Animal Health Research Reviews

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Antimicrobial resistance in *Mannheimia haemolytica*: prevalence and impact

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Review

Cite this article: Credille B (2020). Antimicrobial resistance in *Mannheimia haemolytica*: prevalence and impact. *Animal Health Research Reviews* **21**, 196–199. https://doi.org/10.1017/S1466252320000109

Received: 25 November 2019
Revised: 27 January 2020
Accepted: 5 May 2020
First published online: 2 December 2020

Key words:
Antimicrobial resistance; bovine respiratory disease; *Mannheimia haemolytica*

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Abstract

Bovine respiratory disease (BRD) is the most common cause of morbidity and mortality in North American beef cattle. In recent years, isolation of strains of *Mannheimia haemolytica* that are resistant to multiple different classes of antimicrobials has become commonplace. New research would suggest that the routine use of antimicrobials by some cattle operations might be driving emerging resistance patterns, with the majority of the spread observed due to propagation of strains of *M. haemolytica* that have acquired integrative conjugative elements. To date, there is little information evaluating the impact of antimicrobial resistance on clinical outcome in cattle with BRD.

Introduction

Bovine respiratory disease (BRD) is the most common and costly disease affecting beef cattle in North America (Tennant *et al.*, 2014; Magstadt *et al.*, 2018). Within feedlots, BRD is responsible for approximately 75% of all morbidity and 50% of all mortality. In stocker calves, BRD occurs at a much greater frequency than is commonly seen in feedlot cattle and is estimated to be responsible for 90% of all morbidity and mortality in these operations. While multiple factors play a role in the development of BRD, bacteria, particularly *Mannheimia haemolytica*, are ultimately responsible for the clinical signs observed in affected cattle. For this reason, antimicrobials are a mainstay of BRD therapy. Unfortunately, antimicrobial resistance is an emerging issue in BRD pathogens and isolation of multi-drug resistant (MDR) strains of *M. haemolytica* has become a more frequent occurrence. The purpose of this manuscript is to review the literature as it pertains to antimicrobial resistance in common BRD pathogens, particularly *M. haemolytica*, and how resistance might impact the outcome of therapy in cattle diagnosed with BRD.

Resistance defined

Veterinarians are most concerned about clinical resistance, in other words, what is the probability that a specific antimicrobial will effectively treat an animal infected by a specific pathogen causing a particular disease (Apley, 2003). The concept of clinical resistance is based on clinically derived breakpoints developed by the Clinical and Laboratory Standards Institute Veterinary Antimicrobial Susceptibility Testing Committee (CLSI VAST) using the following criteria (Apley, 2003):

1. Range of *in vitro* minimum inhibitory concentration (MICs) of an antimicrobial for a representative population of a specific bacterial pathogen.
2. Pharmacokinetic/Pharmacodynamic parameters established on the basis of the relationship between drug concentration and microbial susceptibility.
3. Results of clinical trials in the target species.

For BRD, the CLSI has approved BRD-specific breakpoints for penicillin (broth dilution only), ceftiofur, danofloxacin, enrofloxacin, florfenicol, spectinomycin sulfate, tulathromycin, gamithromycin, tildipirosin, tetracycline (broth dilution only), and tilmicosin (Table 1). With these antimicrobial agents, a susceptible result indicates that the likelihood of treatment success is significantly greater than if the result indicated resistance. It is important to remember, however, that the relationship between antimicrobial susceptibility testing and clinical outcome is not perfect and these breakpoints apply only when the antimicrobial is used according to label directions and the susceptibility testing is performed using CLSI-approved methods and interpretive criteria. It is also important to realize that antimicrobial susceptibility testing does not guarantee a specific clinical result in an individual animal. Susceptibility breakpoints attempt to take an *in vitro* test result and extrapolate it to an *in vivo* response and, often, disease outcome is influenced by factors such as host immune status, variations in individual

Table 1. Antimicrobial-pathogen combinations with CLSI-approved breakpoints for bovine respiratory disease

Antimicrobial	Pathogens
Ceftiofur	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni
Danofloxacin	*Mannheimia haemolytica*
	Pasteurella multocida
Enrofloxacin	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni
Florfenicol	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni
Gamithromycin	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni
Penicillin[a,b]	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni
Spectinomycin	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni
Tetracycline[b]	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni
Tildipirosin	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni
Tilmicosin	*Mannheimia haemolytica*
Tulathromycin	*Mannheimia haemolytica*
	Pasteurella multocida
	Histophilus somni

[a]Only applies to the procaine penicillin G formulation used at 22,000 IU kg^{-1} IM q 24 h.
[b]Approved breakpoints only valid for broth dilution.

pharmacokinetic parameters, or increased disease severity/prolonged disease duration. For antimicrobials without CLSI-approved breakpoints, the interpretations have been adapted from interpretive criteria extrapolated from plasma and interstitial fluid in other species. Examples of this approach include penicillin G (disk diffusion), tetracycline (disk diffusion), potentiated sulfonamides, aminoglycosides, and erythromycin. For these antimicrobial agents, a susceptible result is certainly better than a resistant one. However, there are no data available to correlate the results of susceptibility testing and expected outcome in cattle with BRD.

