

## Antimicrobial Susceptibilities of Aerobic Isolates from Respiratory Samples of Young New Zealand Horses

L.J. Toombs-Ruane, C.B. Riley, A.T. Kendall, C.F. Bolwell, J. Benschop, and S.M. Rosanowski

**Background:** Decreased efficacy of antimicrobials and increased prevalence of multidrug resistance (MDR) is of concern worldwide.

**Objectives:** To describe and analyze bacterial culture and antimicrobial susceptibilities from respiratory samples submitted from young horses (4 weeks to 3 years old).

**Animals:** Samples from 289 horses were submitted to a commercial laboratory.

**Methods:** A retrospective database search of submissions made to a New Zealand veterinary laboratory between April 2004 and July 2014. The results of in vitro susceptibility testing by Kirby-Bauer disc diffusion were described and tabulated for the major bacterial species isolated. Multiple correspondence analysis (MCA) was used to describe the clustering of MDR isolates and selected demographic variables.

**Results:** Overall, 774 bacterial isolates were cultured from 237 horses, the majority of these isolates were gram-positive (67.6%; 95% CI 64.3–70.9%). *Streptococcus* spp. were the most common genus of bacteria isolated and were 40.1% (95% CI 36.6–43.5%) of the isolates cultured. Susceptibility of *Streptococcus* spp. to penicillin, gentamicin, and ceftiofur was >85%. Overall, gram-negative susceptibility to ceftiofur, tetracycline, and TMPs was <75%. MDR was defined as resistance to 3 or more antimicrobials, and was found in 39.2% of horses (93/237; 95% CI 33.0–45.5%).

**Conclusions and clinical importance:** Culture and susceptibility results have highlighted that MDR is an emerging problem for young horses in New Zealand (NZ), where a bacterial respiratory infection is suspected. This should be considered when prescribing antimicrobials, and emphasizes the need for submission of samples for culture and susceptibility.

**Key words:** Antimicrobial resistance; Equine; *Streptococcus zooepidemicus*; Thoroughbred.

There has been an increased attention placed upon antimicrobial resistance (AMR) in the medical<sup>1</sup> and veterinary<sup>2</sup> professions. Veterinary use of antimicrobials in horses has recently come under greater scrutiny, with the use of antimicrobials in respiratory disease identified as an area where inappropriate treatment occurs with a relatively high frequency.<sup>3,4</sup> In a survey using clinical case scenarios, 67.4% (763/1128) of UK veterinarians surveyed indicated that they would prescribe a trimethoprim-sulfonamide (TMPs) combination to a coughing pyrexic yearling, whereas 10.4% would prescribe penicillin, 2.9% oxytetracycline, and 5.8% a 3rd or 4th generation cephalosporin.<sup>3</sup> These practices describe antimicrobial treatments that might not be effective,<sup>3</sup> and

### Abbreviations:

AMR	antimicrobial resistance
CI	confidence interval
CLSI	Clinical Laboratory Standards Institute
MCA	multiple correspondence analysis
MDR	multidrug resistance
NZ	New Zealand
NZVP	New Zealand Veterinary Pathology©
TMPs	trimethoprim-sulfonamide combination antimicrobial

have the potential to increase the risk of AMR development and carriage.<sup>5,6</sup> The prescription practices of New Zealand (NZ) equine veterinarians are not known.

Respiratory disease is a source of economic loss, especially for young performance horses.<sup>7</sup> This is not only confined to known contagious pathogens such as *Rhodococcus equi*, *Streptococcus equi* subspecies *equi*, *Streptococcus equi* subspecies *zooepidemicus*, or equine herpes virus (EHV), but includes losses associated with inflammatory respiratory disease.<sup>8–10</sup> It is essential that bacterial respiratory infections are correctly identified and diagnosed,<sup>4,10</sup> because laboratory results should be used in conjunction with the clinical picture to justify the clinical use of antimicrobials.<sup>11</sup>

It is also important to have an understanding of the susceptibility of bacterial pathogens at a regional level, to underpin the development of regionally relevant guidelines for the prudent use of antimicrobials.<sup>11–13</sup> This study aimed to examine the patterns of antimicrobial susceptibility of bacterial isolates from young NZ horses with respiratory disease. The overarching objective of this work is to help provide a rationale for selection of appropriate antimicrobial treatment for suspected or confirmed bacterial respiratory disease.

