Title
Minimum Inhibitory Concentrations of Equine Corynebacterium pseudotuberculosis Isolates (1996-2012)

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Background: Few studies report the minimum inhibitory concentrations for antimicrobials against equine Corynebacterium pseudotuberculosis isolates.

Hypothesis/Objectives: To evaluate trends in the in vitro activities of 20 antimicrobials against equine Corynebacterium pseudotuberculosis isolates from 1996 to 2012 and to determine if a relationship exists between the minimum inhibitory concentration (MIC) and location of the abscess.

Animals: Corynebacterium pseudotuberculosis isolates from 196 horses with naturally occurring disease.

Methods: Retrospective and cross-sectional design. Medical records were reviewed to obtain clinical and MIC data. Minimum inhibitory concentrations were determined by the microdilution technique. The MIC results over 3 periods were compared (1996–2001, 2002–2006, 2007–2012).

Results: The MIC90 values for clinically relevant antimicrobials were as follows: chloramphenicol ≤0.25 μg/mL, enrofloxacin ≤0.25 μg/mL, gentamicin ≤1 μg/mL, penicillin =0.25 μg/mL, rifampin ≤1 μg/mL, tetracycline ≤2 μg/mL, trimethoprim-sulfamethoxazole (TMS) ≤0.5 μg/mL, ceftriaxone =2 μg/mL, and doxycycline ≤2 μg/mL. There were no significant changes in MIC results over the study period. There was no relationship between MIC patterns and abscess location.

Conclusions and Clinical Importance: The MIC90 and MIC50 values of antimicrobials evaluated in this study for equine isolates of C. pseudotuberculosis did not vary over time. Abscess location was not associated with different MIC patterns in cultured isolates. Several commonly used antimicrobials are active in vitro against C. pseudotuberculosis in vitro.

Key words: Antibiotics; Horse; Infection; Susceptibility.

Corynebacterium pseudotuberculosis is a gram-positive pleomorphic intracellular bacterium that causes external abscesses, internal abscesses, and ulcerative lymphangitis in horses. Two genetically distinct biovars exist, biovar equi affects horses and is nitrate positive, whereas biovar ovis affects sheep and goats and is nitrate negative.1 The most common sites of external infection in horses are the ventral abdomen and pectoral region, consequently the disease is often termed “pigeon fever.” Corynebacterium pseudotuberculosis has been implicated in internal or systemic disease conditions including pneumonia, pleuritis, pericarditis, purpura hemorrhagica, abortion, and panniculitis.2–4 Although antimicrobial treatment of external abscesses remains controversial, successful treatment of horses with compromised immune function, recurrent external abscesses, internal abscesses, ulcerative lymphangitis, and other systemic forms of the disease requires early diagnosis and treatment with a long course of antimicrobials (typically 30 days or longer).5 Aleman et al reported a 40.5% mortality rate for horses with internal abscesses.6 In that study, all horses with an internal abscess not treated with antimicrobials did not survive. More recently, a mortality rate of 30% was reported in horses treated for internal C. pseudotuberculosis infection.5 In both reports, antimicrobials were considered crucial to a successful outcome.

Selection of appropriate antimicrobial treatment for bacterial infections depends on a number of factors including the microorganism involved, location of infection, and the pharmacokinetics and pharmacodynamics of antimicrobials.7 Few large reports exist in the literature regarding the antimicrobial susceptibility patterns of equine C. pseudotuberculosis isolates.

A previous study evaluated the susceptibility of clinical isolates of C. pseudotuberculosis from a number of species, including cattle, sheep, goats, and horses. In that study, the most active antimicrobials were penicillins, macrolides, tetracyclines, cephalosporins, chloramphenicol, and rifampin.8 Over the years, increasing antimicrobial resistance has been documented in other gram-positive, closely related bacteria affecting horses.
notably \textit{Rhodococcus equi}.\textsuperscript{9,10} In the human literature, emerging antimicrobial resistance, including multidrug resistance, has been documented among other \textit{Corynebacterium} species.\textsuperscript{11} No data are available regarding long-term temporal patterns in antimicrobial susceptibility of \textit{C. pseudotuberculosis} isolates.

The aims of this study were to evaluate temporal trends in the MIC patterns of 20 antimicrobials against equine isolates of \textit{C. pseudotuberculosis} obtained from 1996 to 2012, and to determine if a relationship exists between antimicrobial MIC\textsubscript{50} and abscess location (whether external or internal). The hypothesis of this study was that the MIC values of \textit{C. pseudotuberculosis} isolates to antimicrobials that are used in the treatment of so-called pigeon fever have increased over time.