The second type of resistance is defined based on data surveilling changes in profiles of susceptibility distributions in wild-type populations of bacteria (Lubbers and Turnidge, 2015). Rather than providing data correlated to clinical outcome, these epidemiologic cut-offs represent deviations of the MIC from the original bacterial population and can be used to indicate the appearance of resistance genes. As a result, epidemiologic cut-offs might declare resistance at an MIC that is different (often lower) than a clinical breakpoint (Lubbers and Turnidge, 2015). For the purposes of this discussion, we will be most concerned about clinical resistance and this definition of resistance will be used throughout the rest of this manuscript.

Table 2. Antimicrobial susceptibility of *M. haemolytica* isolates collected from the lungs of cattle that died of BRD (from Watts *et al.*, 1994)

Organism	# of Isolates	Antimicrobial	% Susceptible
M. haemolytica	461	Tilmicosin	69.1
		Ceftiofur	100
		Tetracycline	57
		Spectinomycin	83.5

Antimicrobial resistance in BRD pathogens

The earliest published MIC distributions for *M. haemolytica* established using modern diagnostic laboratory methodology and CLSI-approved breakpoints were derived from a survey of animals that died of BRD over a several year period from 1988 to 2002 (Watts *et al.*, 1994). In this study, 461 *M. haemolytica* isolates were submitted by numerous veterinary diagnostic laboratories across the USA and Canada (Pennsylvania, Wyoming, Iowa, Washington, California, Missouri, Nebraska, Oregon, Kansas, Arizona, Texas, South Dakota, Montana, Minnesota, Oklahoma, Colorado, Utah, Saskatchewan, Alberta, and Quebec) to an Upjohn laboratory for MIC determination. The results of this study are reported in Table 2. It is important to note that the interpretive criteria for tilmicosin has changed since this study was published and the prevalence of resistance to this drug would be dramatically decreased (>90% susceptible) using currently accepted criteria.

In another study, the susceptibility of 390 *M. haemolytica* isolates obtained from the lungs of beef cattle that died from BRD and submitted to the Oklahoma Animal Disease Diagnostic Laboratory between 1994 and 2002 was investigated (Welsh *et al.*, 2004). This study found that the susceptibility to tetracycline and spectinomycin varied over the course of the study period but was consistently low for each drug. In contrast, the susceptibility to ceftiofur and enrofloxacin remained high and relatively stable throughout the duration of the study (Table 3).

A landmark study from Kansas State University evaluated the prevalence of multi-drug antimicrobial resistance and antimicrobial co-resistance patterns in *M. haemolytica* isolated from the lungs of cattle with BRD over a 3-year period (Lubbers and Hanzlicek, 2013). This work showed that, between 2009 and 2011, the proportion of isolates resistant to five or more antimicrobials increased from 5 to 35%. In addition, isolates resistant to either oxytetracycline or tilmicosin were significantly more likely to be resistant to at least one other antimicrobial class.

Several studies have investigated the prevalence of antimicrobial resistance in feedlot cattle between feedlot arrival and exit. In one study, samples obtained from 10% of animals from 30% of feedlot pens in two feedlots in southern Alberta were submitted for isolation and susceptibility testing of *M. haemolytica* (Klima

https://doi.org/10.1017/S1466252320000109 Published online by Cambridge University Press

Table 3. Susceptibility of *M. haemolytica* obtained from the lungs of Feedlot cattle from 1994 to 2002 (from Welsh *et al.*, 2004)

	Year								
Antimicrobial	1994	1995	1996	1996	1998	1999	2000	2001	2002
Ceftiofur	97	98	100	100	98	100	98	96	97
Enrofloxacin	–	–	–	–	–	96	98	89	98
Florfenicol	–	–	100	96	98	97	96	87	90
Spectinomycin	65	49	71	53	55	63	45	29	51
Tilmicosin	90	78	93	83	80	74	85	71	79
Tetracycline	23	46	74	58	42	63	44	34	54

et al., 2011). Swabs were collected from cattle at the time of feedlot entry and again within 30 days of feedlot exit. Over the course of the study, 409 *M. haemolytica* isolates were obtained and resistance to all antimicrobials tested was low, ranging from 0.2 to 3.9% with resistance to oxytetracycline being most common (Klima *et al.*, 2011). In another study that sampled nearly 5500 cattle from four feedlots in Canada, deep nasopharyngeal swabs (DNP) were collected from enrolled animals at the time of arrival and again at a time point prior to feedlot exit (Noyes *et al.*, 2015). In this study, susceptibility to 21 different antimicrobials was evaluated for 2989 individual *M. haemolytica* isolates. Overall, resistance was rare, with 87% of isolates susceptible to all antimicrobials tested (Noyes *et al.*, 2015).

