Comparison of MALDI-ToF and PFGE for strain-typing *Staphylococcus aureus* isolated from cow's milk

College of Veterinary Medicine
University of Missouri

Geraline T. Arroyo[1], Pamela R. F. Adkins[1], Lawrence K. Fox[2], John R. Middleton[1]
[1]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia
[2]Department of Veterinary Clinical Sciences, Washington State University, Pullman

Veterinary Research Scholars Program
University of Missouri

Background

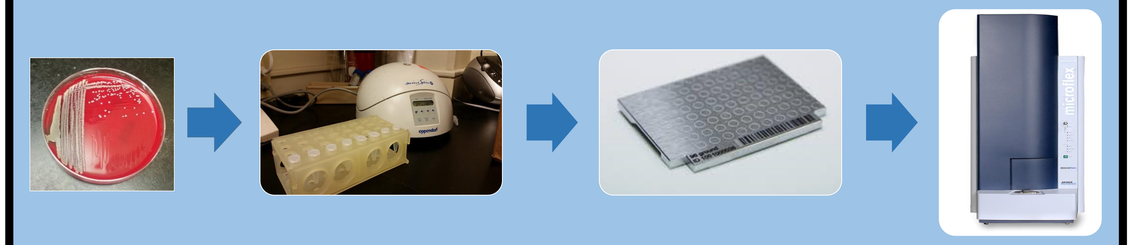
- *Staphylococcus aureus* is one of the most common pathogens associated with contagious mastitis in dairy cattle.
- Subspecies strain-typing is used to identify contagious strains within a herd.
- The current "gold standard" for strain-typing is pulsed-field gel electrophoresis (PFGE), however it is relatively expensive and time consuming; therefore, it is not frequently used in practice.
- Matrix assisted laser desorption ionization time of flight (MALDI-ToF) mass spectrometry identifies bacterial isolates based on each isolate's ribosomal proteome and has been shown to be more cost effective and less time consuming for speciating bacteria than genotypic methods. In addition, recent studies suggest that variability between spectrograms within a given genera and species of bacteria may be used to strain-type isolates.

Objective

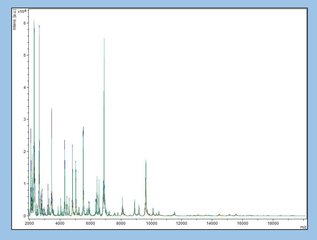
The purpose of this study was to determine whether MALDI-ToF could differentiate strains of *S. aureus* isolated from cow's milk with the same discriminatory power as PFGE.

Material and Methods

- Sixty-five (65) *S. aureus* isolates from 8 dairy herds were selected from a cryopreserved isolate bank. This group of isolates had previously been characterized by PFGE, and the prevalence of each strain type within herd was known. PFGE strain type was based on 100% similarity between PFGE banding patterns.
- Contagious strains were those in which more than one isolate was found within a given PFGE strain-type. Unique strains were defined as those strains that only occurred once within herd. For strains defined as contagious, up to three replicates of that strain-type were evaluated by MALDI-ToF.
- Isolates were cultured on blood agar and incubated at 37°C for 24 hours.
- Colonies were prepared for MALDI-ToF analysis using the manufacturer's described ethanol/formic acid protein extraction method. This method generated a liquid supernatant that was spotted onto 8 locations on the MALDI-ToF plate per isolate. Plates were run on the MALDI Biotyper (Bruker).



- A total of 24 spectra were obtained for each isolate (8 spots run 3 times each). Individual spectra were smoothened by subtracting baseline and removing outliers. From these spectra, a consensus spectra was created using the MALDI Biotyper software.



- MALDI-ToF spectra were then analyzed using Bionumerics 7.5 to generate dendrograms for isolates from within each herd. MALDI-ToF cluster analyses were calculated using the unweighted pair group method with arithmetic mean, and Pearson's correlation was used to measure the linear relationship between the two variables. MALDI-ToF cluster analysis results using similarity coefficients of 95% and 100% were then compared with existing PFGE strain-types.

