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Antimicrobial Multi Drug Resistance and Co-resistance patterns of *Mannheimia*
haemolytica Isolated from Bovine Respiratory Disease Cases – A three year (2009-
2011) Retrospective Analysis

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Running Title: Prevalence of Multi Drug Resistant *Mannheimia haemolytica*

Abstract

Bovine respiratory disease continues to be the most important ailment of feedyard cattle. While the disease is multifactorial in nature, therapy continues to target the primary bacterial pathogens, *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*. A survey of records from a single diagnostic laboratory was conducted to evaluate the percentage of *Mannheimia haemolytica* isolates that were resistant to multiple antimicrobials and if co-resistance patterns could be detected. All susceptibility test results for *Mannheimia haemolytica* recovered from lung tissues of cattle were eligible for inclusion in the survey. There were no isolates over the course of the analysis that were resistant to all 6 antimicrobials, primarily due to a lack of resistance to ceftiofur. In 2009, just over 5% of isolates were resistant to 5 or more antimicrobials (pan-resistant). In 2011, over 35% of the *Mannheimia haemolytica* isolates were characterized as pan-resistant. Significant antimicrobial co-resistance patterns were only seen with oxytetracycline and tilmicosin; bacterial isolates that were resistant to either oxytetracycline or tilmicosin were more likely to be resistant to at least one other antimicrobial. The mechanisms by which *Mannheimia haemolytica* is developing multi drug resistance warrant investigation if antimicrobial utility in the therapy of bovine respiratory disease is to be preserved.

Key Words: Antimicrobial resistance, Bovine Respiratory Disease, *Mannheimia haemolytica*, Susceptibility testing

Bovine respiratory disease (BRD) continues to be one of the most important diseases of feedlot cattle.²⁰ The economic losses due to this disease have been estimated to approach \$1 billion dollars in the United States alone, due to increased drug and labor costs, decreased production and animal death losses.⁹ Applying this estimate today, however, does not account for the increasing pattern, and associated costs, of antimicrobial resistance among the BRD pathogens.¹⁶ While the exact cost of antimicrobial resistance in cases of BRD is unknown, this type of analysis in human cases has shown the economic impact of antimicrobial resistance to be significantly increased, both in terms of dollars and mortality.^{3,19}

It is not uncommon to find large scale summaries of susceptibility data for bovine bacterial pathogens.^{8,10,12,17,23} The data in these summaries are generally presented as either: 1) the percentage of isolates that are susceptible or resistant or 2) the MIC₅₀ / MIC₉₀ for the isolates tested. While this information is useful for evaluating resistance of specific antimicrobials or antimicrobial classes, it does not allow for the evaluation of multi drug resistance for the individual isolates. Data regarding the prevalence of multi drug antimicrobial resistance would clarify the role of susceptibility testing in BRD cases and would allow veterinary clinicians to design more effective empirical treatment protocols.

The primary objective of the current retrospective analysis was to determine the prevalence of multi drug resistant *Mannheimia haemolytica* isolates from bovine respiratory disease cases. The secondary objective was to determine if co-resistance was significantly associated with certain antimicrobials.

All diagnostic records of the Kansas State Veterinary Diagnostic Laboratory from January 1, 2009 through December 31, 2011 were included in the initial search. Records were included in the final analysis if they met the following criteria: 1) specimen was bovine lung; 2)

culture positive for *Mannheimia haemolytica*; 3) susceptibility test results were available; 4) isolate was from a clinical case (research isolates excluded).

