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


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 cefotaxim, trimethoprim, and TMPS was <70%. MDR was defined as resistance to 2 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 and veterinary 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. 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 pyretic yearling, whereas 10.4% would prescribe penicillin, 2.9% oxytetracycline, and 5.8% a 3rd or 4th generation cephalosporin. These practices describe antimicrobial treatments that might not be effective, and have the potential to increase the risk of AMR development and carriage.

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. 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. It is essential that bacterial respiratory infections are correctly identified and diagnosed, because laboratory results should be used in conjunction with the clinical picture to justify the clinical use of antimicrobials. 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. 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.
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: 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,” “thoracic fluid,” “thoracic swab,” and “thoracic 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).

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, and the definition of susceptibility was based on the Clinical Laboratory Standards Institute (CLSI) recommendations for antimicrobial/bacterial isolate combinations.

The antimicrobials examined included cefiotin, enrofloxacin, gentamicin, penicillin, tetracycline, and TMPS. Multidrug resistance was defined as an isolate being resistant to three or more of the following antimicrobials: cefiotin, enrofloxacin, gentamicin, penicillin, tetracycline, and TMPS. One E. coli isolate was removed from the database 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®. 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) 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. 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.

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. All statistical analyses were conducted in using STATA version 13.1® software.

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%) bacteria were cultured from 36/237 (15.2%; 95% CI 10.6–19.8%) of submissions; and 1/237 (0.4%; 95% CI 0.0 to 1.2%) had 11 bacterial species isolated that were tested for antimicrobial susceptibilities.
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 *Pasteurella* spp. accounted for 9/251 (2.3%; 95% CI 1.3–3.9%), 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 <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 χ² test) and date of submission (P = .003, Pearson’s χ² test) were shown to have a significant association with MDR status. Region (P = .60 Fisher’s exact test), sex (P = .40, Pearson’s χ² test), and breed (P = .21, Fisher’s exact test)
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 treatment of equine respiratory tract infections. Table 2. Antimicrobial susceptibility of isolates from 237 equine respiratory submissions (2004–2014).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Ceftiofur</th>
<th>Enrofloxacin</th>
<th>Gentamicin</th>
<th>Penicillin</th>
<th>Tetracycline</th>
<th>TMPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible/Total</td>
<td>Susceptible % (95% CI)</td>
<td>Susceptible/Total</td>
<td>Susceptible % (95% CI)</td>
<td>Susceptible/Total</td>
<td>Susceptible % (95% CI)</td>
</tr>
<tr>
<td>All gram-positive</td>
<td>431/513</td>
<td>84.0 (80.8–87.2)</td>
<td>450/523</td>
<td>86.0 (83.0–89.0)</td>
<td>482/523</td>
<td>92.2 (89.9–94.5)</td>
</tr>
<tr>
<td>Staph spp.</td>
<td>102/118</td>
<td>86.4 (80.2–92.6)</td>
<td>116/119</td>
<td>97.5 (94.7–100.3)</td>
<td>111/119</td>
<td>93.3 (88.8–97.8)</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>294/303</td>
<td>97.0 (95.1–98.9)</td>
<td>248/310</td>
<td>80.0 (75.5–84.5)</td>
<td>284/310</td>
<td>91.6 (88.5–94.7)</td>
</tr>
<tr>
<td>Rest gram-positive</td>
<td>35/92</td>
<td>38.0 (28.1–47.9)</td>
<td>86/94</td>
<td>91.5 (85.9–97.1)</td>
<td>87/94</td>
<td>92.6 (87.3–97.9)</td>
</tr>
<tr>
<td>All gram-negative</td>
<td>138/248</td>
<td>55.6 (49.4–61.8)</td>
<td>237/250</td>
<td>94.8 (92.0–97.6)</td>
<td>216/250</td>
<td>86.4 (82.2–90.6)</td>
</tr>
<tr>
<td>E. coli</td>
<td>41/61</td>
<td>67.2 (55.4–79.0)</td>
<td>60/60</td>
<td>100</td>
<td>52/60</td>
<td>86.7 (78.1–95.3)</td>
</tr>
<tr>
<td>Rest gram-negative</td>
<td>97/187</td>
<td>51.9 (44.7–59.1)</td>
<td>177/190</td>
<td>93.2 (89.6–96.8)</td>
<td>164/190</td>
<td>86.3 (81.4–91.2)</td>
</tr>
</tbody>
</table>

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

Figure 1. Multiple correspondence analysis (MCA) in 2 dimensions, using a joint method, of 773 bacterial isolates cultured from NZ horses 2004–2014. Dimension 1 is shown on the x-axis, dimension 2 on the y-axis. The plot 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 explained in dimension 1. Variables contributing most to the variation in the plot include age (dimension 1) and geographic region (dimension 2). Variables with most of the variance explained in dimension 2 were not significantly associated with the occurrence of MDR in this dataset.

Antimicrobial Resistance in Young NZ Horses
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 enrofloxacins commonly found to be effective in vitro against gram-negative bacteria.

Streptococcus spp. accounted for a high proportion of all isolates in this study, 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), Actinobacillus/Pasteurella spp., and Actinobacillus/Pasteurella spp. in descending frequency of occurrence were most commonly isolated from the respiratory tract. These bacteria have a known or potentially causal role in equine respiratory disease. 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. However, the results of this study do not support a therapeutic advantage over the use of penicillin. Streptococcus spp. are generally susceptible to penicillin, and there was little appreciable difference between penicillin and ceftiofur in vitro susceptibilities in this study (ceftriaxone 98.9% versus penicillin 95.2–99.0%). In addition, it has been recommended that ceftiofur, a 3rd generation cephalosporin, and enrofloxacins should not be used as first-line treatments as they have been identified as critically important antimicrobials by the World Health Organization. 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. Staphylococcus spp. are more likely to be associated with contamination from the upper respiratory tract. 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 and based upon a recent survey, its misuse on-farm is suspected.

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. 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%. It is possible that this similarity is coincidental, and reflects the classification of bacteria, as there is intrinsic variation in the susceptibilities to antimicrobials that different species of Streptococcus exhibit. 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 is does not appear to reach therapeutic concentrations in respiratory secretions. 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.

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. 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, from 5-year-old horses in the submission years 2009–2014. There was no association 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 with the NZ horses is variable, and conclusions cannot be made based upon these results. Further investigation of this is warranted from both a treatment and public health perspective.

The overuse and misuse of antimicrobials in equine medicine has been described in Canada and the United Kingdom, especially in what is termed respiratory conditions. 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. 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 when standards are used, reflect the definition of susceptibility by the standards (e.g., CLSI). This contrasts with studies where MIC data are used, 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.\textsuperscript{31}

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.\textsuperscript{35,36} 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” isolation of the greatest concentration of horses in the commercial Thoroughbred population in NZ.\textsuperscript{35,36}

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

Footnotes

\textsuperscript{a} Microsoft Corporation, Redmond, WA.
\textsuperscript{b} StataCorp, College Station, TX.

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