distinct to either the persistently high or low SCC IMI group.

The gene *coa*, encoding for the staphylocoagulase enzyme, was identified in two *Staph. chromogenes* isolates belonging to ST25. *coa* has not been widely reported to be present in *Staph. chromogenes* of bovine IMI origin. It would be interesting to screen more *Staph. chromogenes* from the current study for presence of *coa,* whether by whole genome sequencing or PCR. Both isolates with *coa* belonged to ST25. Identifying more isolates by MLST and screening for *coa* could determine if carriage of this notable staphylococcal virulence factor was more likely for isolates belonging to this particular ST. Additionally, performing a coagulase test for *coa* positive isolates in this collection could yield interesting insight between carriage of this gene and phenotypic ability to clot serum *in vitro.*

*6.2.3 Further work exploring antimicrobial resistance for Staph. chromogenes isolates from the current study*

Ten of the 30 isolates *Staph. chromogenes* isolates described in Chapter 4 were positive for carriage of the *blaZ* gene, with *in silico* analysis of data from whole genome sequencing. *blaZ* encodes a β-lactamase enzyme which hydrolytically destroys β-lactam antibiotics, and is the primary determinant of phenotypic resistance to benzylpenicillin in staphylococci (Pinho, 2008). However, carriage of a particular resistance gene does not always translate into phenotypic resistance. Inconsistencies exist between phenotypic and genotypic resistance results, due either to 1) detection of phenotypic resistance in the absence of expected genotypic determinants, or 2) phenotypic susceptibility despite the presence of genotypic determinants. For staphylococciassociated with bovine mastitis, both of these types of discrepancies have been reported for penicillin resistance (Sampimon, 2009; Taponen et al., 2023). In a study by Taponen et al. (2023) comparing methods of testing for β-lactamase mediated resistance, overall agreement between phenotypic and genotypic resistance tests was moderate to substantial for staphylococci from bovine IMI. However, some inconsistencies were found between phenotypic susceptibility by disk diffusion method, the nitrocefin test to assess β-lactamase production, and PCR to detect the presence of the *blaZ, mecA*, and *mecC* genes encoding the β-lactamase gene.

Performing phenotypic antibiotic susceptibility testing for the 30 *Staph. chromogenes* isolates described in Chapter 4 could further inform agreement between genotypic and phenotypic susceptibility of bovine staphylococci causing IMI. Preliminary results for 12 of the 30 isolates show 100% agreement between *blaZ* carriage and phenotypic susceptibility to penicillin (using the agar dilution method, Table 1). All 8 isolates negative for *blaZ* based on whole genome sequencing were susceptible to penicillin *in vitro,* while the 4 *blaZ-*positive isolates were determined to be resistant. Although no other ARG were identified from genotypic data for these isolates, we may potentially see phenotypic resistance to other antimicrobial compounds when they are tested *in vitro.* Databases identifying ARG are only looking for previously-described genes and mechanisms of resistance in bacteria; the process of identifying novel mechanisms of resistance begin by observing phenotypic resistance in the absence of previously-described resistance determinants.

Resistance determinants in bacteria can be carried either as part of a genetic mobile element (usually a plasmid) or chromosomally. Location of *blaZ* carriage is not well characterized for *Staph. chromogenes*, but a study of *Staph. aureus* IMI isolates in Finland and Norway found that 26 out of 34 Finnish isolates (76.5%) and 25 out of 44 Swedish isolates (56.8%) carried *blaZ* on a plasmid (vs. chromosomally) (Bagcigil et al., 2012). Extrapolating from these results, *blaZ* could potentially be carried either chromosomally or on a plasmid for the *Staph. chromogenes* isolates in the current study. Preliminary work exploring location of *blaZ* gene for the 10 positive isolates in the current study found that 4 were negative for plasmid carriage (using Plasmid Finder from the Center for Genomic Epidemiology, Technical University of Denmark, <https://cge.food.dtu.dk/services/PlasmidFinder/>). One of these 4 was from ST6, 1 was from a different farm and belonged to ST51, and 2 from a third farm both belonged to ST5. As *blaZ* carriage appeared to be primarily a function of ST in Chapter 4, and consistent carriage of *blaZ* from ST originating from different farms is observed, this may suggest that *blaZ* is primarily located chromosomally for this population of *Staph. chromogenes*. The only ST identified from Chapter 4 which did not have consistent carriage of *blaZ* was ST6. It may be that these isolates of ST6 carry *blaZ* on a plasmid. If resistance genes are harbored on horizontally transmissible elements (such as plasmids), strains carrying these elements can successfully disseminate them to new, previously-susceptible bacteria, belonging to both the same species and different species. Location of resistance determinants on plasmids generally promotes more efficient spread of resistance genes (Malachowa and DeLeo, 2010). Studies exploring whether *blaZ* is more likely to be carried chromosomally or on a plasmid for *Staph. chromogenes* from bovine IMI would be useful in understanding transmission of penicillin resistance for this predominant mastitis pathogen.