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## Materials and methods

### Data collection

Records of antimicrobial susceptibilities (in vitro) of bacterial isolates cultured from equine samples submitted to New Zealand Veterinary Pathology (NZVP© Ltd, Auckland, Hamilton and Palmerston North laboratories, NZ) between April 2004 and July 2014 were assessed. All equine culture and susceptibility records submitted to the laboratory between the study dates were available for selection. Unique accession numbers were used to identify samples, and each accession number was assumed to be from different animals. Clinical case histories were not available in the database, so samples submitted from the same horse on different occasions were not able to be identified.

### Case selection

The susceptibilities of the bacterial isolates from equids were selected from horses between 4-weeks of age and 3-years old as defined by and listed in the NZVP database. Three age categories were defined according to the definitions used by the laboratory submission forms, with the age group of 1–23 months collapsed to include submissions listed as “weaner (weaning)” and “yearling” age categories, 2-year olds and 3-year olds.

Only samples described as likely respiratory (ie, “bronchoalveolar lavage,” “lung,” “lung swab,” “lymph node swab,” “nasal discharge,” “nasal swab,” “pharyngeal swab,” “pleural fluid,” “respiratory swab,” “sinus,” “sinus swab,” “throat swab,” “thoracic fluid,” “tracheal swab,” and “tracheal wash”) were included for analyses. Lymph node samples were included because of the abscessation of respiratory-associated lymph nodes with *Streptococcus equi* subspecies *equi* infection (strangles or metastatic strangles).<sup>14</sup>

Horses with more than 1 sample submitted were evaluated for similarity of bacterial culture, and exclusion of an isolate was made if two bacterial isolates cultured from the same horse had an identical antibiogram. The exclusion of apparently identical bacteria (from the same horse) resulted in one sample per horse assessed. For the purposes of comparing demographic information, only one sample per horse (if only negative culture results were obtained) was included in the dataset for analysis.

### Culture and susceptibility, identification and classification

The laboratory selection of antimicrobials for testing was based either on standardized NZVP protocols, individual microbiologist selection, or submitting clinician request. Aerobic culture results were selected for analysis; anaerobic and fungal isolates were not assessed. Disc-diffusion tests of the cultured isolates were based on a standardized protocol,<sup>15</sup> and the definition of susceptibility was based on the Clinical Laboratory Standards Institute (CLSI) recommendations for antimicrobial/bacterial isolate combinations.<sup>16,17</sup>

The antimicrobials examined included ceftiofur, enrofloxacin, gentamicin, penicillin, tetracycline, and TMPS. Multidrug resistance was defined as an isolate being resistant to three or more of the following antimicrobials:<sup>18</sup> ceftiofur, enrofloxacin, gentamicin, penicillin, tetracycline, and TMPS. One *E. coli* isolate was removed from the dataset before analyses attributable to the results of testing with three of these antimicrobials not being available.

### Data analysis

Data were stored and manipulated in Microsoft Excel® and Microsoft Access®.<sup>a</sup> Demographic and signalment variables included region of origin, age, and breed. The anatomic origin (if

known) and type of submitted sample was tabulated. The data for bacterial isolates described in the records were then examined with respect to susceptibility to antimicrobials and demographic factors, in particular age and region. Data were described by using counts, percentages, and 95% confidence intervals (CI). Pearson’s chi-squared or Fisher’s exact tests were performed on isolates with respect to the MDR status and selected submission factors to determine p-values for significant associations. Multiple correspondence analysis (MCA)<sup>19</sup> was performed to visualize multidrug resistance on a two-dimensional plot. Multiple correspondence analysis is a unique way to describe ordinal and categorical data, as it allows for the visualization of associations between multiple variables.<sup>19</sup> In this study, MCA was used to visually describe demographic factor variables, and the way these variables were related to the MDR status. A two-dimensional plot was generated from a matrix of bacterial isolate data (including MDR status). Variables clustering about the center of the plot are considered average for the data set.<sup>19</sup>

For each bacterial isolate, the demographic factors of “region,” “age,” and “date” were included, as was the MDR status. The MDR status was assessed at the isolate level. The dates were recorded into 2 time span categories: April 2004–2008 (inclusive), and July 2009–2014 (inclusive). These time spans were chosen based on the total number of submissions per year, so that within each group the years were relatively similar. The analysis was adjusted to account for the inflation of the Burt Matrix using the joint method.<sup>19</sup> All statistical analyses were conducted in using STATA version 13.1® software.<sup>b</sup>

## Results

Over the 10-year study period, records were available for 289 horses from which respiratory samples were submitted for culture. Aerobic bacteria were cultured from 237 (82.0%) of these submissions, antimicrobial susceptibilities were recorded, and demographic information summarized (Table 1).