During the survey period, all susceptibility testing was performed using broth microdilution methods as recommended by Clinical and Laboratory Standards Institute (CLSI).⁶ Briefly, growth from an overnight culture was used to directly suspend colonies into cation-adjusted Mueller-Hinton broth. Suspensions were adjusted to deliver approximately 5×10^5 CFU/mL per well to the plates.^a Inoculation was performed using an automated delivery device^b and plates were incubated for 18 to 24 hours in a 35°C, non-CO₂ incubator. In 2009 and 2010, plates were read using a manual system.^c In 2011, plates were read using a fully automated reading system.^d

Only antimicrobials with CLSI approved interpretive criteria for *Mannheimia haemolytica* isolated from bovine respiratory disease were evaluated in this study.⁶ These antimicrobials included: ceftiofur, danofloxacin, enrofloxacin, florfenicol, oxytetracycline, spectinomycin, tilmicosin, and tulathromycin.

Descriptive analysis was completed using a commercial spreadsheet program.^e Data analysis in the odds ratio portion was completed using a commercial statistical software program.^f Logistic regression (generalized mixed) models were used to analyze the probability that resistance to a given agent was associated with resistance to at least one other antimicrobial (co-resistance). The random effect was animal-owner within year to reflect the lack of independence between samples. A *P*-value of 0.10 was considered significant for all models.

The search yielded 55, 155, and 179 eligible bacterial isolates from years 2009, 2010, and 2011, respectively. Following the initial analysis of 2011 data, strong relationships within drug class were noted for the fluoroquinolones and macrolides. The susceptibility test results for

enrofloxacin and danofloxacin were equivalent within the error of the test (± 1 dilution) for 177 of the 179 isolates. For the macrolide class, the interpretation was “resistant” for both tilmicosin and tulathromycin in 153 of 179 (85.5%) isolates. Of the remaining 26 isolates, 14 were interpretation discrepancies of “intermediate” and “susceptible”, which had no effect on the outcome variable of concern (“resistant”) in this report. Seven of the remaining isolates were “resistant” to tulathromycin and “intermediate” or “susceptible” to tilmicosin. The remaining 5 isolates were “resistant” to tilmicosin and “intermediate” or “susceptible” to tulathromycin. To eliminate the antimicrobial class effect, danofloxacin and tulathromycin were excluded from the final analysis. The class effect was not evaluated for 2009 and 2010 as the objective was to compare the same antimicrobials across the three year period.

The contribution of isolates from individual premises was evaluated to control for bias in the data due to clonal isolates. In this data set, the 389 isolates originated from 266 unique premises. The majority of these premises (75.2%) are represented by only a single isolate in the three year data set (Fig. 2). Less than 10% of the premises are represented by 3 or more isolates. The highest number of isolates from a single premise was nine ($n=1$). Removing isolates from the same premise with identical susceptibility profiles in a given calendar year had minimal effects on the outcomes; 2011 isolates characterized as “resistant to 5 antimicrobials” would decrease by 3.75%, all other year-resistance classifications were affected by less than 2% (data not shown). No isolates were excluded from the data set because of their premise origin. Doing so for these particular isolates had minimal effects on outcomes and represents an overly conservative approach to estimating multi drug resistance.

Over the three year period, there were no bacterial isolates that were resistant to all 6 antimicrobials. This was primarily due to a general lack of resistance to ceftiofur. Only 2

Mannheimia haemolytica isolates were classified as resistant to ceftiofur during the entire surveyed period (these isolates were susceptible to other antimicrobials). In 2009, almost 35% of *Mannheimia haemolytica* isolates were susceptible to all 6 antimicrobials tested (pan-susceptible). Isolates resistant to 1, 2, 3, 4 and 5 antimicrobials made up 9%, 15%, 13%, 24% and 5% of recovered *Mannheimia haemolytica*, respectively. In 2011, 17%, 8%, 12%, 3%, 25% and 35% of isolates were resistant to 0, 1, 2, 3, 4, and 5 antimicrobials, respectively. (Fig. 1). Using resistance to 3 or more antimicrobials as the definition for multi drug resistance, 42%, 46% and 63% of the isolates would be classified as multi drug resistant in 2009, 2010, and 2011, respectively.

