Identifying coagulase negative staphylococcal species in dairy goats using MALDI-ToF mass spectrometry

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Background

Mastitis, an inflammation of the udder usually caused by an intramammary infection (IMI), can be a limiting factor in dairy goat milk production.

Intramammary infection is often caused by coagulase negative staphylococci (CNS) with different CNS species potentially having different impacts on udder health and duration of IMI.

Previously, PCR amplification and sequencing of housekeeping genes has been used to identify isolates by genus and species; however, it can be time-consuming and costly.

Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-ToF) may be a much more efficient technique to identify bacterial isolates by genus and species.

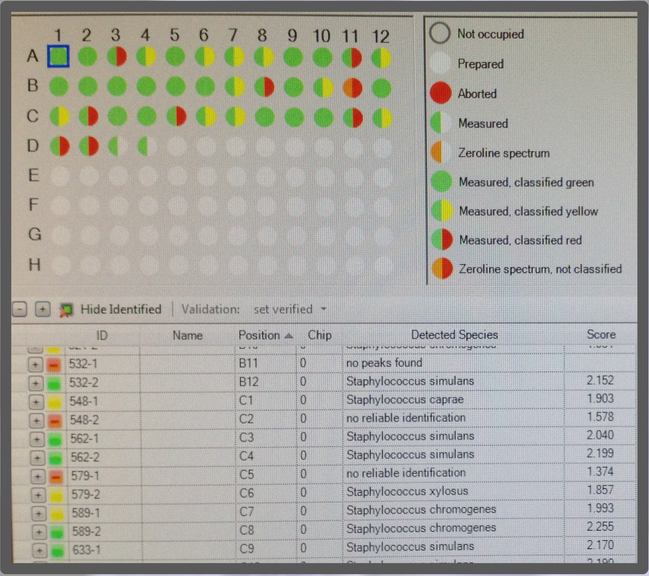


Figure 1 – MALDI-ToF rapidly analyzes microorganism protein profiles and compares results to a database. A genus and species is assigned with a confidence score.

Objective

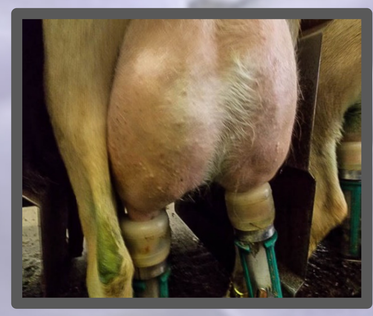
The goal of the present study was to determine the diagnostic utility of MALDI-ToF mass spectrometry in identifying CNS staphylococcal species isolated from cases of dairy goat mastitis.

Methods

184 isolates obtained from dairy goats with CNS intramammary infection were assigned a genus and species by sequencing of *rpoB* and *tuf* genes.

To date, 131 of these isolates also have been analyzed in duplicate by the Bruker Autoflex MALDI-ToF machine.

Results of MALDI-ToF were compared to genotyping and agreement was determined.



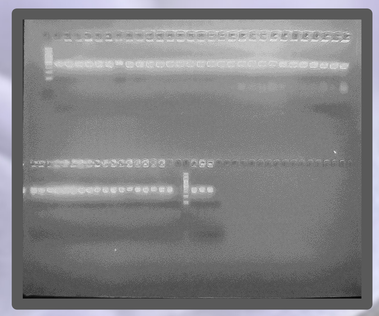


Figure 2 – (above right) An electrophoresis gel confirms the presence of a PCR amplicon of the *rpoB* gene. Only samples successfully amplified via PCR are sequenced to ensure proper identification.

Conclusions

To date, the results of this work are encouraging and suggest that MALDI-ToF may be a viable alternative to housekeeping gene sequencing as a method to speciate CNS isolated from goat's milk. This has the potential to reduce research costs and improve laboratory throughput when conducting mastitis research on dairy goats. Future aims are to improve sample preparation of MALDI-ToF samples to hopefully reduce the rate of misdiagnosis.

Results

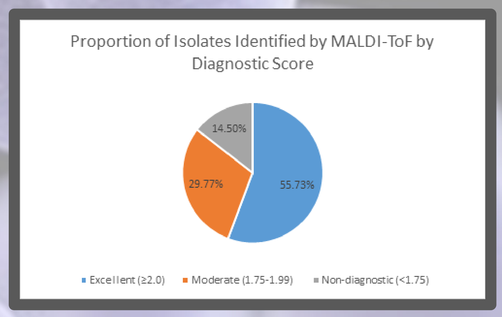


Figure 3. Each result produced by the MALDI-ToF mass spectrometry machine is awarded a score rating the confidence of the results. Overall, 85.5% of isolates had at least one of the duplicate samples with a score ≥1.75, which was considered diagnostic for a given genus and species.

To be an acceptable result, one of the two MALDI-ToF samples run for each isolate had to receive a confidence score at or above 1.75.

The final MALDI-ToF diagnosis (genus and species) was compared with the genus and species diagnosed by housekeeping gene sequence analysis to determine the sensitivity (Se) and accuracy of MALDI-ToF for making a genus and species identification. Nineteen isolates could not be identified by MALDI-ToF (14.5%). Of the remaining 112 isolates only one isolate was incorrectly identified by MALDI-ToF; hence, when a diagnosis was made by MALDI-ToF it was 99% accurate. If all isolates are considered the sensitivity and accuracy of MALDI-Tof was 85%. The lack of a definitive diagnosis for 19 of the isolates may be due to errors in plating and this hypothesis will be explored in the next phase of this research.

Acknowledgements

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