### Samples

Samples for which susceptibilities were not recorded included those from which an anaerobic bacterium or fungus was cultured (without also culturing aerobic bacteria), those that yielded mixed bacterial growth were not subsequently speciated and susceptibility tested, and those for which selective culture for *Rhodococcus equi* and *Streptococcus equi* subspecies *equi* were negative. In total, 52/289 (18.0%; 95% CI 13.6–22.4%) of the respiratory samples submitted from horses were not culture-positive (ie, had no susceptibilities to antimicrobials recorded); 6/52 (15.4%; 95% CI 5.6–25.2%) had two submitted samples that were both culture-negative. Of the samples resulting in a positive culture, 119/237 (50.2%; 95% CI 43.8–56.6%) were from nasal swabs and tracheal samples (“swab” or “wash”) accounted for 98/237 (41.4%; 95% CI 35.1–47.6%) of the samples. A single bacterial species was cultured from 26/237 (11.0%; 95% CI 7.0–14.9%) submissions; 2 to 4 bacterial species were cultured from 175/237 (73.8%; 95% CI 68.2–79.4%); 5-to-7 bacterial species were cultured from 36/237 (15.2%; 95% CI 10.6–19.8%) of submissions; and 1/237 (0.4%; 95% CI –0.4 to 1.2%) had 11 bacterial species isolated that were tested for antimicrobial susceptibilities.

Demographic Groups		Culture Positive n	Total Submissions n	Proportion Positive % (95% CI)
Region	Auckland	61	70	87.1 (79.2–95.0)
	Waikato	135	166	81.3 (75.4–87.2)
	North Island (other)	29	37	78.4 (65.1–91.6)
	South Island (other)	12	16	75.0 (53.8–96.2)
Year	2004	6	6	100
	2005	8	8	100
	2006	8	8	100
	2007	13	13	100
	2008	18	19	94.7 (84.6–104.8)
	2009	33	46	71.7 (58.7–84.7)
	2010	25	45	55.6 (41.1–70.1)
	2011	34	38	89.5 (79.8–99.2)
	2012	26	26	100
	2013	50	56	89.3 (81.2–97.4)
Age	1–23 months	129	162	79.6 (73.4–85.8)
	2 years	56	67	83.6 (74.7–92.5)
	3 years	52	60	86.7 (78.1–95.3)
Breed	Standardbred	19	22	86.4 (72.0–100.7)
	Thoroughbred	172	216	79.6 (74.3–85.0)
	Other breed	13	16	81.3 (62.1–100.4)
	Unknown	33	35	94.3 (86.6–102.0)
Sex	Female	97	115	84.3 (77.7–91.0)
	Male	113	131	86.6 (80.4–92.2)
	Unknown	27	43	62.8 (48.3–77.2)
All Submissions	Total	237	289	82.0 (77.6–86.4)

Region “South Island (other)” includes all regions in the South Island; Region “North Island (other)” includes Bay of Plenty, Manawatu–Wanganui, Northland, Wellington; 2014 is January–July 2014. Proportion positive indicates Culture positive/Total submissions, as a percent.

### Bacterial culture

A total of 774 unique bacterial isolates were cultured from 237 horses with positive growth from the submitted samples. Of these isolates, 523/774 (67.6%; 95% CI 64.3–70.9%) were gram-positive; *Staphylococcus* spp. accounted for 119/523 (22.8%; 95% CI 19.2–26.3%) of these isolates, of which 65/119 (54.6%; 95% CI 45.7–63.6%) were *Staphylococcus aureus*. *Streptococcus* spp. constituted 310/523 (59.3%; 95% CI 55.1–63.5%) of all gram-positive isolates. Of these 125/310 (40.3%; 95% CI 34.9–45.8%) were identified as *Streptococcus equi* subspecies *zooepidemicus*. *Enterococcus* spp. accounted for 18/523 (1.6%; 95% CI 1.9–5.0%) of cultured isolates. Gram-negative bacterial isolates accounted for 251/774 (32.4%; 95% CI 29.1–35.7%) of the isolates. Enterobacteriaceae constituted 164/251 (65.3%; 95% CI 59.5–71.2%) of all gram-negative isolates. Of these 61/164 (37.2%; 95% CI 29.8–44.6%) were identified as *Escherichia coli*. *Pseudomonas* spp. accounted for 32/251 (4.1%; 95% CI 8.6–16.9%) of gram-negative isolates, and *Actinobacillus* spp. and *Pasturella* spp. accounted for 9/251 (2.3%; 95% CI 1.3–5.9%), and 2/251 (1.1%; 95% CI –0.3 to 1.9%), respectively.

