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Antimicrobial multidrug resistance and coresistance patterns of *Mannheimia haemolytica* isolated from bovine respiratory disease cases—a three-year (2009–2011) retrospective analysis

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Abstract. Bovine respiratory disease continues to be the most important ailment of feed yard 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 *M. haemolytica* isolates that were resistant to multiple antimicrobials and if coresistance patterns could be detected. All susceptibility test results for *M. 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, more than 35% of the *M. haemolytica* isolates were characterized as pan-resistant. Significant antimicrobial coresistance 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 *M. haemolytica* is developing multidrug 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 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 minimal inhibitory concentration for either 50% or 90% of isolates tested. While this information is useful for evaluating resistance of specific antimicrobials or antimicrobial classes, it does not allow for the evaluation of multidrug resistance for the individual isolates. Data regarding the prevalence of multidrug 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 multidrug-resistant *Mannheimia haemolytica* isolates from BRD cases. The secondary objective was to determine if coresistance was significantly associated with certain antimicrobials.

All diagnostic records of the Kansas State Veterinary Diagnostic Laboratory (Manhattan, Kansas) 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 *M. haemolytica*, 3) susceptibility test

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results were available, and 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 the 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 18–24 hr in a 35°C, non-CO_2 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 *M. haemolytica* isolated from BRD were evaluated in the current study.[6] The 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 1 other antimicrobial (coresistance). 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 susceptibility interpretation was the same 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 the present 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 3-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 3-year data set (Fig. 1).

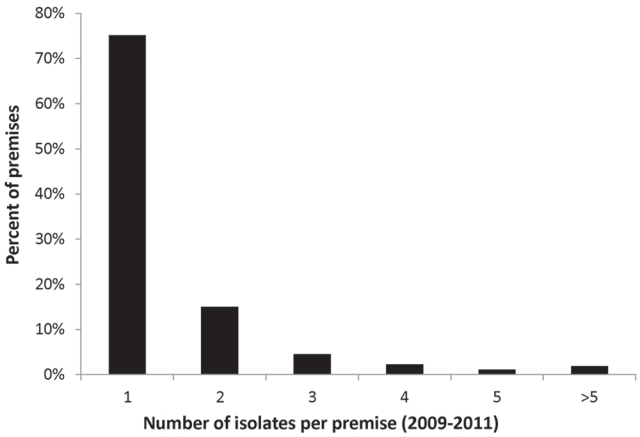

Figure 1. The number of isolates recovered per premise during the 3-year survey period. The 389 *Mannheimia haemolytica* isolates originated from 266 unique premises. The highest number of isolates recovered from a single premise was 9.

Less than 10% of the premises are represented by 3 or more isolates. The highest number of isolates from a single premise was 9 (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 multidrug resistance.

Over the 3-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 *M. 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 *M. 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 *M. 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. 2). Using resistance to 3 or more antimicrobials as the definition for multidrug resistance, 42%, 46%, and 63% of the isolates would be classified as multidrug resistant in 2009, 2010, and 2011, respectively.

In determining coresistance patterns, isolates found to be resistant to oxytetracycline were 3.52 times more likely (P = 0.04) to be resistant to 1 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 1 other antimicrobial (Table 1). There were no statistically significant coresistance patterns for enrofloxacin, florfenicol, or spectinomycin over

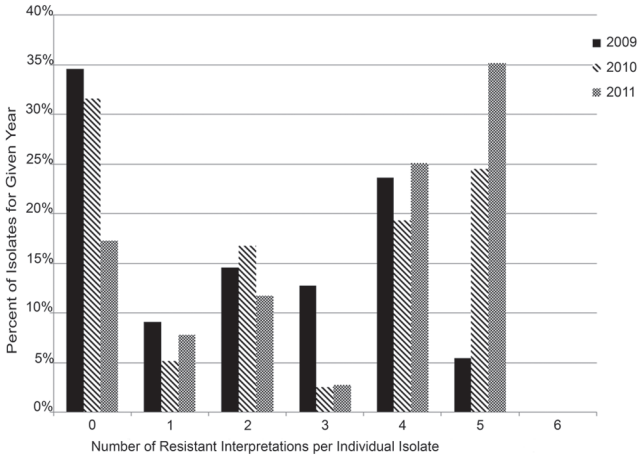

Figure 2. The percentage of *Mannheimia haemolytica* isolates, by year, that were resistant to 0, 1, 2, 3, 4, and 5 antimicrobials, respectively. Isolates in the 0 column would be considered pan-susceptible isolates. There were no isolates resistant to all 6 antimicrobials over the course of the survey.

Table 1. Antimicrobial coresistance patterns of *Mannheimia haemolytica* isolates in the current study.

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 1 or more other antimicrobial given resistance to the antimicrobial listed compared to the odds of an isolate being resistant to 1 or more other antimicrobials given the isolate is not resistant to the antimicrobial listed.

the 3-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 multidrug resistance to limited or failed therapy in veterinary patients has received much less attention.[15] Previous reports on multidrug resistance in *M. 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 published in 2011 evaluating resistance in *M. haemolytica* reported very low rates (1.2%) of multidrug resistance.[13] In that 2011 study, isolates were obtained from the nasopharynx of cattle upon entry into, and exit from, 2 Canadian feed yards from September 2008 to February 2009. The higher rates of multidrug resistance reported in the current study 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 *M. haemolytica* isolates recovered from bovine lung at the Kansas State Veterinary Diagnostic Laboratory are multidrug resistant. Because there are a limited number of antimicrobial classes indicated for treatment of BRD and restrictions on the extralabel use of therapeutics in food animals, multidrug 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 BRD. In 2011, the majority (82.7%) of isolates were resistant to at least 1 antimicrobial. Although ceftiofur was generally susceptible, and oxytetracycline and tilmicosin were associated with coresistance, 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 the current report. As with all retrospective surveys of diagnostic submissions, the isolates selected for testing are not a random sample of all *M. haemolytica* isolates.[11] As the isolates in the present 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 such data likely reduces 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. The data presented in the current study would support the need for similar analyses in other regions of the United States.

In the present 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 herein. Although the effect of prior treatment could not be evaluated in the current study, 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 herein, the presence of these isolates cannot be dismissed.

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

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Sources and manufacturers

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b. Sensititre AIM, Trek Diagnostic Systems Inc., Cleveland, OH.
c. Sensititre Vizion System, Trek Diagnostic Systems Inc., Cleveland, OH.
d. Sensititre ARIS 2X, Trek Diagnostic Systems Inc., Cleveland, OH.
e. Excel 2010, Microsoft Corp., Redmond, WA.
f. Stata v. 12.0, StataCorp LP, College Station, TX.

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