Results

- PFGE identified 17 unique strain-types and 14 contagious strain-types within herd; 4 strain-types were found in >1 herd. Using a 100% similarity, MALDI-ToF identified 65 unique strain-types; whereas with a 95% similarity cut-off MALDI-ToF identified 24 strain-types (10 unique; and 14 contagious).
- While MALDI-ToF considered 14 strain-types contagious, 13 of these 14 stain-types contained multiple PFGE strain-types. In only 1 instance in Herd 2 was there 100% agreement between MALDI-ToF and PFGE (Table 1).

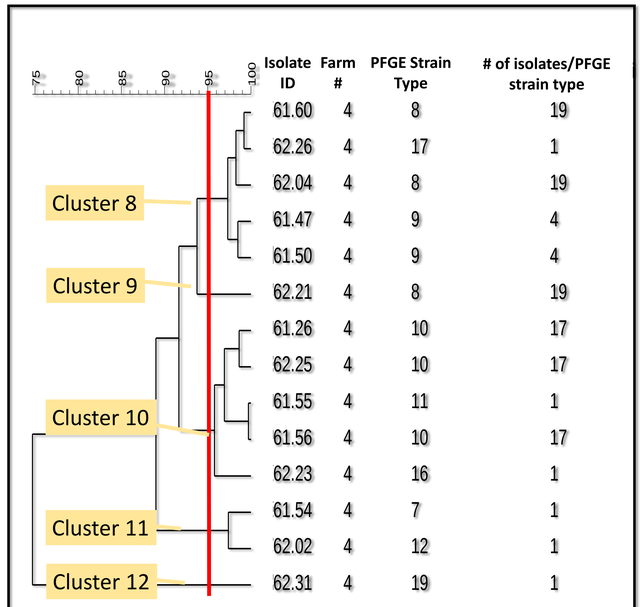


Figure 1. MALDI-ToF Dendrogram for Herd #4 using a 95% similarity cut-off. A total of 5 clusters are identified. Within some MALDI-ToF strain types (clusters), there was clustering of PFGE strain types (ex: PFGE strain-type 8 and 9 within MALDI cluster 8).

Herd	# of Isolates	MALDI Strain ID	# of isolates per MALDI strain	PFGE Strain Types
1	8	#1 #2 #3	2 1 5	1, 2 3 1, 2, 3
2	3	#4	3	4
3	4	#5 #6 #7	2 1 1	5,6 7 6
4	14	#8 #9 #10 #11 #12	5 1 5 2 1	8, 9, 17 8 10, 11, 16 7, 12 19
5	10	#13 #14 #15 #16	6 1 1 2	21, 22, 23, 24 22 22 24, 26
6	9	#17 #18 #19	2 1 6	28, 30 29 23, 27, 31
7	13	#20 #21 #22	7 1 5	32, 34, 37, 38, 39 35 8, 23, 31
8	4	#23 #24	3 1	40, 41, 42 43

Table 1. Distribution of MALDI-ToF strain-types and PFGE strain-types within each herd.

Conclusions

- A total of 65 isolates were evaluated which comprised 35 different PFGE strain types. At a 100% similarity cut-off, MALDI-ToF was more discriminatory than PFGE and showed no clustering with 65 unique strain-types, while at a 95% similarity cut off only 24 strain-types were identified making it less discriminatory than PFGE.
- While some isolates clustered according to the previously determined PFGE strain-type, most did not.
- Further work is necessary to determine the best way to analyze MALDI-ToF data.
- Future work will include a comparison of PFGE and MALDI-ToF data at different similarity cut-offs to see if agreement can be optimized.
- Ultimately, if MALDI-ToF can discriminate between contagious and unique (sporadic) strains within herd, it may prove useful in designing mastitis pathogen control programs and making culling decisions on the dairy farm..

Acknowledgements

Research funding and stipend support were provided by the USDA National Institute of Food and Agriculture, Animal Health project # 1008624.