### Antimicrobial susceptibility

Susceptibility results are described in Table 2. Antimicrobial susceptibility of gram-positive isolates were

**Table 1.** Demographic submission information from 289 horses from which respiratory samples for culture and susceptibility were submitted to a New Zealand laboratory (2004–2014).

<75% for tetracycline and TMPS, and >90% for gentamicin alone. *Streptococcus* spp. susceptibility to penicillin was >97%. The lowest overall susceptibility found for a gram-negative bacterium was to ceftiofur (55.6%). Multidrug resistance was recorded among the 773 eligible isolates. Of these, 120/773 (15.5%; 95% CI 13.0–18.1%) were resistant to 3 or more antimicrobials. Multidrug-resistant isolates were cultured from 93/237 (39.2%; 95% CI 33.0–45.5%) horses (range 1–4 MDR isolates per horse). Of all gram-positive isolates, 55/523 were MDR (10.5%; 95% CI 7.9–13.1%). Within a specific genera of gram-positive isolates, *Enterococcus* spp. included 3/18 MDR isolates (16.7%; 0–33.9%), *Staphylococcus* spp. 12/119 MDR isolates (10.1%; 95% CI 4.7–15.5%), and *Streptococcus* spp. 12/310 MDR isolates (3.9%; 95% CI 1.7–6.0%). Overall 65/250 (26.0%; 95% CI 20.6–31.4%) gram-negative bacteria cultured were MDR. In the family Enterobacteriaceae there were 39/163 MDR isolates cultured (23.9%; 95% CI 17.4–30.5%).

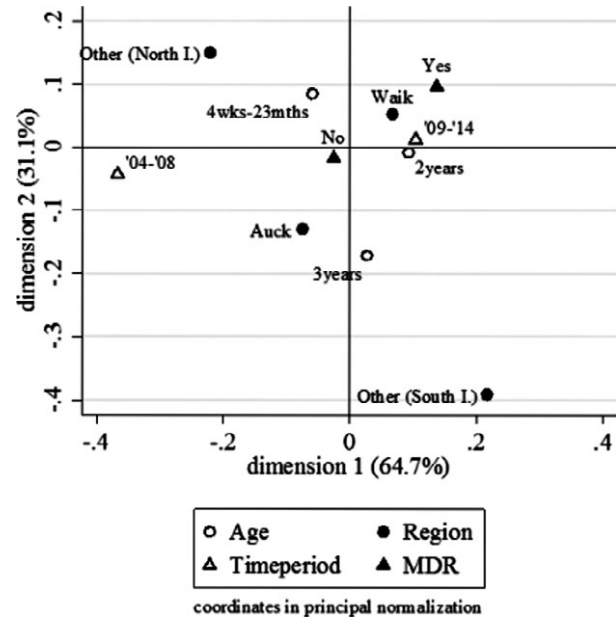
### Statistical analyses

The age of horse ( $P = .05$ , Pearson's  $\chi^2$  test) and date of submission ( $P = .003$ , Pearson's  $\chi^2$  test) were shown to have a significant association with MDR status. Region ( $P = .60$  Fisher's exact test), sex ( $P = .40$ , Pearson's  $\chi^2$  test), and breed ( $P = .21$ , Fisher's exact test)

