Determining the ability of MALDI-TOF mass spectrometry to appropriately categorize *Staphylococcus chromogenes* MLST clonal complex subtypes using isolates from dairy farms in the United States

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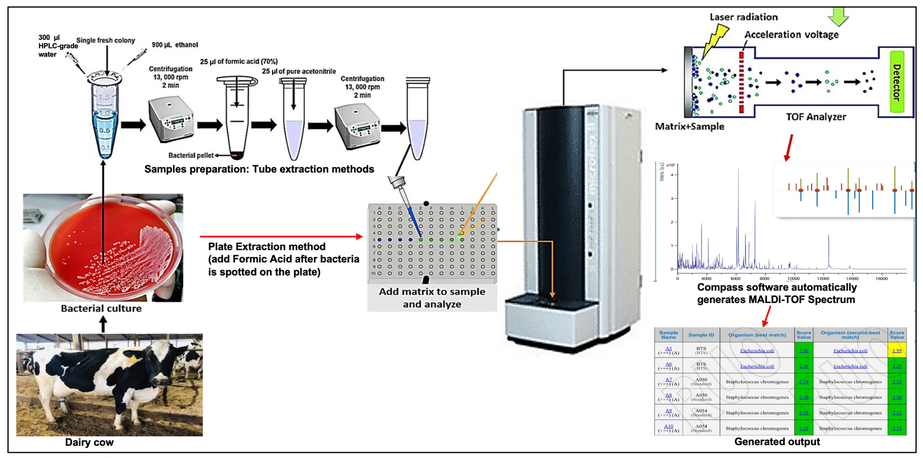
Introduction

- Mastitis is one of the most common and costly diseases impacting dairy cattle globally.
- Non-*aureus* staphylococci (NAS) have become the most frequently identified bacteria isolated from milk samples collected from dairy cows globally.
- Among the NAS species, *Staphylococcus chromogenes* is most frequently identified.
- Studies have confirmed that *S. chromogenes* has been associated with persistent intramammary infections and elevated somatic cell counts.
- Others have identified this species to have minimal impact on the gland or potentially protective effects against major mastitis pathogens.
- Identifying simple ways to differentiate *S. chromogenes* strains is important to understanding these differences.
- **Therefore, the goal of this study is to determine if MALDI-TOF mass spectrometry can be used to determine the MLST clonal complex subtypes of *S. chromogenes* isolates collected from dairy farms in the United States.**

Methods

Figure 1: Diagram of study materials and methods



- A collection of *S. chromogenes* isolates (n = 46) with known MLST clonal complex (CC) determinations was tested.
- A total of 15 *S. chromogenes* isolates were selected as representatives of each clonal complex and were added to the MALDI-TOF database after tube extraction (**Figure 1**) to create the *S. chromogenes* study MALDI library.
- Four MALDI-TOF spots were run for each isolate, including two tube extraction spots and two direct plate extraction spots (**Figure 1**).
- Collected data was evaluated in 5 ways. In all methods, 2 MALDI results were used to compare to the test isolate. The MLST CC type of the test isolate was compared with the *S. chromogenes* study MALDI library CC types by comparing the CC type of:
  1. The top 2 matches within 1 spot run for the tube extraction method
  2. The top 2 matches within 1 spot run for the plate extraction method
  3. The top match of the 2 spots run for the tube extraction method
  4. The top match of the 2 spots run for the plates extraction method
  5. Only compared if the MALDI-TOF cut-off value was >2.30
- Results were defined as <u>correct</u> if both CC types within the evaluations matched the known test isolates CC type. Results were defined as <u>incorrect</u> if the CC types within the evaluation method were the same but did not match the test isolate CC type. Results were defined as <u>unknown</u> if 2 different CC types were identified within the results of the comparison method.

Results

Figure 2: Matching Percentage of 46 MALDI-TOF-identified isolates using the *S. chromogenes* study MALDI library using 2 Plate Spots using the Extraction and Direct Plate Methods (Evaluation Methods #1 and #2). For this analysis, each isolate was analyzed twice.

