Genetic characterization of *Mannheimia haemolytica* isolated from high risk stocker cattle

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Introduction

Antimicrobial resistance in *Mannheimia haemolytica* (*Mh*) is an emerging issue. Documenting the resistance genes present in this pathogen, and the role a gene may or may not play in a resistant phenotype is an important step in understanding the epidemiology of multi-drug resistant *Mh*. Therefore, our objectives were to identify and map the resistance genes present in *Mh* isolates collected from calves prior to and following treatment with the antimicrobial drug tulathromycin, and assess the concordance between genotype and resistance phenotype.

Materials and Methods

Deep nasopharyngeal swabs (NPS) were collected from 169 bull and steer calves at arrival to a stocker facility. Calves were processed following standard industry protocol and received a metaphylactic dose of tulathromycin. A second NPS was collected 10 to 14 days later at revaccination. For samples culture positive for *Mh*, a maximum of 3 colonies from each sample were subcultured and submitted for antimicrobial susceptibility testing. DNA was extracted and whole genome sequencing was performed on isolates from calves *Mh* positive at both time points. Phylogenetic trees were constructed to illustrate the phylogenetic relationships between these "matched" isolates. The sequences were also BLASTed against resistance genes documented in the Comprehensive Antibiotic Resistance Database (CARD) and the Microbial Ecology Group Resistance Database (MEGARes).

Results

There were 22 calves culture positive at both time points, and a total of 50 isolates with unique susceptibility profiles; 27 from arrival samples and 23 from revaccination. Of all arrival isolates, 15 (55.6%) were susceptible to all antimicrobials tested, 10 (37%) were intermediate or resistant to 1 class of antimicrobial, and 2 (7.4%) were intermediate or resistant to 2 classes of antimicrobials. Of the revaccination isolates, none (0%) were susceptible to all antimicrobials tested, 11 (47.8%) were resistant or intermediate to 3 classes of antimicrobials, and 12 (52.2%) were resistant or intermediate to 4 classes of antimicrobials. Phylogenetic analysis of the sequences of these 50 isolates revealed 5 genetically distinct groups. All of the isolates collected at revaccination belonged to 1 group and were clonal. The remainder of the arrival isolates comprised 4 distinct clonal groups. There were a total of 16 resistance genes detected in all 50 isolates, representing 7 different classes of antimicrobials. In the isolates from revaccination, many of these genes were located very closely together on the same contig within each genome. Point mutations resulting in amino acid changes were identified in the genes *gyrA* and *parC*, which are involved in fluoroquinolone susceptibility. In all isolates resistant to fluoroquinolones, there was an amino acid change from S to F at amino acid 83 and from D to N at amino acid 87 in *gyrA*. In *parC*, there was a change from E to K at amino acid 84. Concordance between genotype and resistance phenotype was 94% for *floR* and florfenicol susceptibility, and 100% for *tetH* and tetracycline. For the macrolides, to be concordant, an isolate must be susceptible to a given macrolide and negative for all macrolide resistance genes or, resistant/intermediate and positive for any 1 macrolide resistance gene.For tilmicosin, concordance was 80%, for gamithromycin 96%, and for tulathromycin, 90%.

Significance

There was an increase in the number of resistance genes identified in isolates from arrival to revaccination. In addition, there was a decrease in the genetic diversity of the isolates from arrival to revaccination. Furthermore, the presence or absence of a resistance gene is not always the best indicator of phenotypic resistance. It is important that we continue to study the role of antimicrobial resistance genes in the *Mh* genome on phenotypic resistance, and to assess the role of antimicrobial exposure to the development of multi-drug resistance.