**Table 2.** Antimicrobial susceptibility of isolates from 237 equine respiratory submissions (2004–2014).

Bacteria	Ceftiofur		Enrofloxacin		Gentamicin		Penicillin		Tetracycline		TMPS	
	Susceptible/ Total	% (95% CI)	Susceptible/ Total	% (95% CI)	Susceptible/ Total	% (95% CI)	Susceptible/ Total	% (95% CI)	Susceptible/ Total	% (95% CI)	Susceptible/ Total	% (95% CI)
All gram-positive	431/513	84.0 (80.8–87.2)	450/523	86.0 (83.0–89.0)	482/523	92.2 (89.9–94.5)	411/523	78.6 (75.1–82.1)	359/523	68.6 (64.6–72.6)	297/523	56.8 (52.6–61.0)
<i>Staph</i> spp.	102/118	86.4 (80.2–92.6)	116/119	97.5 (94.7–100.3)	111/119	93.3 (88.8–97.8)	68/119	57.1 (48.2–66.0)	97/119	81.5 (74.5–88.5)	98/119	82.4 (75.6–89.2)
<i>Strep</i> spp.	294/303	97.0 (95.1–98.9)	248/310	80.0 (75.5–84.5)	284/310	91.6 (88.5–94.7)	301/310	97.1 (95.2–99.0)	183/310	59.0 (53.5–64.5)	163/310	52.6 (47.0–58.2)
Rest gram-positive	35/92	38.0 (28.1–47.9)	86/94	91.5 (85.9–97.1)	87/94	92.6 (87.3–97.9)	42/94	44.7 (34.6–54.8)	79/94	84.0 (76.6–91.4)	36/94	38.3 (28.5–48.1)
All gram-negative	138/248	55.6 (49.4–61.8)	237/250	94.8 (92.0–97.6)	216/250	86.4 (82.2–90.6)	–	–	173/250	69.2 (63.5–74.9)	147/250	58.8 (52.7–64.9)
<i>E. coli</i>	41/61	67.2 (55.4–79.0)	60/60	100	52/60	86.7 (78.1–95.3)	–	–	42/60	70.0 (58.4–81.6)	32/60	53.3 (40.7–65.9)
Rest gram-negative	97/187	51.9 (44.7–59.1)	177/190	93.2 (89.6–96.8)	164/190	86.3 (81.4–91.2)	–	–	131/190	68.9 (62.3–75.5)	115/190	60.5 (53.5–67.5)

*Staph* spp., *Staphylococcus* spp.; *Strep* spp., *Streptococcus* spp.; *E. coli*, *Escherichia coli*; TMPS, trimethoprim-sulfonamide; –, testing not indicated.



**Fig 1.** Multiple correspondence analysis (MCA) in 2 dimensions, using a joint method, of 773 bacterial isolates cultured from NZ horses 2004–2014. Dimension 2 is shown on the y-axis, dimension 1 on the x-axis. For interpretation, the dimension that a variable contributes to can be visually assessed, for example year ('04-'08; '09-'14) is shown as different by the spread of the variables on the x-axis (dimension 1). Age = age of horse (variables are 4 weeks to 23 months; 2 years; 3 years) associated with each bacterial isolate; MDR = multidrug resistant to  $\geq 3$  antimicrobials (variables are yes; no); Region = region of origin of the bacterial isolates (variables are Auck = Auckland; Other (North I.) = North Island (Other); Other (South I.) = South Island (Other); Waik = Waikato); Time period = year of submission (grouped) (variables are '04-'08 = 2004–2008; '09-'14 = 2009–2014).

were not significantly associated with the occurrence of MDR in this dataset.

**Multiple correspondence analysis**

Figure 1 shows the results of multiple correspondence analysis, which was used to graphically depict associations between selected demographic factors and MDR. The plot shows that non-MDR isolates (“No”) lie close to the center, and this represents the most common (or average) result, indicating that most isolates were not MDR. Also shown in the plot is a clustering of 2-year-olds, submission years 2009–2014, and the Waikato region of NZ with MDR isolates (“Yes”). In total, 95% of the variance is explained in 2 dimensions, with most of the variance shown in dimension 1. Variables contributing most to the variation in the analysis were date (dimension 1) and geographic region (dimension 2).

**Discussion**

Based on respiratory sample culture and susceptibility results from 2004 until 2014, in vitro resistance was found to the antimicrobials that are used for the treat-



ment of respiratory infections in young NZ horses. Treatments such as TMPS were not effective in vitro against bacterial respiratory pathogens in many cases. However, penicillin, gentamicin, and ceftiofur were effective in vitro against multiple species of gram-positive bacteria in most cases, with gentamicin and enrofloxacin commonly found to be effective in vitro against gram-negative bacteria.