In determining co-resistance patterns, isolates found to be resistant to oxytetracycline were 3.52 times more likely ($P = 0.04$) to be resistant to one or more additional antimicrobials compared to non-oxytetracycline resistant isolates (Table 1). Isolates resistant to tilmicosin were 2.64 times more likely ($P = 0.06$) to be resistant to at least one other antimicrobial (Table 1). There were no statistically significant co-resistance patterns for enrofloxacin, florfenicol or spectinomycin over the three year period. Due to low numbers of ceftiofur resistant isolates, the odds ratio was not calculated for this antimicrobial.

Antimicrobial resistance in veterinary medicine has received a considerable amount of recognition as a potential factor leading to antimicrobial resistance in human medicine.^{1,2,22} However, the contribution of multi drug resistance to limited or failed therapy in veterinary patients has received much less attention.¹⁵ Previous reports on multi drug resistance in *Mannheimia haemolytica* from cattle have been limited by low numbers (<30) of isolates or testing antimicrobials without CLSI approved interpretive criteria.^{4,5,7,18,24} A comprehensive study evaluating resistance in *Mannheimia haemolytica* reported very low rates (1.2%) of multi

drug resistance.¹³ In that study, isolates were obtained from the nasopharynx of cattle upon entry into and exit from 2 Canadian feedyards from September 2008 to February 2009. The higher rates of multi drug resistance reported here could be a result of geographical and/or temporal factors. These differences might also be explained by the methods used to select isolates; the Canadian study surveyed nasal flora of healthy animals, while the current study retrospectively analyzed isolates from lung tissue of deceased animals.

The results of the current report indicate that a high percentage of *Mannheimia haemolytica* isolates recovered from bovine lung at the Kansas State Veterinary Diagnostic Laboratory are multi drug resistant. Because there are a limited number of antimicrobial classes indicated for treatment of BRD and restrictions on the extra-label use of therapeutics in food animals, multi drug antimicrobial resistance in the BRD pathogens poses a severe threat to the livestock industry.

The findings of the present study also emphasize the importance of antimicrobial susceptibility testing in the management of bovine respiratory disease. In 2011, the majority (82.7%) of isolates were resistant to at least one antimicrobial. Although ceftiofur was generally susceptible and oxytetracycline and tilmicosin were associated with co-resistance, the patterns of resistance to other antimicrobials were largely unpredictable. Together, these factors would support the justification of susceptibility testing both from efficacy and antimicrobial stewardship standpoints. The turnaround time of traditional culture and susceptibility testing makes it impractical for individual case management. However, if used in early BRD cases, it can be useful for justifying treatment protocol deviations in future cases within the herd or group setting.

There are several limitations to the data summarized in this report. As with all retrospective surveys of diagnostic submissions, the isolates selected for testing are not a random sample of all *Mannheimia haemolytica* isolates.¹¹ As the isolates in this report are primarily from Kansas and Nebraska, there is the potential for geographical bias. Similar retrospective analyses of human clinical isolates have shown strong regional distribution patterns of antimicrobial resistance phenotypes.²¹ The overrepresentation of feed yard submissions in these data likely reduce the external validity of the results to different cattle industry subgroups, but these limitations do not limit the importance of these findings, especially to feed yard veterinarians in the Midwest. These data would support the need for similar analyses in other regions of the United States.

In the current study, the selection criteria used (only isolates from lung tissue were included) creates a bias toward isolates causing clinical disease. Although unknown in the majority of submissions, the assumption with regard to isolates from lung tissue is that the animal died from BRD. The correlation between virulence of the bacterial organism and antimicrobial resistance phenotype is unknown, however isolates from clinical cases are more likely to have had previous exposure to antimicrobials. Whether an animal had received prior antimicrobial treatments and/or the timing of therapy relative to death of the animal are potential confounding factors that are unknown in the majority of cases reported here. Although the effect of prior treatment could not be evaluated here, it may be of minimal importance as a previous study reported that the antimicrobials used antemortem had little impact on the postmortem susceptibility patterns.¹⁴ While selection pressure from prior antimicrobial therapy may or may not impact the *percentages* of isolates reported here, the *presence* of these isolates cannot be dismissed.

