***Discussion***

- ST diversity

*MLST and strain type diversity*

In the current study, 10 different strain types determined by MLST were identified for the 30 *S. chromogenes* isolates. As the MLST scheme for *S. 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). In the phylogenetic analysis, study isolates belonging to ST174, ST175, and ST176 were identified as being closely related to ST1 isolates from PubMLST, and were identified as single locus variants of ST1 by the MLST 2.0 tool. ST1 was the most commonly found strain type in the current study (11/30 isolates, 36.7%). This agrees closely with the work of Huebner et al. (2021), who determined MLST for 120 *S. chromogenes* isolates from Belgium, Vermont (US), and Washington state (US). They found 39/120 (32.5%) of isolates strain-typed belonged to a nodal cluster centered around ST1. For the 48 isolates in Huebner et al. (2021) from Vermont, 36 (75%) belonged to nodal cluster 1. ST1 was also commonly found in 118 *S. chromogenes* isolates from bovine subclinical IMI in Sweden, in a study from Persson Waller et al. (2023), although ST6 and a related novel ST (ST109) were more predominant. For Huebner et al. (2021), ST1 was the only strain type found in all three geographical locations. ST6 was the second most commonly found ST in the current study (9/30 isolates, 30%), and the third most common (15/120, 12.5%) in Huebner et al. (2021). Persson Waller et al. (2023) identified 47 different strain types among 105 isolates from Sweden. Huebner et al. (2021) found a similar degree of diversity, with 47 ST identified from 120 isolates from three geographical locations. After ST1, ST15 was the second most commonly identified by Hubener et al. (2021), with 17/120 (14.2%) of isolates belonging to this strain type. ST15 was primarily identified in isolates from Vermont and Washington state (16/17 isolates), which Huebner et al. (2021) highlight as an example of how the distribution of different strain types varies geographically. Interestingly, 2 of 30 isolates belonged to ST15 in the current study, while only 1 ST15 was found in Sweden (Persson Waller et al., 2023).

Both Persson Waller et al. (2023) and Huebner et al (2021) saw a pattern where ST6 and ST1 as central nodes of ST clusters, with single- and double-locus variants surrounding these two ST. Both authors suggest this indicates a clonal expansion is occurring for *S. chromogenes* isolates belonging to these 2 ST. Results of the current study would support this, as the 3 ST in ST cluster 1 (ST174, ST175, ST176) were all newly-identified single-locus variants of ST1. Describing the diversity of strain type using MLST is an incredibly active area of research. Four of the 10 ST in the current study had previously not been described, while 43% of 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 *S. chromogenes.*

* 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
  + 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)

- AMR/blaZ

PW found blaZ in 22% of isolates, they found strpS194, which confers resistance to streptogramin in some of their chromogenes (7%)

When considered as a group, resistance to β-lactam antibiotics is the predominant type of AMR present in staphylococci. The reported proportion of NASM isolates with β-lactamase resistance can be fairly high, with 51.6% phenotypically resistant to penicillin in Argentina (Raspanti et al. 2016), 63% phenotypically resistant to penicillin in South Africa (Phophi et al. 2019), and 80% of CNS isolates positive for the *blaZ* gene (encoding the production of a β-lactamase enzyme) in a study from the Netherlands (Sampimon 2009). Proportion of phenotypically penicillin-resistant NASM seems to vary geographically, with Nordic countries reporting 34% (Nyman et al. 2018), 23% (Fergestad et al. 2021), and 29% (Persson Waller et al. 2011), while a Korean study found 14% of NASM isolates were resistant to penicillin (Kim et al. 2019) and Nobrega et al. (2018) report a prevalence of 10% in Canada. β-lactam antibiotics are among the few choices for treating mastitis in the US, with first- and third-generation cephalosporins being the most commonly-used mastitis treatments (USDA 2016; de Campos et al. 2021). Moderate resistance has been observed in NASM against tetracycline, another highly important antimicrobial frequently used in dairy herds, with 30.1%, 20.9%, and 10% of isolates reported to be resistant in Argentina, India, and Canada, respectively (Raspanti et al., 2016; Mahato et al., 2017; Nobrega et al. 2018). This marked geographic variation in resistance patterns may likely be due to differing selective pressure in dairy farm systems around the world. Which specific antimicrobials are most typically used to treat mastitis and in what amount, as well as the various regulation around their usage, varies from country to country.

Studies comparing NASM at the species level have consistently shown that AMR profile varies between species (Sampimon 2009; Persson Waller et al. 2011; Taponen et al. 2016; Nobrega et al. 2018; Fergestad et al. 2021; Taponen et al. 2023). Overall, both phenotypic resistance and resistance genes are relatively rare in the most common species, *S. chromogenes,* in comparison to other NASM (Sampimon 2009; Persson Waller et al. 2011). A notable exception is the presence of the *blaZ* gene, which was found in 80% of all 170 CNS isolates and 87% of *S. chromogenes* specifically in a Flemish study (Sampimon 2009). β-lactamase production was significantly lower for *S. chromogenes* vs. *S. epidermidis* and *S. haemolyticus* in Sweden (Persson Waller et al. 2011). Although a smaller-scale study in Argentina found a relatively high proportion of *S. chromogenes* were resistant to penicillin (45.1%), both *S. haemolyticus* and *S. xylosus* had an even higher proportion of penicillin-resistant isolates (58.6% and 92.9%, respectively; Raspanti et al., 2016).

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