*Streptococcus* spp. accounted for a high proportion of all isolates cultured, suggesting that streptococcal infections are more common than other causes of bacterial respiratory infection (or colonization) in NZ. In a study of British National hunt racehorses, *Streptococcus* spp. (nonhemolytic and Viridans group), *Staphylococcus* spp., *Bacillus* spp., and *Actinobacillus/Pasteurella* spp. in descending frequency of occurrence were most commonly isolated from the respiratory tract.<sup>20</sup> These bacteria have a known or potentially causal role in equine respiratory disease.<sup>3</sup> In contrast, *Actinobacillus* and *Pasteurella* were not cultured in high proportions in this study. These differences could reflect the diagnostic practices of veterinarians in NZ, and further investigation into this is warranted. As nothing was known regarding previous treatment with antimicrobials or clinical history of horses from which samples were submitted to NZVP laboratories, results presented in this study should be interpreted with caution.

Ceftiofur has been recommended for the treatment of respiratory streptococcal infections in horses.<sup>21</sup> However, the results of this study do not support a therapeutic advantage over the use of penicillin. *Streptococcus* spp. are generally susceptible to penicillin,<sup>22,23</sup> and there was little appreciable difference between penicillin and ceftiofur in vitro susceptibility in this study (ceftiofur 95.1–98.9% versus penicillin 95.2–99.0%). In addition, it has been recommended that ceftiofur, a 3rd generation cephalosporin, and enrofloxacin should not be used as first-line treatments as they have been identified as critically important antimicrobials by the World Health Organization.<sup>13</sup>

Overall, the in vitro susceptibility of bacteria to TMPS was low for both gram-positive and gram-negative isolates. Staphylococcal isolates were susceptible to TMPS (82.4%); however, their association with clinical respiratory infections is not as common and that of Streptococcal species.<sup>20</sup> *Staphylococcus* spp. are more likely to be associated with contamination from the upper respiratory tract.<sup>24,25</sup> In NZ, TMPS is one of the few antimicrobials that is available in an oral formulation and accounts for a high proportion of antibiotic sales<sup>26</sup> and based upon a recent survey, its misuse on-farm is suspected.<sup>27</sup>

In a recent retrospective study of diagnostic samples of beta-hemolytic *Streptococcus* submitted to a US laboratory over a 10-year period, a statistically significant increase in resistance of *Streptococcus equisimilis* to both gentamicin and tetracycline was noted.<sup>23</sup> In the current study, the overall susceptibility of *Streptococcus* spp. to gentamicin was 88.5–94.7% (95% CI), and overlaps with the susceptibility reported in the US study of between 83.3% and 91.2%.<sup>23</sup> It is possible that this sim-

ilarity is coincidental, and reflects the classification of bacteria, as there is intrinsic variation in the susceptibilities to antimicrobials that different species of *Streptococcus* exhibit.<sup>22</sup> However, gentamicin is not an appropriate first choice for the treatment of respiratory infections in horses, despite bacteria cultured in this study having overall high susceptibility to the antimicrobial, as it does not appear to reach therapeutic concentrations in respiratory secretions.<sup>28,29</sup> However, in the presence of inflammation and increased blood flow, systemic administration of gentamicin is likely to penetrate affected lung tissue and have chemotherapeutic effects.<sup>30,31</sup>

Bacteria that were resistant to 3 or more antimicrobials appeared to be relatively common in the study population, with at least one MDR isolate cultured from 39.2% of horses. The age of the horse and the year of submission were both significantly associated with MDR status and this was reflected in the MCA plot. Multiple correspondence analysis is a method not commonly reported in veterinary research, although it has been effectively utilized to describe focal bone mineral density patterns.<sup>32</sup> Based on the number of different variables potentially associated with MDR, it was used here to assess multiple factors for associations, without assessment of statistical significance. In this dataset, there was a clustering of isolates from the geographic region of Waikato, with 2-year-old horses and in the submission years 2009–2014. This is an indication that submission of MDR isolates was common in this age group of animals, and these could be potential risk factors for MDR carriage and infection. Although there might be a geographical association between veterinarian submission patterns and the results of this study, veterinarian involvement on NZ farms is varied,<sup>27</sup> and conclusions cannot be made based upon these results. Further investigation of this is warranted from both a treatment and public health perspective.<sup>18</sup>

The overuse and misuse of antimicrobials in equine medicine has been described in Canada and the United Kingdom, especially in the treatment of respiratory conditions.<sup>3,4</sup> There was no knowledge of pretreatment or overuse of antimicrobials in the animals from which samples were taken for this study, although increased antimicrobial resistance among host bacteria after the use of antimicrobials has been described in other equine populations.<sup>6,33</sup> This might have been a contributing factor to the high proportion of horses culturing an MDR isolate, but confirmatory data were not available in this retrospective record-based study.