Despite these limitations, this report provides a regional perspective on multi drug antimicrobial resistance in *Mannheimia haemolytica* isolates from cattle. This survey was not designed to determine the clinical implications of multi drug resistance or the mechanisms by which these isolates are developing resistance. However, these would be critical topics for further investigation if antimicrobial utility in food animals is to be preserved. This study also highlights the need for continuing, prospective monitoring programs.

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Sources and manufacturers

^a BOPO6F, Thermo Scientific – Trek Diagnostic Systems, Cleveland, OH.

^b Sensititre AIM, Thermo Scientific – Trek Diagnostic Systems, Cleveland, OH.

^c Sensititre Vizion System, Thermo Scientific – Trek Diagnostic Systems, Cleveland, OH.

^d Sensititre ARIS 2X, Thermo Scientific – Trek Diagnostic Systems, Cleveland, OH.

^e Excel 2010, Microsoft Corporation, Redmond, WA.

^f STATA v.12.0, StataCorp, College Station, TX.

Declarations of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Brian Lubbers has an active consulting agreement with Merck Animal Health.

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Tables

Table 1. Antimicrobial co-resistance patterns of *Mannheimia haemolytica* isolates.

| Antimicrobial | Odds Ratio [‡] | 95% Confidence Interval | P- Value |
|-----------------|-------------------------|-------------------------|----------|
| Enrofloxacin | 0.71 | 0.32 - 1.58 | 0.41 |
| Florfenicol | 1.43 | 0.63 - 3.25 | 0.40 |
| Oxytetracycline | 3.52 | 1.07 - 11.61 | 0.04 |
| Spectinomycin | 1.08 | 0.48 - 2.44 | 0.86 |
| Tilmicosin | 2.64 | 0.97 - 7.17 | 0.06 |

‡ Odds ratio is the odds of an isolate being resistant to one or more other antimicrobials given resistance to the antimicrobial listed compared to the odds of an isolate being resistant to one or more other antimicrobials given the isolate is not resistant to the antimicrobial listed.

