Analyses of Bordetella isolates collected from turkeys with respiratory disease using MALDI-TOF mass spectroscopy and comparison to a Bordetella avium vaccine

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ABSTRACT

Bordetella avium has been isolated from turkeys showing clinical signs of respiratory disease and increased mortality in Sanpete County Utah, despite receiving the B. avium vaccine. To determine if recent B. avium isolates are related, or unrelated to the vaccine strain, twenty-five isolates from different time periods and different locations in the U.S. were collected for comparison by MALDI-TOF mass spectroscopy. Spectra were evaluated by MALDI Biotyper software (Bruker Co.) to determine relationships among the clinical isolates. Cluster analysis of the spectra showed four major clusters using the principle component scores for the three spectral peaks in highest abundance. These clusters also accounted for >70% of the variability in the data based on identification score values. Four of five Utah isolates were in the same cluster as the vaccine strain. However, one isolate from Utah and isolates from other locations did not cluster with the vaccine strain.

INTRODUCTION

Tracheal swab cultures were used to isolate Bordetella avium from turkeys showing clinical signs of respiratory disease and increased mortality in Sanpete County Utah, despite receiving the B. avium vaccine. To determine if recent B. avium isolates are related, or unrelated to the vaccine strain, twenty-five isolates from different time periods and different locations in the U.S. were collected for comparison by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectroscopy.

MALDI-TOF mass spectroscopy is a powerful new tool for identification of pathogens in clinical specimens. Compared with other methods, including 16S rRNA and rpoB gene sequencing, reported accuracy of identification of bacteria, fungi or parasites has been 95.4% to 99.5% (Seng, et al., 2009). Bacterial colonies are mixed with a matrix solution and placed and dried onto a target plate. The matrix solution (cinnamic acid) co-crystallizes with the bacterial sample on the target plate (96 plate sample). Samples are then exposed to short laser pulses under high vacuum, vaporizing the bacteria and the matrix. Bacterial proteins are ionized, and an electromagnetic field accelerates the ions as they enter the flight tube. The time of flight (TOF) required for analytes to reach the detector at the end of the flight tube is measured and recorded. A “characteristic spectrum” based on the TOF provides a specific sample fingerprint, considered unique for each bacterial species resulting in precise identification (Clark, et al., 2013). Software compares the collected spectra with a databank of reference spectra of bacterial isolates. A numerical score value of similarity to known isolates’ spectra is calculated. A score value above 2.0 is defined as a valid species level identification, while values between 2.0 and 1.7 represent reliable genus level identifications.

METHODS

Protocol for MALDI-TOF Mass Spectroscopy

Spectra were obtained from clinical isolates collected from turkeys, grown overnight on blood agar plates, and evaluated by MALDI-TOF mass spectroscopy. The MALDI-TOF spectra from the clinical isolates were evaluated using the Bruker MALDI Biotyper software (ID2 MALDI Biotyper 2.3) to determine relationships among the clinical isolates. This was done by comparing the MALDI-TOF results to the database of microorganisms using the Biotyper software for peak-matching and by multivariate analyses using the three principal component scores (Ringer, 2008). After completion of the peak-matching algorithm, the score value was evaluated for the suggested matches. A score between 2.3 and 3.0 had a high degree of confidence that the correct species was identified. A score between 2.0 and 2.29 was also reliable, although it indicates a lower level of confidence in the species identification. We manually selected samples with high scores and processed the data sets into both dendrograms and cluster charts. Types of analyses included: presumptive species, location of collection, and year of collection. In the cluster charts, the relative distance between points is indicative of bacterial similarity.

RESULTS

Spectra were evaluated by MALDI Biotyper software to determine relationships among clinical isolates. Cluster analysis of the spectra showed four major clusters using the principle component scores for the three spectral peaks in highest abundance. These clusters also accounted for >70% of the variability (considered significant) in the data based on identification score values (Figure 1). Four of five Utah isolates were in the same cluster as the vaccine strain. However, one isolate from Utah and isolates from other locations did not cluster with the vaccine strain.

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