Some of the limitations of this study are inherent with using retrospective data from disc-diffusion susceptibility testing. Results of disc-diffusion testing, even where standards are used,<sup>16</sup> are subject to changes in the definition of susceptibility by the standards (eg, CLSI). This contrasts with studies where MIC data are used,<sup>34</sup> and antimicrobial concentrations are stated for a given susceptibility breakpoint, and thus could improve the quality of retrospective temporal analysis. Another limitation of using data from commercial laboratories includes the inability to relate antimicrobial sus-

ceptibilities to an accurate and well described case history.<sup>11</sup>

It should also be noted that the isolates included in this study were submitted to one of two commercial diagnostic microbiology laboratories in NZ, and therefore not necessarily representative of the broader population. In addition, a likely bias exists from the origin of samples, with a majority of samples submitted from the Waikato region of NZ (57.4%). This bias reflects the location of the greatest concentration of horses in the commercial Thoroughbred population in NZ.<sup>35,36</sup> The laboratories from which the data were obtained are situated in the North Island of NZ, and although a small proportion of samples originated from the South Island (Table 1), any true regional differences in antimicrobial susceptibility were not able to be accurately described. Breed variations are likely to be emphasized by this same regional bias, as well as the economic utility of racehorse breeds in the age range chosen for this study. Data from respiratory samples were likely lost from this study because of incomplete information regarding the source of samples, information that was not completed by the submitting veterinarian or clinic. Although an attempt was made to describe one sample per horse, the potential inclusion of samples from the same horse on multiple occasions is a limitation in this study and has a potential to bias the results by the inclusion of potentially more resistant isolates. As there was no evidence of association between “unknown” submission information and the culture of MDR isolates, it is possible that the missing susceptibility data would not have been from the MDR isolates. Provisions of clinical history, especially previous treatment, as well as signalment information are important not only so the laboratory can provide comprehensive feedback, but also to allow for the continued monitoring of antimicrobial susceptibility patterns.<sup>37</sup>

The samples in this study came predominantly from nasal (50.2%) and tracheal (41.4%) samples, and although respiratory disease is typically stratified into “upper” and “lower” airway disease, the exact mode of collection of the samples used in this study was not specified. The implication is that even if a sample has been labeled as tracheal, bronchial, or lung, there is a significant likelihood of contamination from the upper airway flora to occur.<sup>20</sup> Bacterial isolates from all sample sources were described together, as the potential clinical implications to keeping them separate are lost in the limitations of the study methodology. Even a stringent laboratory protocol is also subject to variations year-to-year and between laboratories; there are inherent limitations to any retrospective study of antimicrobial susceptibility or resistance.<sup>38</sup> Nevertheless the authors suggest that the data described here provide a valuable and necessary contribution to understanding of general clinical antimicrobial susceptibility and resistance in NZ.

The results of this study confirm that penicillin is an appropriate first-line antimicrobial for use in most horses in NZ where a streptococcal respiratory infection

is suspected, when results of the samples submitted for bacterial culture and susceptibility are pending. A suspected decrease of in vitro bacterial susceptibility to commercially available veterinary antimicrobials (including MDR) is of concern, and culture and susceptibility should be included in the appropriate diagnosis of bacterial disease. Ongoing monitoring of culture and susceptibility results at a local level should be performed to ensure guidelines reflect regional antimicrobial susceptibilities, and therefore inform appropriate antimicrobial selection in the future. The continued monitoring and surveillance of antimicrobial susceptibility and resistance in NZ is warranted.

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*Conflict of Interest Declaration:* Authors disclose no conflict of interest.

*Off-label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

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### Footnotes

<sup>a</sup> Microsoft Corporation, Redmond, WA.

<sup>b</sup> StataCorp, College Station, TX.

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