Comparative *in vitro* Susceptibility of Bacterial Isolates from Horses to Trimethoprim/Sulfadiazine and Trimethoprim/Sulfamethoxazole

W. David Wilson, BVMS, MS, HonDACVIM*; and Judy E. Edman, BS

In vitro susceptibility testing of 479 bacterial isolates from horses, including *Streptococcus equi* subsp. *zooepidemicus* (n = 282), *S. equi* subsp. *equi* (n = 55), *Corynebacterium pseudotuberculosis* (n = 96), and *Actinobacillus equuli* (n = 46) revealed that 478 (99.7%) were highly susceptible to both trimethoprim/sulfadiazine (TMP-SDZ) and trimethoprim/sulfamethoxazole (TMP-SMZ). Minimum inhibitory concentration (MIC) values for all susceptible isolates were between ≤0.12/2.4 μg/mL and 1/19 μg/mL for both drug combinations and most isolates were susceptible to the lowest concentration tested (≤0.12/2.4 μg/mL). Whereas 52.5% of *S. zooepidemicus* isolates and 60% of *S. equi* isolates had an MIC value for TMP-SDZ that was one concentration higher than for TMP-SMZ, this result is unlikely to be of clinical significance and does not justify the extra-label use of TMP-SMZ in preference to available FDA approved oral TMP-SDZ formulations. Authors' address: Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California–Davis, Davis, CA 95616; e-mail: wdwilson@ucdavis.edu. *Corresponding and presenting author.

1. Introduction

Bacterial infections are common in adult horses and foals and are associated with substantial morbidity and mortality. *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*), *S. equi* subsp. *equi* (*S. equi*), *Actinobacillus equuli* (*A. equuli*) and, in some geographic areas, *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*), are among the most prevalent and important bacterial pathogens, causing disease in the respiratory tract and other body systems.[1] Potentiated sulfonamides (trimethoprim/sulfonamide combinations) are frequently included in treatment protocols for infections with these and other bacteria because a broad spectrum of pathogens are susceptible to these drugs.[2] Additionally, potentiated sulfonamides are well absorbed following oral administration to both adult horses and foals.[3–7] Of the potentiated sulfonamides, only trimethoprim/sulfadiazine (TMP-SDZ) formulations are currently FDA approved for use in horses; the label indication being treatment of lower respiratory tract infections caused by *S. zooepidemicus*. For many years, TMP-SDZ formulations for oral use were not available in the United States; therefore, practitioners instead used the human-label trimethoprim/sulfamethoxazole (TMP-SMZ) formulations in an extra-label manner, in compliance with the provisions of the Animal Medicinal Drug Use Clarification Act (AMDUCA).[8] Now that several FDA-approved TMP-SDZ products are available, there is minimal justification for use of the human-label TMP-SMZ formulation because such use is rarely justified under the provisions of AMDUCA.

NOTES

These include the provision that there is no approved new animal drug that is labeled for such use and that contains the same active ingredient, which is in the required dosage form and concentration, except where a veterinarian finds, within the context of a valid veterinarian-client-patient relationship, that the approved new animal drug is clinically ineffective for its intended use.[8] Two important determinants of clinical efficacy of antibiotics are pharmacokinetics, including bioavailability following administration by routes other than intravenous, and susceptibility of commonly encountered bacteria to the antimicrobial drug or combination in question.[1,9] Several studies have shown that TMP-SDZ is well absorbed after oral administration to horses and has a pharmacokinetic profile that is consistent with efficacy following oral dosing at 12-hour intervals.[4–7,10] To date, there is a paucity of data regarding the comparative *in vitro* susceptibility of *S. zooepidemicus* and other equine-origin bacterial pathogens to TMP-SDZ and TMP-SMZ. The objective of this study was therefore to generate such data for *S. zooepidemicus* and other common equine pathogens.

2. Methods

Antimicrobial susceptibility testing was performed on equine-origin isolates of *S. zooepidemicus* (n = 282), *S. equi* (n = 55), *C. pseudotuberculosis* (n = 96), and *A. equuli* (n = 46). The *Actinobacillus* spp. tested included *A. equuli* subsp. *equuli* (n = 14), *A. equuli* subsp. *hemolyticus* (n = 14), *A. equuli* subsp. *hemolyticus* biovar 1 (n = 14), and *A. equuli* subsp. *hemolyticus* biovar 2 (n = 5). These bacteria had been collected between 1986 and 2016 from adult horses and foals with clinical disease, and had been stored as frozen stabilates at −80°C in skim milk or on glass beads.[a] The identity of each bacterial isolate was confirmed based on colony morphology, Gram-staining characteristics, biochemical characteristics, and results of genetic testing using the matrix-assisted laser desorption ionization–time of flight mass spectrometry system. Susceptibility testing was performed using the broth microdilution procedure[b], following Clinical Laboratory Standards Institute protocols.[11] Briefly, one bacterial colony was inoculated into brain heart infusion broth and incubated for 4 hours at 35°C. A small amount of this inoculated broth was then added to 0.85% NaCl solution to achieve a 0.5 McFarland Standard concentration, as measured using a nephelometer. Ten microliters of this suspension were added to Mueller Hinton broth, and plates[b] were inoculated with 100 μL of the Mueller Hinton broth in each well. The following bacterial strains were run weekly as controls in accordance with the standard quality control procedures in place at the Veterinary Medical Teaching Hospital Microbiology Laboratory (MDL): *Staphylococcus aureus* America Type Culture Collection (*ATCC*) *29213*, *Enterococcus faecalis ATCC 29212*, *E. coli ATCC 25922*, *E. coli ATCC 35218*, and *Pseudomonas aeruginosa ATCC 27853*.