*6.2.4 Further work exploring the intraspecies diversity of Staph. chromogenes*

Among the 30 *Staph. chromogenes* strain-typed using MLST in Chapter 4, there were 10 different ST identified. As the MLST scheme for *Staph. chromogenes* was described fairly recently (Huebner et al., 2021), the number of studies describing strain-typing results using this scheme to date is limited (Petzer et al., 2022; Persson Waller et al., 2023). Describing the diversity of *Staph. chromogenes* using MLST is a rapidly growing area of research. Four of the 10 ST in the current study had previously not been described, and 43% of all isolates belonging to 33 new ST were identified by Persson Waller et al. (2023). These results highlight the importance of contributing to publicly-available databases in order to improve our ability to better understand the diversity of this common mastitis pathogen. Some overlapping ST (those related to ST1 and ST6) were observed between the Swedish isolates in Persson Waller et al. (2023), the Vermont isolates in the current study, and the isolates from Washington State, Vermont, and Belgium described in Huebner et al. (2021). However, distribution of different *Staph. chromogenes* ST also seems to be a function of geographical location. ST15 was the second most commonly identified by Hubener et al. (2021) after ST1, with 17/120 (14.2%) of isolates belonging to this ST. In their study, ST15 was primarily identified in isolates from Vermont and Washington State (16/17 isolates), and less frequently in Belgium. Interestingly, although all isolates in the current study are from Vermont, only 2 of 30 isolates belonged to ST15. A study using MLST to strain-type larger number of *Staph. chromogenes* associated with bovine IMI from numerous countries across multiple continents would help us to better understand how the distribution and diversity of this pathogen varies with geography.

In the longitudinal study described in Chapters 3-5, MALDI-TOF mass spectrometry was used to identify bacterial isolates to species level. Isolates were sent to the University of Missouri to be run on the MALDI-TOF. Generated spectra were assigned a score based on similarity with spectra in the manufacturer’s database, as well as the University of Missouri laboratory custom database, which has been specifically informed to better identify isolates important to bovine mastitis. In total, 488 bacterial isolates were identified by the MALDI-TOF as *Staph. chromogenes.* Three isolates later identified to be *Staph. chromogenes* (using sequencing of the *tuf* gene) were called “no ID” by MALD-TOF. These 3 isolates are all from the same farm, and 2 were isolated from the same quarter over sequential visits. It is interesting to consider why these 3 particular *Staph. chromogenes* isolates out almost 500 were unable to be identified with MALDI-TOF. Unfortunately, none of these isolates were strain-typed using RAPD or MLST, so whether or not they are the same ST is unknown. However, it is tempting to wonder if they belong to a certain ST which is different enough from the others within *Staph. chromogenes,* preventing them from being identified in the same manner. Further, if this was the case, it may lead to the question of whether MALDI-TOF could be used for strain-typing isolates of *Staph. chromogenes.* If so, it would be significantly easier and faster than any current method of strain-typing used for this organism. In order to explore this question, one would need a large set of *Staph. chromogenes* isolates, with a diversity of MLST identified and represented, and a substantial number of isolates belonging to each ST.