**Materials and Methods**

**Isolate Sources**

A total of 207 bacterial isolates from 196 horses were available for inclusion in this study. All samples were collected from naturally infected horses and were submitted to the UC Davis William R. Pritchard Veterinary Medical Teaching hospital (VMTH) microbiology laboratory for culture and identification by standard methods. Susceptibility testing of \textit{C. pseudotuberculosis} isolates submitted to the microbiology laboratory was not routinely performed before 2005. Isolates obtained from necropsy samples also had not been tested. Isolates collected before 2005 and those samples collected at necropsy therefore required prospective susceptibility testing. Minimum inhibitory concentration data were already available for 146 isolates, thus requiring MIC determinations on 61/207 isolates.

**MIC Determination**

A broth microdilution method was used to assess MICs according to the Clinical and Laboratory Standards Institute guidelines with few exceptions.\textsuperscript{12,13} Sixty-one isolates without pre-existing MIC data were obtained from a bacterial bank, where they had been stored at \textsuperscript{\textdegree}C until analysis. The beads were recovered under aseptic conditions and used to inoculate a 5% sheep blood agar plate.\textsuperscript{14} Inoculated sheep blood agar plates were incubated at 37\textsuperscript{\textdegree}C for 24 hours and then inspected for purity and colony morphology.

Once isolates were confirmed to be pure culture, 10-mL brain heart infusion (BHI) broth with 0.2\% Tween 80\textsuperscript{a} was inoculated with a 10-\textmu L loop (Fisherbrand Flexible loop\textsuperscript{b}) from the blood agar plate and incubated at 37\textsuperscript{\textdegree}C for 24 hours. The supernatant was used to inoculate a second 10-mL tube containing BHI-Tween, which was incubated under the same previously described conditions. An aliquot of the BHI broth was used to inoculate 0.85\% sodium chloride to a 0.5 McFarland turbidity standard as determined by nephelometer. A 10-\textmu L aliquot of saline was used to inoculate cation-adjusted Mueller-Hinton Broth with lysed horse blood (BMH) (Cation-adjusted Blood Mueller-Hinton Broth\textsuperscript{b}), and 100 \textmu L of broth was used to inoculate each well of an Equine Sensititre tray\textsuperscript{d} (a 96-well plate containing various antimicrobial concentrations) by an automated technique (SensititreAutoInoculator\textsuperscript{c}). The plates were sealed with a nonperforated adhesive seal and then placed in a non-CO\textsubscript{2} incubator at 35\textsuperscript{\textdegree}C for 48 hours, after which time, the MIC was determined for the available antimicrobials with a SensititreSensiTouch plate reader.\textsuperscript{4} Plates were read at 48 hours because of the slow growth typical of \textit{C. pseudotuberculosis}.

The antimicrobials and the concentrations tested (\mu g/mL) were those on the Equine Sensititre tray. Antimicrobials and range of concentrations tested included: amikacin (4–32 \mu g/mL), ampicillin (0.25–32 \mu g/mL), azithromycin (0.25–4 \mu g/mL), cefazolin (4–16 \mu g/mL), cefotaxime (0.5–64 \mu g/mL), cefotaxime (0.5–64 \mu g/mL), chloramphenicol (4–32 \mu g/mL), clari

**Isolates with Pre-existing MIC Data**

Minimum inhibitory concentration data were available for 146 isolates. The MIC data were determined using the aforementioned broth microdilution technique, performed at the UC Davis Veterinary Medical Teaching Hospital Microbiology Laboratory. Over the study period, different customized Sensititre trays\textsuperscript{d} have been used by the VMTH microbiology laboratory. Therefore, the types of antimicrobials as well as their dilutions were not standard throughout the study period. As an example, the Trek Equine Sensititre tray\textsuperscript{d} used for prospective MIC determinations has different minimal dilutions for amikacin, cefazolin, chloramphenicol, doxycycline, gentamicin, and rifampin. The minimum dilutions used for MIC interpretation were those present on the commercially available Equine Sensititre tray\textsuperscript{d} described above, unless otherwise indicated.