Sensititre™ plates[b] were custom made for the MDL by the manufacturer. The range of TMP-SDZ or TMP-SMZ concentrations tested was 0.12/2.4 μg/mL to 8/152 μg/mL for each antimicrobial combination. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration of antimicrobial drug combination (TMP-SDZ or TMP-SMZ) that inhibited visible growth of bacteria. An isolate was considered to be susceptible to TMP-SDZ or TMP-SMZ if its MIC value was ≤ 2/38 μg/mL, as recommended by the Clinical Laboratory Standards Institute.[11] The respective concentrations at which 50% (MIC50) and 90% (MIC90) of isolates of a particular bacterial species were susceptible to TMP-SDZ and TMP-SMZ, were also determined. In order to create numerical data that could be analyzed, only the concentration of TMP in the fixed ratio combination was used. Concentrations that were at or below the lower limit of quantitation of the MIC test (i.e., ≤ 0.12 μg/mL) were ascribed a value of 0.12 μg/mL to facilitate statistical analysis using the Wilcoxon signed-rank test for paired data. A P-value of ≤ .05 was used to ascribe statistical significance to differences between groups (TMP-SDZ vs TMP-SMZ).

3. Results

Overall Findings

The MIC values for both TMP-SDZ and TMP-SMZ against isolates of *S. zooepidemicus*, *S. equi*, *C. pseudotuberculosis*, and *A. equuli* are shown in Table 1. Of the 479 isolates tested, only 1 (0.21%), a *S. zooepidemicus* isolate, was resistant to TMP-SDZ. The same isolate was also resistant to TMP-SMZ.

S. equi subsp. *zooepidemicus*

Of the 282 *S. zooepidemicus* isolates tested, 281 (99.6%) were susceptible to both TMP-SDZ and TMP-SMZ (MIC ≤ 2.0/38 μg/mL), whereas 1 (0.4%) isolate was resistant to both drug combinations (MIC = 8/152 μg/mL). With the exception of the resistant isolate, all *S. zooepidemicus* isolates were highly susceptible, with MIC values ranging between ≤0.12/2.4 μg/mL and 1/19 μg/mL for both TMP-SDZ and TMP-SMZ (Table 1). One hundred thirty-four of the 282 *S. zooepidemicus* isolates (47.5%) had an MIC value that was the same for both TMP-SDZ and TMP-SMZ, whereas 148 isolates (52.5%) had an MIC value for TMP-SDZ that was one concentration higher than for TMP-SMZ. In other words, 52.5% of isolates were one dilution less susceptible to TMP-SDZ than to TMP-SMZ. Statistical analysis showed this difference to be highly significant ($P < .0001$). The MIC_{50} values for TMP-SDZ and TMP-SMZ were 0.25/4.75 μg/mL and 0.12/2.4 μg/mL, respectively. The MIC_{90} value for both drug combinations was 0.25/4.75 μg/mL.

S. equi subsp. *equi*

Of the 55 *S. equi* isolates, all (100%) were highly susceptible to both drug combinations (MIC range of

Table 1. MIC Values for TMP-SDZ and TMP-SMZ Against Isolates of *Streptococcus. equi* subsp. *zooepidemicus*, *S. equi* subsp. *equi*, *Corynebacterial pseudotuberculosis* and *Actinobacillus equuli*

		No. of Isolates Susceptible at Each Antimicrobial Dilution (μg/mL)*						
Organism (No. of Isolates)	Antimicrobial*	≤0.12/2.4	0.25/4.75	0.5/9.5	1.0/19	2.0/38	4.0/76	8.0/152
S. zooepidemicus (282)	TMP-SDZ[a]	101	175	4	1			1
	TMP-SMZ[a]	243	37		1			1
S. equi (55)	TMP-SDZ[b]	21	33	1				
	TMP-SMZ[b]	50	5					
C. pseudotuberculosis (96)	TMP-SDZ	95	1					
	TMP-SMZ	95	1					
A. equuli (46)	TMP-SDZ	43	2	1				
	TMP-SMZ	43	2	1				

*Mean MIC values for antimicrobials (TMP-SDZ and TMP-SMZ) identified with the same superscript letter are significantly different from each other ($P < .001$) for the bacterial species included in each row.