Although we did not find any support for an association between carriage of *blaZ* and the associated SCC category of an IMI, results from Chapter 4 suggest that *blaZ* carriage is likely a function of ST in *Staph. chromogenes*. As mentioned above, for all but 1 of the 5 MLST identified, *blaZ* carriage was uniform across a ST. Similar to *blaZ*, the carriage of virulence factors by isolates in the current study also appears to be a function of ST. The cumulative number of virulence factors identified belonging to the 5 ST with multiple isolates showed little to no variation, and many of the limited differences in presence or absence of virulence factors occurred at the ST level. Association between ST and carriage of ARG has been demonstrated for bovine *Staph. aureus.* Evidence for association between ST and virulence potential, as well as ST and carriage of ARG, was observed in Persson Waller et al. (2023) for *Staph. chromogenes.* When Naushad et al. (2019) applied various clustering approaches in order to determine whether particular virulence factor distributions had any association with SCC category or clinical mastitis, NASM isolates instead grouped together by their respective species. Resistance profiles for NASM are species-specific, and it appears that the pattern of virulence genes carried by NASM isolates is also likely species-dependent. Therefore, this genetic basis for carriage of virulence factors and ARG may also extend to the strain level.Our ability to explore if pattern and number of virulence factors or carriage of *blaZ* varies by ST in the current study was limited, both by the relatively small number of isolates assigned to most ST, as well as complete uniformity in the number and type of virulence factors and *blaZ* presence for a particular ST. Future studies with larger isolate collections collected from a greater number of farms and the ability to sequence more *Staph. chromogenes* isolates may be better able to explore associations of ARG and virulence factor carriage by ST (as determined by MLST). Larger sample sizes would likely result in a greater diversity of ST, with more isolates assigned to each ST, and a greater ability to account for the effect of clustering by herd.

Although no evidence was found that the number of virulence factors or ARG would predict if a *Staph. chromogenes* isolate would be associated with a persistently high or low SCC IMI, it may be interesting to these attributes between isolates associated with clinical vs. non clinical mastitis. In a study of 217 *Staph. aureus* IMI isolates typed using pulsed-field gel electrophoresis, Haveri et al. (2005) identified a particular pulsotype which was significantly associated with severe clinical mastitis symptoms but reduced persistence when compared to the 4 other commonly identified pulsotypes. This association between specific clinical traits associated with an IMI (persistency and clinical severity) has not yet been widely described for *Staph. chromogenes*, but may help us better understand if particular pathotypes or ST exist in the population which are of greater concern.

*6.2.5 Further work to better understand differences between organic and conventional dairy farm systems*

While Chapters 3-5 described the species-specific prevalence of IMI pathogens, as well as the virulence potential and ARG carriage of *Staph. chromogenes* from organic dairy farms, the longitudinal study described was not designed to identify differences between conventional and organic dairy farms. Although Chapters 3 and 5 found that the species diversity and prevalence of various NASM were similar to what has previously been described for conventional farms (and 1 study of organic farms), it would be interesting to carry out research directly comparing NASM causing IMI in both types of system. Research summarized in Chapter 1 compared prevalence of IMI due to “coagulase negative staphylococci” generally, but these isolates were not identified to species level. Differences in management practices, facility types, and cow demographics between conventional and organic dairies was summarized in Chapter 5. Given that variation in NASM species distribution and diversity is associated with a variety of herd and cow-level effects, it is possible that these differences may lead to dissimilar relative distributions or diversity of NASM causing IMI between the two farm types. Furthermore, although comparison of resistance profiles of staphylococci causing IMI between organic and conventional dairies has generally has been well-described (Chapter 1), these studies are again limited by a lack of identification to species level. As antimicrobial resistance (AMR) patterns are species-specific, potential exists for research comparing the both genotypic and phenotypic species-specific resistance profiles for NASM causing IMI on organic and conventional dairies. Exploring this topic would identify what kind of resistance was prevalent in NASM not experiencing selective pressure from antibiotic usage, which would be the first step in understanding why resistance is maintained in these populations, what type of resistance is prevalent.

Related to this, potential exists for research comparing the most common ST of *Staph. chromogenes* causing IMI on organic and conventional farms. Comparison of predominant ST within a given species causing IMI could further our understanding of the complex interplay between phylogeny and selection pressures resulting from management factors on the resistance profiles of these mastitis pathogens. Previous findings suggests that ST may vary between organic and conventional farms, although researchers were studying fecal *E. coli* and not mastitis pathogens. Walk et al. (2007) found that phylogenetic groupings varied between organic and conventional dairies, suggesting there may be differences between lineages of *E. coli* in their ability or likelihood of acquiring resistance genes. Based on their findings, the authors conclude that “organic farming practices not only change the frequency of resistant strains but also impact the overall population genetic composition of the resident *E. coli*flora.”