Figure 1

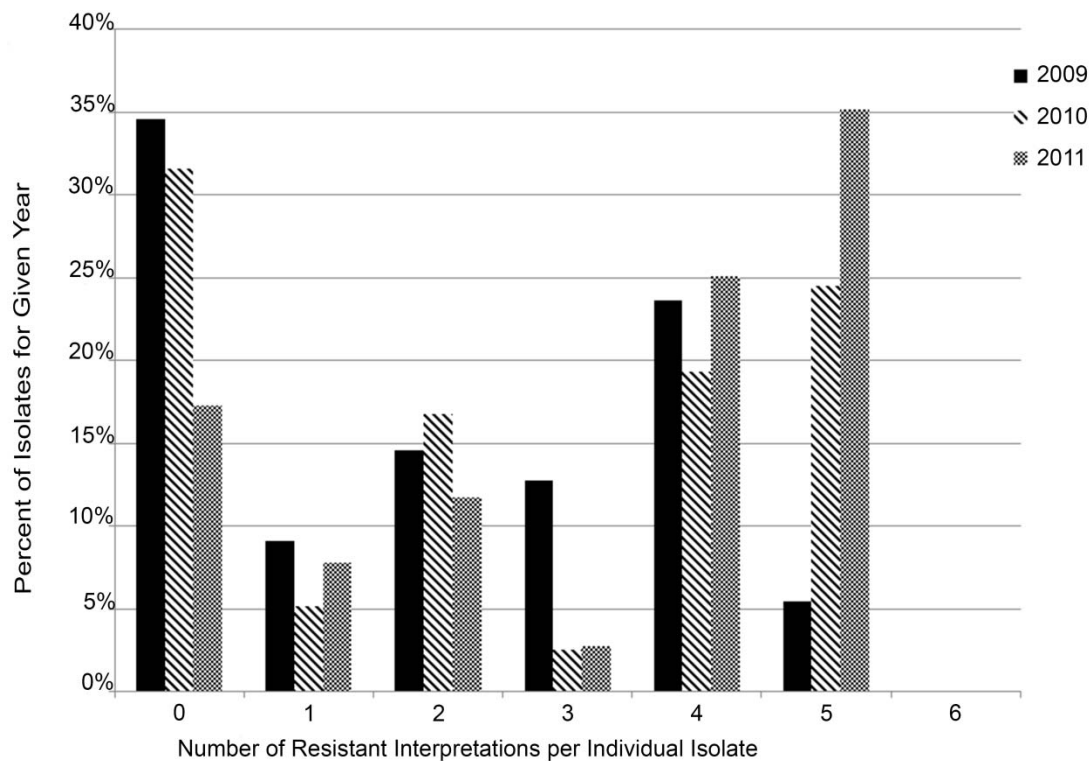


Figure 1. The percentage of *Mannheimia haemolytica* isolates, by year, which were resistant to 0, 1, 2, 3, 4, and 5 antimicrobials, respectively. Isolates in the 0 column would be considered pan susceptible isolates. There were no isolates resistant to all 6 antimicrobials over the course of the survey.

Figure 2

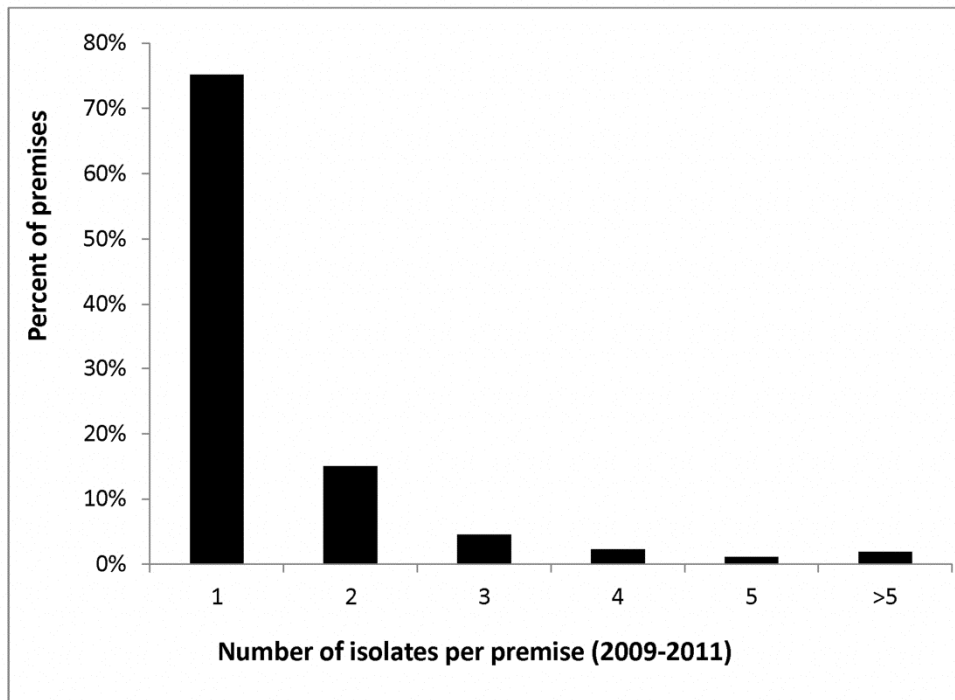


Figure 2. The number of isolates recovered per premise during the 3 year survey period. The 389 *Mannheimia haemolytica* isolates originated from 266 unique premises. The highest number of isolates recovered from a single premise was nine.