Rapid typing of *Mannheimia haemolytica* major genotypes 1 and 2 using MALDI-TOF mass spectrometry

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Introduction

Bovine respiratory disease (BRD) is one of the most costly diseases to cattle production throughout the globe, and losses in the US are estimated at one billion dollars annually. *Mannheimia haemolytica* is the most commonly detected bacterial agent associated with BRD. Recently, whole genome sequencing was employed on 1,133 *M. haemolytica* isolates from North American cattle and a nucleotide polymorphism typing system was developed. Two major genotypes were discovered (1 and 2). Genotype 1 *M. haemolytica* were mostly isolated from the nasopharynx of cattle without BRD. In contrast, genotype 2 *M. haemolytica* predominantly associated with the lungs of cattle with BRD and integrative conjugative elements that contained antimicrobial resistance determinants. The objective of this study was to develop a rapid matrix-assisted laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF MS) assay for the detection of *M. haemolytica* genotypes 1 and 2. MALDI-TOF MS is an emerging technology in diagnostic microbiology and is most frequently used for bacterial identification.

Materials and Methods

Thirty four isolates were used in this study that had been subjected to whole genome sequencing and nucleotide polymorphism typing, and that represented the major *M. haemolytica* genotypes 1 (n=23) and 2 (n=11) and subtypes 1b (n=6), 1b recombinant (n=1), 1c (n=3), 1e (n=2), 1f (n=4), 1i (n=7), 2b (n=6), 2c (n=2), 2d (n=1) and 2e (n=2). Biological (n=3) and technical (n=12) replication were utilized to collect 36 independent spectra from each isolate. Spectra were collected via the manufacturer's recommended procedures of formic and acetonitrile extraction with automated detection in linear mode between 2 K and 20 K *m/z*. Peak comparisons were made between each major genotype and subtype and a "Peak Statistic Table" was generated for each comparison which was used to develop a biomarker based model using the Quick Classifier tool to objectively and repeatably resolve genotypes.

Results

A 9,494 Da peak was detected for all *M. haemolytica* genotype 2 isolates and absent for all *M. haemolytica* genotype 1 isolates. Conversely, a 9,523 Da peak was detected for all *M. haemolytica* genotype 1 isolates and absent for all *M. haemolytic*a genotype 2 isolates. The biomarker based model was able to correctly classify all isolates into genotype 1 or genotype 2 when the majority of spectra were used for overall classification. Additionally, the direct smear extraction method, which is routinely used in diagnostic workflows for bacterial identification was able to generate spectra that enabled all isolates to be correctly classified.

Significance

A MALDI-TOF MS assay was developed that provides an effective, simple, and cost effective way to rapidly type *M. haemolytica* into clinically and biologically relevant genotypes. This assay should greatly enhance the ability of veterinary diagnostic labs to interpret *M. haemolytica* cultures, especially from nasal swabs or the upper respiratory tract where both genotypes are likely to be present and interpretation of culture results can be challenging. Assay data can be rapidly gathered in routine diagnostic workflows and the results integrated into diagnostic testing reports for enhanced bovine respiratory disease diagnosis.