**Trends in MIC\textsubscript{50} and MIC\textsubscript{90}**

The study period included samples submitted between 1996 and 2012. Because of variable numbers of isolates per year, the study period was divided into 3 time periods to assess trends in MIC data: 1996–2001, 2002–2006, and 2007–2012. For all antimicrobials, the concentrations at which 50 or 90\% of isolates were inhibited (MIC\textsubscript{50} and MIC\textsubscript{90}, respectively) were determined over the 3 time periods. The antimicrobials that were considered for evaluation in the final analysis included amikacin, ampicillin, azithromycin, cefazolin, cefotaxime, cefotaxime, chloramphenicol, clarithromycin, doxycycline, enrofloxacin, erythromycin, gentamicin, imipenem, oxacillin, penicillin, rifampin, tetracycline, ticarcillin, and TMS. For descriptions other than temporal changes, the overall MIC\textsubscript{50} and MIC\textsubscript{90} for the entire study period were used (ie, the MIC\textsubscript{50} and MIC\textsubscript{90} of all 207 isolates).

**Clinical Data**

When available in the medical records, clinical data collected included breed, age, sex, abscess location (internal versus external infection or ulcerative lymphangitis), days of antimicrobial treatment before presentation, antimicrobials used for treatment, and whether the animal survived, died, or was euthanized because of the disease or was euthanized or died for reasons unrelated to clinical \textit{C. pseudotuberculosis} infection. Of the 196 horses, 166 belonged to clients of the UC Davis VMTH. The remaining 30 consisted of cases submitted by practitioners from California (12/30), Colorado (12/30), and Utah (6/30).
Statistical Analysis

Trends in MIC\textsubscript{50} and MIC\textsubscript{90} were analyzed using an exact Coehran-Armitage test for each antimicrobial. Fisher’s exact test for independent binomial proportions was used to determine the relationship between changes in MICs and abscess location (internal versus external). \( P < .05 \) was considered statistically significant.

Results

Clinical Data

Breeds represented included Quarter Horses (49), Arabians (30), Thoroughbreds (21), American Paint Horses (21), Warmbloods (13), mixed breeds (7), draft breeds (6), Appaloosas (5), Peruvian Pasos (3), Mustangs (2), Standardbreds (2), Friesians (1), Andalusians (1), Morgans (1), Norwegian Fjords (1), and Saddlebreds (1). There also were 2 donkeys. The breed was not recorded for 30 horses. Of the 196 horses, 83 (45%) were treated with antimicrobials at some point in the course of their disease. Antimicrobial treatments before presentation (median duration, 6 days). Administered antimicrobials included TMS, oxytetracycline, doxycycline, penicillin, gentamicin, amikacin, metronidazole, cefiourf, rifampin, chloramphenicol, enrofloxacin, and erythromycin. Many of these drugs were used in combination. The median number of antimicrobials administered per horse was 2 (range, 1–6).

One hundred and seventy-two horses (87.7%) had external abscesses, which included those horses with concurrent internal infection. One hundred and forty (71.4%) horses had only external abscesses. Of those cases with external abscesses, 62/172 had a single abscess, 19/172 had 2 abscesses, 13/172 had 3, and 47/172 had =4 abscesses. The number of external abscesses was not recorded for 31 horses. External abscess locations included the pectoral region, ventral abdomen, inguinal region, sheath, mammary gland, ventral abdomen, triceps, neck, face, gluteal, axilla, tuber coxae, and submandibular regions.

Fifty-two horses (26.5%) had internal abscesses, 32 had concurrent external abscesses, and 20 horses had internal abscesses only. Four horses (2%) were diagnosed with ulcerative lymphangitis. Location of internal infection included liver (n = 15), lungs (n = 15), kidney (n = 10), spleen (n = 3), and colonic mesentery (n = 1). Corynebacterium pseudotuberculosis also was cultured from a joint (n = 1), inner ear (n = 1), and peritoneal fluid (n = 6).

Of the 196 horses in the study, only 42 (21.4%) were not treated with antimicrobials compared to 125 (63.7%) that were treated with antimicrobials at some time point in the course of their disease. Antimicrobial history was unknown for 29 horses (14.7%). The majority of horses were not treated before presentation to the VMTH (127/196). Thirty-nine horses had been treated with antimicrobials before presentation (median duration of treatment, 6 days). Administered antimicrobials included TMS, oxytetracycline, doxycycline, penicillin, gentamicin, amikacin, metronidazole, cefiourf, rifampin, chloramphenicol, enrofloxacin, and erythromycin. Many of these drugs were used in combination. The median number of antimicrobials administered per horse was 2 (range, 1–6).