A few studies describe resistance patterns of mastitis pathogens before and after transitioning to organic status, and these are summarized in Chapter 1. However, most were (understandably) limited in both the number of herds enrolled and the amount of time farms were followed. Although likely logistically difficult and expensive, a long-term study of farms transitioning from conventional to organic status would be incredibly valuable in understanding what types of AMR is maintained in organic dairy herds and for how long. This research could help us start understanding where the resistance genes in these bacteria came from, how long they maintain them in the absence of selective antimicrobial pressure, and specifically what types of resistance are maintained.

An extremely unique opportunity exists for research using IMI isolates from the longitudinal study described in Chapters 3-5. Staphylococci causing IMI from a particular farm enrolled in the current study were collected and stored from a previous study, approximately 25 years earlier. Identifying these isolates to the species level and then comparing resistance profiles across 25 years could give insight into trends in AMR prevalence over time. This particular farm was founded in the 1970s and has no history of antibiotic use. The 3 *Staph. chromogenes* isolates included from this farm in Chapter 4 (assemblies 1-3, Table 6.1) were found to be penicillin susceptible and *blaZ-*negative. However, 19 *Staph. aureus* and 92 NASM isolates belonging to 8 different species (including 50 additional *Staph. chromogenes*) have been identified and stored from this farm in the current study. This collection of historical and new isolates from the same farm held by our research group would be limited to a case study. However, potential exists for collaboration with other research groups who may happen to have a similar collection of isolates. A study like this may help shed light on what different types of resistance persist long-term among mastitis pathogens in a dairy farm environment in the absence of antimicrobial use. As summarized in Chapter 1, some degree of AMR was found in isolates despite decreased (EU) or absence (US) of selective pressure of antimicrobial use. Additionally, isolate collections like this would allow for comparing what version of an ARG was present in bacteria at different points in time. Specifically, one could compare type of carriage of *blaZ* (chromosomally vs. plasmid) between old and new isolates, or the particular protein signature of the β-lactamase enzyme encoded by *blaZ*. One could then determine whether the same type of penicillin resistance has been maintained in staphylococci for a particular farm, or if the predominant ARG conferring resistance to penicillin has shifted over time. Assuming there is a fitness cost to bacteria for maintaining AMR genes (Vanacker et al., 2023), this certainly begs the question of why resistance genes have been maintained to any degree in the absence of selective antimicrobial pressures. If particular ARG were shown to be maintained in mastitis pathogens from organic farms for 25 years, identifying where these ARG are carried in bacterial genomes could help determine why they have been maintained. Are these ARG close to genes associated with processes necessary for survival, and therefore get “carried along?” Or, are they linked to a different trait which is locally advantageous to survival in that particular organic dairy farm? Improving our understanding of how AMR is transmitted or retained in dairy farm systems is essential for developing effective strategies to mitigate resistance among mastitis pathogens.

* 1. ***General conclusions and overall significance***

The research presented in this dissertation indicates that the species distribution and prevalence of staphylococcal species causing IMI on organic dairy farms in Vermont are similar to what has been described previously for conventional farms.Furthermore, the inflammatory reaction in the mammary gland associated with IMI due to different staphylococcal species is also similar to that which has been previously described. The impact of IMI caused by staphylococci seems to be consistent across differing facility types used by organic farms, as shown by comparison of bulk tank milk data presented in Chapter 2 and the diversity and relative proportion of species causing IMI at the quarter-level presented in Chapter 5.

This work underscores that NASM are indeed worthy of consideration as significant mastitis pathogens, and are not “minor” mastitis pathogens as some have previously described them. As a group, NASM were clearly the predominant pathogen causing IMI, with a 20% median quarter-level prevalence described in Chapter 5. Nine of the ten species analyzed in Chapter 3 species elevated quarter SCC above healthy quarters. Although this increase in quarter SCC was modest for most species observed, their widespread nature can still result in an increased bulk tank SCC. This work also underscores the importance of *Staph. chromogenes* specifically as a mastitis pathogen. I found a prevalence of 33% prevalence penicillin resistance in *Staph. chromogenes*, which is especially noteworthy as these isolates originate from organic farms. Findings from our longitudinal study show that this mastitis pathogen has a great ability to persist in the mammary gland, as well as cause a number of infections associated with a persistently high SCC. Furthermore, findings from this work also suggest that particular strains of *Staph. chromogenes* behave in a contagious manner within a herd, as the same RAPD type was found causing IMI in multiple animals on a farm, and particular strains as identified by MLST appear to be dominant at different farms.