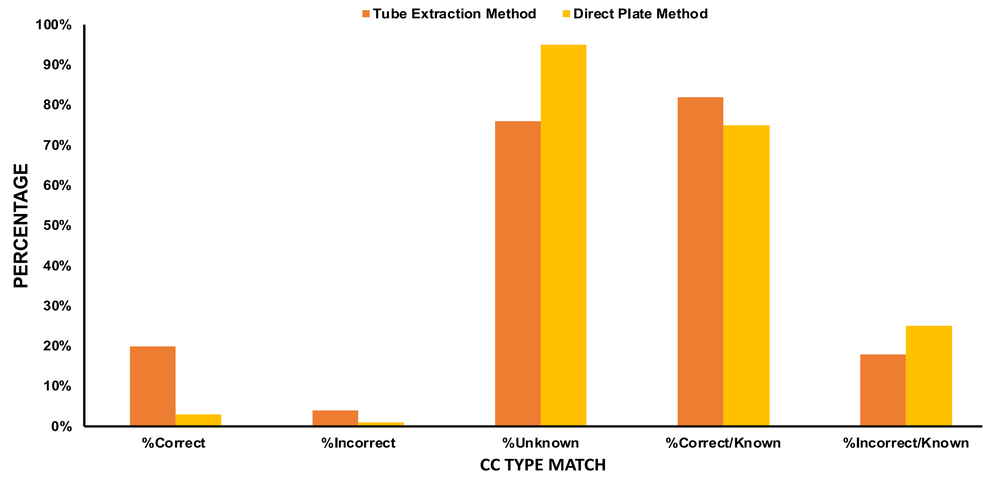


Figure 3: Matching Percentage of 46 MALDI-TOF-identified isolates using the *S. chromogenes* study MALDI library and 2 Plate Spots using the Extraction and Direct Plate Methods (Evaluation Methods #3 and #4). For this analysis, each isolate was analyzed once.

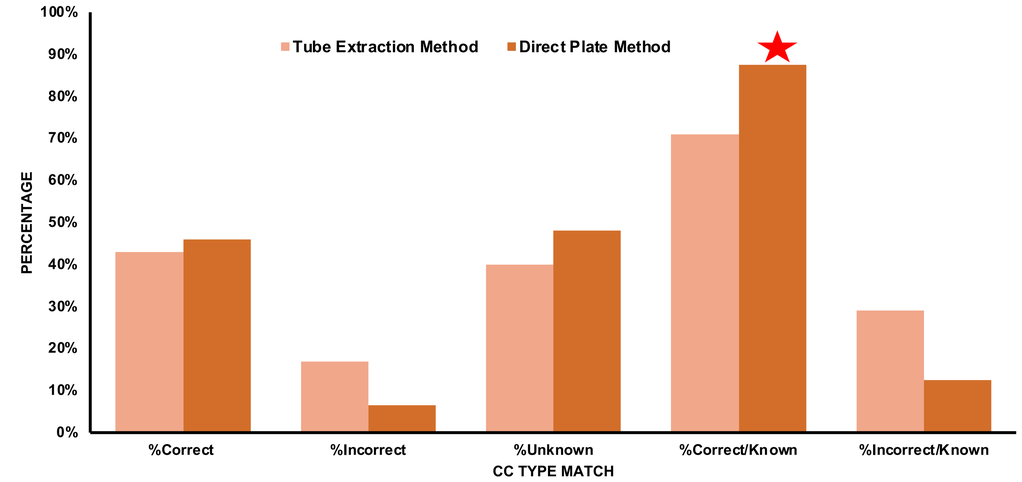
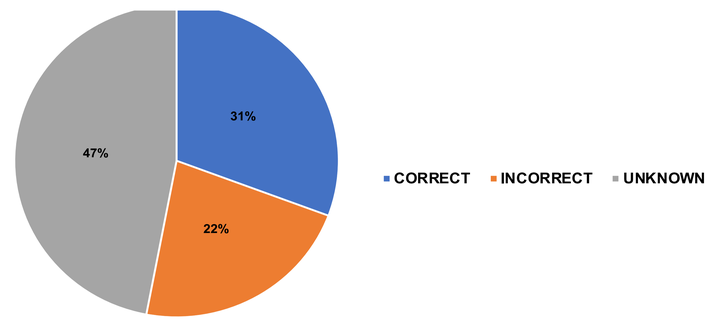


Figure 4: Matching Percentage of 46 MALDI-TOF-identified using the *S. chromogenes* study MALDI library and a cut-off score value of 2.30 of the isolates within 4 Plate Spots of Extraction and Direct Plate Methods. For this analysis, each isolate was analyzed twice. Unknown in this analysis was any MALDI run result of < 2.3.



Conclusions

- Direct plate method of MALDI-TOF mass spectrometry provides more correct matches with MLST clonal complex (CC) subtypes.
- A matching algorithm that uses 2 plate spots on the MALDI-TOF provides a better matching score with MLST CC subtypes.
- MALDI-TOF is capable of determining the MLST CC of *S. chromogenes* isolates, although further research is needed to identify the best matching algorithm to use for the comparison.

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