One hundred and sixty-two horses had outcome recorded. Of these, 134 (82.7%) survived, 12 horses were euthanized and 1 died because of disease attributable to \textit{C. pseudotuberculosis}. An additional 15 horses died or were euthanized because of unrelated causes, whereas the outcome was unknown for 34 horses. With these 15 horses excluded, the survival rate was 91.2%. Of the 13 horses with nonsurvival attributable to \textit{Corynebacterium} infection, all had confirmed or suspected internal abscesses.

Trends in Minimum Inhibitory Concentrations

There were 32 isolates from 1996 to 2001, 83 from 2002 to 2006, and 92 from 2007 to 2012. Twenty-six of the 32 isolates from 1996 to 2001, 30/83 from 2002 to 2006, and 5/92 from 2007 to 2012 had to be prospectively tested. The MIC for cefotaxime was not determined for the 1996–2001 period because of a paucity of data. There were no significant changes in the MIC\textsubscript{50} and MIC\textsubscript{90} values over time. The MIC\textsubscript{90} results determined using data from entire study period (ie, all isolates obtained from 1996 to 2012) are presented in Table 1.

MIC\textsubscript{50} and MIC\textsubscript{90} Trends and Abscess Location

No significant relationships were detected between MIC patterns and abscess location.

Table 1. Cumulative MIC\textsubscript{90} data for equine \textit{Corynebacterium pseudotuberculosis} isolates obtained from horses.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>n</th>
<th>MIC\textsubscript{50} (\mu g/mL)</th>
<th>MIC\textsubscript{90} (\mu g/mL)</th>
<th>Range (\mu g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>207</td>
<td>4</td>
<td>8</td>
<td>=0.25–32</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>204</td>
<td>&lt;0.25</td>
<td>0.5</td>
<td>=0.25–16</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>120</td>
<td>&lt;0.25</td>
<td>=0.25</td>
<td>=0.25–1</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>202</td>
<td>2</td>
<td>4</td>
<td>=2–8</td>
</tr>
<tr>
<td>Cefoxatime</td>
<td>57</td>
<td>1</td>
<td>1</td>
<td>=0.5–4</td>
</tr>
<tr>
<td>Cefiourf</td>
<td>205</td>
<td>2</td>
<td>2</td>
<td>=0.25–4</td>
</tr>
<tr>
<td>Cefizoxime</td>
<td>81</td>
<td>1</td>
<td>2</td>
<td>=0.5–4</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>203</td>
<td>2</td>
<td>=4</td>
<td>=0.25–4</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>65</td>
<td>≤1</td>
<td>=1</td>
<td>=0.25–1</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>124</td>
<td>≤2</td>
<td>=2</td>
<td>=0.12–2</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>182</td>
<td>0.25</td>
<td>=0.25</td>
<td>=0.06–4</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>146</td>
<td>≤0.12</td>
<td>=0.25</td>
<td>=0.12–2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>206</td>
<td>1</td>
<td>2</td>
<td>=0.25–8</td>
</tr>
<tr>
<td>Imipenem</td>
<td>122</td>
<td>≤1</td>
<td>=1</td>
<td>=0.12–1</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>38</td>
<td>2</td>
<td>4</td>
<td>=0.12–4</td>
</tr>
<tr>
<td>Penicillin</td>
<td>178</td>
<td>0.25</td>
<td>0.25</td>
<td>=0.06–4</td>
</tr>
<tr>
<td>Rifampin</td>
<td>203</td>
<td>≤0.12</td>
<td>=1</td>
<td>=0.12–1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>148</td>
<td>0.5</td>
<td>2</td>
<td>=0.25–2</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>66</td>
<td>≤8</td>
<td>=8</td>
<td>≤8</td>
</tr>
<tr>
<td>TMS</td>
<td>203</td>
<td>0.25</td>
<td>0.5</td>
<td>=0.25–4</td>
</tr>
</tbody>
</table>

TMS, trimethoprim-sulfamethoxazole.
**Discussion**

To the authors' knowledge, this is the first large study of the MICs of antimicrobial agents of equine *C. pseudotuberculosis* isolates and temporal changes in MICs values. Currently, there are no specific Clinical Laboratory Standard Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints published for *C. pseudotuberculosis*, which prevents the classification of isolates as susceptible or resistant. The CLSI guidelines vary, even among the same genus of bacteria, thus extrapolating susceptibility patterns across bacterial species is not possible. For example, the CLSI susceptibility breakpoint for penicillin is ≤0.12 μg/mL for beta-hemolytic streptococci (excluding *S. pneumoniae*), ≤0.06 μg/mL for *S. pneumoniae*, and ≤1 μg/mL for isolates of *Corynebacterium* sp from humans. As a result, the MIC90 data, combined with data from published pharmacokinetic studies in horses, can be used to guide treatment of *C. pseudotuberculosis* infections in horses.