This work contributes to contributes to the understanding of intraspecies diversity within *Staph. chromogenes,* the leading cause of IMI in dairy animals worldwide. Four novel ST were identified using MLST, and 10 MLST were identified to be causing 30 persistent IMI. The AMR and virulence profile for 30 *Staph. chromogenes* were described from whole genome sequence data, with the identification of two virulence genes not widely reported for bacteria of this species (*coa*, encoding staphylocoagulase, and *etc,* encoding exfoliative toxin type C). Although no association was found between virulence and AMR carriage and whether or not the associated persistent IMI would have a high or low SCC, findings seem to indicate to a genetic basis for virulence and AMR carriage in *Staph. chromogenes.*

Taken together, my research generated foundational knowledge about the epidemiology of staphylococci causing mastitis on organic dairy farms in Vermont. While all dairy producers rely on best management practices to support cow health, mastitis control is of the utmost importance in the prevention of IMI on organic farms. Better understanding the epidemiology of mastitis pathogens leads to more effective measures which prevent or limit transmission of IMI. Mitigating the effect of mastitis caused by staphylococci through more effective, targeted prevention and control measures helps dairy producers achieve quality price premiums and results in a higher-quality product for consumers.

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| --- | --- | --- | --- | --- |
| **Table 6.1** Agreement between *blaZ* gene carriage and phenotypic susceptibility (using agar dilution) to penicillin by strain type (ST; as determined by MLST) for 30 *Staphylococcus chromogenes* isolates associated with persistent bovine intramammary infections. Isolates were defined as susceptible to penicillin if their MIC was 0.12 µg/mL or lower, and resistant if their MIC was 0.25 µg/mL (2018 Clinical & Laboratory Standards Institute guidelines). N = no *blaZ* carriage; Y = positive for *blaZ* carriage; S = susceptible to penicillin; R = resistant to penicillin. | | | | |
| Isolate | ST | ST cluster | *blaZ* carriage | Susceptibility phenotype (penicillin) |
| assembly\_3 | 175 | 1 | **N** | S |
| assembly\_2 | 174 | 1 | **N** | S |
| assembly\_20 | 176 | 1 | **N** | - |
| assembly\_21 | 176 | 1 | **N** | - |
| assembly\_22 | 176 | 1 | **N** | - |
| assembly\_23 | 176 | 1 | **N** | - |
| assembly\_14 | 176 | 1 | **N** | - |
| assembly\_15 | 176 | 1 | **N** | - |
| assembly\_16 | 176 | 1 | **N** | - |
| assembly\_17 | 176 | 1 | **N** | - |
| assembly\_19 | 176 | 1 | **N** | - |
| assembly\_10 | 5 | 5 | **Y** | - |
| assembly\_24 | 5 | 5 | **Y** | - |
| assembly\_12 | 5 | 5 | **Y** | R |
| assembly\_1 | 6 | 6 | **N** | S |
| assembly\_6 | 6 | 6 | **N** | S |
| assembly\_9 | 6 | 6 | **N** | S |
| assembly\_13 | 6 | 6 | **N** | S |
| assembly\_30 | 6 | 6 | **N** | - |
| assembly\_25 | 6 | 6 | **Y** | - |
| assembly\_26 | 6 | 6 | **Y** | - |
| assembly\_27 | 6 | 6 | **Y** | - |
| assembly\_29 | 6 | 6 | **Y** | - |
| assembly\_5 | 25 | 25 | **N** | S |
| assembly\_7 | 25 | 25 | **N** | S |
| assembly\_4 | 48 | 48 | **Y** | R |
| assembly\_11 | 48 | 48 | **Y** | R |
| assembly\_8 | 51 | 51 | **Y** | R |
| assembly\_28 | 136 | 136 | **N** | - |
| assembly\_18 | 177 | 177 | **N** | - |

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