In a study from Canada evaluating resistance patterns in *M. haemolytica* isolated from healthy cattle and cattle with BRD, a resistant phenotype was found in 18% of *M. haemolytica* isolates tested (Klima *et al.*, 2014a). Overall, resistance was more common in isolates collected from cattle with BRD (32%) than isolates collected from healthy cattle (2%). Resistance to tetracycline was the most common phenotype observed and, generally speaking, if an isolate was resistant to one drug, it was also resistant to at least one other antimicrobial class (Klima *et al.*, 2014a).

Work from our laboratory evaluating the prevalence of resistance in *M. haemolytica* after metaphylaxis with the long-acting macrolide tulathromycin has yielded surprising results (Snyder *et al.*, 2017). In this study, lightweight, unweaned calves entering a stocker facility in Northeast Georgia were given tulathromycin at the time of arrival to the facility to prevent BRD. DNP were collected from each animal at the time of arrival and again 10–14 days later. For all antimicrobials except ceftiofur, there was a significant increase in the proportion of isolates classified as intermediate or resistant at the time of second sampling compared to samples collected at arrival (Snyder *et al.*, 2017). Of the 123 calves with *M. haemolytica* cultured at the time of second sampling, one (0.8%) had only pan-susceptible isolates, 30 (24.4%) had at least one isolate classified as intermediate or resistant to two antimicrobial classes (fluoroquinolones and macrolides), and 92 (74.8%) had at least one isolate classified as intermediate or resistant to three antimicrobial classes (fluoroquinolones and macrolides in addition to either phenicols or cephalosporins) (Snyder *et al.*, 2017). Additional work by our group evaluating efficacy and resistance in both enrofloxacin and tulathromycin in high-beef calves produced similar results (Crosby *et al.*, 2018).

Similar work by researchers at Mississippi State University produced comparable results. In this study, DNP were collected from calves at day 0 and then 7, 14, and 21 days after arrival processing and mass medication with tildipirosin (Woolums *et al.*, 2018). In these calves, nearly 100% of *M. haemolytica* isolates collected from calves at 7, 14, and 21 days after arrival processing and exposure to tildipirosin were classified as MDR and were resistant to all drugs tested except ceftiofur (Woolums *et al.*, 2018).

Mechanisms of multi-drug antimicrobial resistance

It is clear that resistance in BRD pathogens, particularly *M. haemolytica*, is on the rise. It is also clear that MDR is becoming more commonplace as well. The question is then, how does resistance to multiple antimicrobials arise after exposure to only one drug? In *M. haemolytica*, the primary driver for the increase in MDR strains is the integrative conjugative element (ICE) (Clawson *et al.*, 2016). ICEs are mobile genetic elements that integrate into the bacterial chromosome and, under the right conditions, transfer to neighboring bacterial cells (Wozniak and Waldor, 2010). These ICEs can carry multiple genes associated with antimicrobial resistance and, with exposure to one antimicrobial drug, the ICE and the rest of the resistance genes carried can be transferred to other bacterial cells (Snyder *et al.*, 2019). It is important to note that ICEs have been documented to move between different bacterial pathogens associated with BRD and are not just limited to *M. haemolytica* (Klima *et al.*, 2014b).

Antimicrobial resistance: impact on therapeutic outcome

First treatment success, defined as the proportion of animals successfully responding to antimicrobial therapy at the time of first pull, has historically been high in most populations in cattle on feed. Generally, a first treatment success risk of >80% is considered acceptable. However, a recent retrospective study evaluating risk factors for treatment failure found that over 30% of cattle failed to respond to first treatment (Avra *et al.*, 2017). In this study, high-risk calves demonstrated a greater risk of treatment failure than low-risk calves (Avra *et al.*, 2017). Unfortunately, little work has been done to evaluate the impact of antimicrobial resistance on clinical outcome in cattle with BRD. In the one published study that the author is aware of, 62% of cattle infected with susceptible *M. haemolytica* isolates ($n = 688$) responded to treatment with tilmicosin compared to 38% of animals ($n = 6$) with resistant isolates (McClary *et al.*, 2011).

Conclusions

Despite the importance of BRD to the North American cattle industry, there are few well-designed studies that evaluate

https://doi.org/10.1017/S1466252320000109 Published online by Cambridge University Press

antimicrobial resistance in bacterial pathogens important to this disease syndrome. The majority of published literature includes diagnostic laboratory submissions obtained from dead cattle that have been treated multiple times with multiple different antimicrobials. Nevertheless, general trends would suggest that a decrease in the susceptibility of *M. haemolytica* has occurred over time. Recent work suggests that antimicrobial use practices that are common within certain cattle operations might be the primary factor driving selection of resistant clones. As a result, it is critical that clinicians working with cattle recognize the importance of antimicrobial resistance in BRD pathogens and how this might affect the treatment efficacy in animals with clinical disease.

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