Macrolides, including erythromycin and clarithromycin, as well as the closely related azolides (azithromycin) are highly lipophilic and conducive for use in the treatment of abscesses. Plasma concentrations above the MIC determined in this study are achievable in horses, making these drugs attractive for the treatment of *Corynebacterium* abscesses or lymphangitis. The risk of severe colitis in adult horses, however, precludes the common use of macrolides in equine practice.

Ceftiofur is labeled for use in horses and is commonly used in equine practice. The MIC90 was found to be 2 μg/mL. This MIC suggests that ceftiofur would be a poor choice for treatment of *Corynebacterium pseudotuberculosis* in horses, because plasma concentrations of 2 μg/mL would be unachievable for >50% of the dosing interval in adult horses using the labeled and commonly used dosage of 2.2 mg/kg IM q24h for ceftiofur sodium (Naxcel®) or 6.6 mg/kg for ceftiofur crystalline free acid (Excede®). The use of ceftiofur should be dictated by MIC testing of specific isolates.

Previously published studies suggested that *C. pseudotuberculosis* isolates were highly susceptible to beta lactam antimicrobials such as ampicillin in vitro. IV administered ampicillin could have potential value in treating *C. pseudotuberculosis* infections in hospitalized horses, but abscess penetrability should be considered because ampicillin has low lipid solubility. Penicillin administered IM at a dosage of 22,000 IU/kg produces plasma concentrations of 0.86 μg/mL for 24 hours. The MIC90 for penicillin in our study was 0.25 μg/mL, indicating that attainable plasma penicillin concentrations are above the MIC for *C. pseudotuberculosis* for adequate duration. A potential disadvantage of penicillin is its lack of lipid solubility in the presence of well-encapsulated abscesses typical of *C. pseudotuberculosis* infections.

The tetracycline class of drugs is attractive for the treatment of this disease because of their lipid solubility. Based on the pharmacokinetics of PO administered doxycycline in horses, the recommended MIC targets have included 0.25 μg/mL because plasma concentrations in horses after PO administration rarely exceed 0.48 ± 0.11 μg/mL. The drug should not be administered IV because it is associated with fatal dysrhythmias in horses. The MIC90 of doxycycline in our study was ≤2 μg/mL for all isolates combined, because 2 μg/mL was the lowest dilution on the trays used in the prospective component of the study. However, during the period 2007–2012, the trays used included a concentration 10-fold lower than the achievable peak plasma concentrations in horses. Minimum inhibitory concentrations values >0.5 μg/mL would not be conducive to this result. Another measure of potential for efficacy of the fluoroquinolones is the area under the curve: MIC (AUC:MIC) ratio. Using reported AUCs of 21.03 and 16.3 μg h/mL for IV and intragastric enrofloxacin administration in horses, respectively, and the MICs obtained in this study, the calculated AUC:MIC ratios for enrofloxacin against *C. pseudotuberculosis* are 65.2–84.1. These are within the suggested targets (45–95) used for fluoroquinolones in human patients and are consistent with an expectation for efficacy.

This observation also highlights the need for MIC testing of *C. pseudotuberculosis* isolates, because individual strains may have MIC above achievable plasma concentrations of enrofloxacin in horses. Potentiated sulfonamides are widely used PO antimicrobials in horses. Similar to enrofloxacin, the MIC90 of TMS was below achievable plasma concentrations reported in horses. The MIC of gentamicin was approximately 10-fold lower than achievable peak plasma concentrations reported in horses. The range of MIC for gentamicin in our study was 0.25–8 μg/mL, with those isolates having MIC ≤2 μg/mL having potentially achievable peaks. However, aminoglycosides do have decreased activity in vivo to closely related microbes such as *Rhodococcus equi*. In addition, aminoglycoside uptake is decreased in anaerobic or acidic environments, and aminoglycosides may be inactivated by purulent material, 2 conditions that exist in the environment of *C. pseudotuberculosis* abscesses. Finally, aminoglycosides are hydrophilic, making them poor candidates to
penetrate abscesses. All of these factors suggest that aminoglycosides may not be a first line choice in the treatment of horses with *C. pseudotuberculosis* infections. The MIC\(_{90}\) of amikacin was 8 µg/mL. Peak plasma concentrations of 64–80 µg/mL (8 to 10-fold the MIC) would be difficult to achieve using commonly used dosage in horses.\(^{28}\)