≤0.12/2.4 μg/mL to 1/19 μg/mL). For TMP-SDZ, 21 isolates had an MIC of ≤0.12/2.4 μg/mL, 33 had an MIC of 0.25/4.75 μg/mL, and 1 had an MIC of 0.5/9.5 μg/mL (Table 1). For TMP-SMZ, 50 isolates had an MIC of ≤0.12/2.4 μg/mL and 5 had an MIC of 0.25/4.75 μg/mL. Twenty-three (42%) of the 55 *S. equi* isolates had the same MIC for both TMP-SDZ and TMP-SMZ, 33 (60%) had an MIC for TMP-SDZ that was one concentration higher than for TMP-SMZ and 1 (1.8%) had an MIC value for TMP-SMZ that was 1 concentration lower than for TMP-SMZ. Statistical analysis showed MIC values for TMP-SMZ to be significantly lower than those for TMP-SDZ ($P < .0001$). The MIC_{50} and MIC_{90} for TMP-SDZ were both 0.25/4.75 μg/mL, whereas the MIC_{50} and MIC_{90} for TMP-SMZ were both ≤0.12/2.4 μg/mL.

A. equuli

All *A. equuli* isolates were highly susceptible to both TMP-SDZ and TMP-SMZ; MIC values ranged from ≤0.12/2.4 μg/mL to 0.5/9.5 μg/mL for both drug combinations (Table 1). MIC values for TMP-SDZ were identical to those of TMP-SMZ. Forty-four of the 47 isolates (93.6%) had MIC values of ≤0.12/2.4 μg/mL for both drug combinations, 2 isolates (4.3%) had MIC values of 0.25/4.75 μg/mL and 1 isolate (2.1%) had an MIC value. of 0.5/9.5 μg/mL. No significant differences were observed in MIC values between TMP-SDZ and TMP-SMZ.

C. pseudotuberculosis

All isolates of *C. pseudotuberculosis* were highly susceptible to both TMP-SDZ and TMP-SMZ and the two drug combinations showed equal antimicrobial activity (Table 1). The MIC value for 95 of 96 isolates was ≤0.12/2.4 μg/mL for both TMP-SDZ and TMP-SMZ. The remaining isolate had an MIC value of 0.25/4.75 μg/mL for both drug combinations. The MIC_{50} and MIC_{90} values were ≤0.12/2.4 μg/mL for both TMP-SDZ and TMP-SMZ and no significant differences were observed between them.

4. Discussion

The almost universal susceptibility of the commonly encountered equine bacterial pathogens tested in this study to the potentiated sulfonamide antimicrobials attests to their potential utility for treating a range of Gram-positive and Gram-negative bacterial infections in horses. Of the potentiated sulfonamide antimicrobials, only the TMP-SDZ combination is licensed for use in horses; the label indication being treatment of respiratory infection caused by *S. zooepidemicus*. In addition to the favorable pharmacokinetic profile of TMP-SDZ after oral administration to both adult horses and foals, the label indication is supported by the findings of this antimicrobial susceptibility study. Two hundred eighty-one of 282 (99.6%) *S. zooepidemicus* isolates were found to be susceptible to TMP-SDZ (MIC ≤2/38 μg/mL). Of these, 276 (97.2%) were susceptible at concentrations ≤ 0.25/4.75 μg/mL. Although statistical analysis using the Wilcoxon signed-rank test showed that MICs for TMP-SMZ were significantly lower than those for TMP-SDZ against *S. zooepidemicus* and *S. equi*, this difference was typically one dilution and is unlikely to be of clinical significance because the MIC was approximately 10-fold lower than the cut-off for susceptibility (2/38 μg/mL). Additionally, those isolates that had MIC values greater than 0.25/4.75 μg/mL for TMP-SDZ also had higher MIC values for TMP-SMZ. Furthermore, Diagnostic Laboratories rarely include concentrations lower than 0.5/9 μg/mL in quantitative (MIC) susceptibility tests on clinical isolates or use the non-quantitative Kirby-Bauer test. Under these circumstances, reported susceptibility profiles for TMP-SDZ and TMP-SMZ would typically be identical.

The above results do not support the extra-label use of TMP-SMZ in preference to approved formulations of TMP-SDZ; in fact, such use of TMP-SMZ could be interpreted as violating the provisions of the AMDUCA and should be discouraged.

Acknowledgments

Declaration of Ethics

The Authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

This study was funded by Aurora Pharmaceutical, Inc., 1196 Highway 3 South, Northfield, MN 55057. Although the funding source poses a potential conflict of interest, the Authors have no financial interest in Aurora Pharmaceutical, Inc.

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[a]Microbank™, Pro Lab Diagnostics, Inc., Richmond Hill, ON L4B 1K3, Canada.
[b]Sensititre™, Thermo Fisher Scientific, Waltham, MA 02451.