Application of the matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry method to identify *Moraxella* spp isolated from cattle eyes

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Introduction

Infectious bovine keratoconjunctivitis (IBK) is an economically significant disease caused by the Gram-negative bacterium *Moraxella bovis*. *Moraxella bovoculi* also secretes virulence factors and is the most frequently isolated species from bovine eyes during IBK outbreaks. Whole genome sequencing has presented extensive genetic differences between *M. bovoculi* isolated from the eye and nasopharynx (NP) of cattle with and without IBK. At present, distinguishing between *Moraxella* species in clinical laboratory specimens is time consuming and relies on expensive and complex biochemical testing, of which reactions are not always consistent. Nucleic acid assays (PCR RFLP) can distinguish species, but can be costly and do not work on all strains. Identification of the specific etiologic agent during an IBK outbreak is critical to assist veterinarians implement the proper prevention and treatment strategies. Matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry is an emerging tool in bacterial identification that is rapid, inexpensive, and provides advantages over current identification methods. The overall goal of this study was to determine the accuracy of the MALDI-TOF MS method as applied to *Moraxella* spp isolated from bovine eyes and compare it to existing identification methods.

Materials and Methods

Moraxella bovoculi (n=240) and *Moraxella bovis* (n=22) were used in this study that had been subjected to whole genome sequencing using MiSeq (Illumina) and subsequent 16S rDNA analysis (*M. bovis*) or whole genome sequence identification (*M. bovoculi)*. Isolates represented included isolates from both outbreaks of clinical IBK and those from normal eyes in adults and young cattle. Isolates were subjected to the PCR RFLP test (Angelos 2007) and band sizes were evaluated using capillary gel electrophoresis. For MALDI-TOF mass spectrometry, spectra were collected using manufacturer's recommended procedures for the direct smear method and automated detection in linear mode between 2 K and 20 K m/z (Bruker Biotyper). Identifications were determined using commercial software (Bruker Biotyper) and the manufacturer's database (BDAL) or a modified database developed by the authors (UNL VDC) with additional spectra from reference isolates added, including additional *M. bovis*, *Moraxella ovis*, and *M. bovoculi* isolates. Comparisons were conducted using sequence information as the gold standard for identification.

Results

MALDI-TOF MS with the UNL VDC database was able to correctly classify 100% (250/250) of *M. bovoculi* and 91% (20/22) of *M. bovis*. The BDAL database correctly classified 99% (249/250) of the M. bovoculi and 45% (10/22) of the *M. bovis* isolates. In comparison, the PCR RFLP test was able to correctly classify 87.5% (210/250) of the *M. bovoculi* and 68% (15/22) of the *M. bovis*. Most of the disagreement was isolates identified as *M. bovoculi* by sequencing and MALDI-TOF MS that did not demonstrate a restriction site with RFLP and had an amplicon size consistent with *M. bovis*. Overall agreement between the MALDI-TOF-MS method and PCR RFLP for *M. bovoculi* was moderate with a kappa statistic of 0.4, and agreement between MALDI-TOF MS and sequencing was nearly perfect with a kappa statistic of 1.0

Significance

In conclusion, MALDI-TOF MS provides an effective, simple, and cost effective tool to rapidly identify *Moraxella* spp isolated from cattle eyes. This method has diagnostic utility and workflow advantages over the PCR RFLP method, and it was able to correctly classify all of the isolates of *M. bovoculi*. Although *M. bovis* was under-represented in the study, over 90% of the isolates tested were correctly classified by MALDI-TOF MS. Improvement of classification was also seen when additional reference spectra were added to the database, where 10 of the *M. bovis* isolates and an additional *M. bovoculi* isolate were correctly classified. This enhanced MALDI-TOF MS database with inclusion of additional reference spectra enhances the ability to identify bovine *Moraxella* and database sharing may enable users of the platform to further enhance identification capabilities.