Chloramphenicol is highly lipophilic. Because of the relatively poor oral bioavailability after repeat administration, the high frequency of administration required, and risk of aplastic anemia in humans handling the drug, the use of chloramphenicol for treatment of *C. pseudotuberculosis* infection in horses may be less desirable than other antimicrobials.\(^{29}\) Because of the variable absorption of chloramphenicol in horses and the MIC values determined in this study, chloramphenicol is not ideal for treatment of most infections of *C. pseudotuberculosis* in horses; use in individual cases should be dictated by MIC results.\(^{30}\)

Rifampin is commonly used in the treatment of *C. pseudotuberculosis* infections in horses, because of its high oral bioavailability and intercellular penetration.\(^{5,6}\) The MIC results of this study would support its use.\(^{31}\) However, because of concerns over acquired resistance, it likely should be administered with another effective and compatible antimicrobial.\(^{32}\)

The classification of the abscess as internal or external did not correlate with differences in MIC\(_{90}\) results over time. When combining isolates from all time periods, there were no significant differences between MICs of *C. pseudotuberculosis* isolates cultured from external versus internal abscesses.

Limitations of this study were largely a result of the retrospective and cross-over design of the study. The antimicrobials and their respective dilutions on the commercially available microtiter Sensititre tray used by the microbiology laboratory at the VMTH have changed over the study periods. This resulted in a paucity of data for some antimicrobials because the specific antimicrobials as well as the dilutions included on the trays varied over the years. This may have resulted in statistically significant differences being missed when the data were evaluated for temporal changes in MIC because some of the trays used relatively high antimicrobial concentrations. For example, the standard trays used 2 µg/mL as the lowest dilution for doxycycline, but because of relatively high oral bioavailability in horses, 0.25 µg/mL would be a more appropriate dilution to use, and was used in the more recent trays. Despite these different lowest dilutions used for some of the antibiotics across time, no statistically significant differences were detected in MIC results over the 3 time periods. The MIC results obtained from records and those obtained prospectively were within 1 dilution of one another for all of the studied antimicrobials, with the exception of rifampin and doxycycline for which the lowest concentration available on the specific trays used differed widely. Another limitation was that the MIC data were based on only 1 selected colony, rather than multiple colonies. Variations in antimicrobial susceptibility may be present in bacteria of the same species isolated from 1 source.\(^{33}\) Another potential limitation of the study is that the MICs were determined at 48 hours because corynebacteria are slow-growing microbes.\(^{11,34}\) However, 48 hours has been used for MIC determination of other corynebacteria.\(^{31}\)

No CLSI or EUCAST clinical breakpoints or consensus guidelines exist for *C. pseudotuberculosis* isolates. Therefore, it was not possible to classify isolates as susceptible or resistant. Instead, we utilized published knowledge of pharmacokinetic and pharmacodynamic properties of the tested antimicrobials, such as oral bioavailability and lipophilicity, to hypothesize whether the determined MIC would be conducive to the use of these drugs in the treatment *C. pseudotuberculosis* infection in horses.

In conclusion, this study suggests that many commonly used antimicrobials in equine practice are effective against *C. pseudotuberculosis* isolates in vitro and that changes in MIC were not identified over the study period. Antimicrobial susceptibility testing of isolates as well as oral bioavailability of antimicrobials should be considered in horses when interpreting potential for clinical efficacy. Currently, MIC testing of *C. pseudotuberculosis* isolates is not routinely performed by all commercial microbiology laboratories. Based on the results of this study, MIC testing of cultured isolates should be strongly recommended.

**Footnotes**

\(^a\) Microbank system, Pro-Lab Diagnostics, Round Rock, TX
\(^b\) Blood agar plate, 5% sheep blood in tryptic soy agar base, Hardy Diagnostics, Santa Maria, CA
\(^c\) Brain Heart Infusion broth with 0.2% Tween 80, Veterinary Medical Biologic Media Services, Davis, CA
\(^d\) Thermo Fisher Scientific, Waltham, MA
\(^e\) Zoetis, Florham Park, NJ

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**Conflict of Interest Declaration:** The authors disclose no conflict of interest.

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

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