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Original Research

Antimicrobial Use for and Resistance of Zoonotic Bacteria Recovered from Nonhuman Primates

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As a growing threat to human and animal health, antimicrobial resistance (AMR) has become a central public-health topic. Large-scale surveillance systems, such as the National Antimicrobial Resistance Monitoring System (NARMS), are now established to monitor and provide guidance regarding AMR, but comprehensive literature on AMR among NHP is sparse. This study provides data regarding current antimicrobial use strategies and the prevalence of AMR in zoonotic bacteria recovered from NHP within biomedical research institutions. We focused on 4 enteric bacteria: *Shigella flexneri*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Campylobacter jejuni*. Fifteen veterinarians, 7 biomedical research institutions, and 4 diagnostic laboratories participated, providing susceptibility test results from January 2012 through April 2015. Veterinarians primarily treated cases caused by *S. flexneri*, *Y. enterocolitica*, and *Y. pseudotuberculosis* with enrofloxacin but treated *C. jejuni* cases with azithromycin and tylosin. All isolates were susceptible to the associated primary antimicrobial but often showed resistance to others. Specifically, *S. flexneri* isolates frequently were resistant to erythromycin (87.5%), doxycycline (73.7%), and tetracycline (38.3%); *Y. enterocolitica* isolates to ampicillin (100%) and cefazolin (93.6%); and *C. jejuni* isolates to methicillin (99.5%) and cephalothin (97.5%). None of the 58 *Y. pseudotuberculosis* isolates was resistant to any tested antimicrobial. Notably, resistance patterns were not shared between this study's NHP isolates and human isolates presented by NARMS. Our findings indicate that zoonotic bacteria from NHP diagnostic samples are broadly susceptible to the antimicrobials used to treat the clinical infections. These results can help veterinarians ensure effective antimicrobial therapy and protect staff by minimizing occupational risk.

Abbreviations: AMR, antimicrobial resistance; NARMS, National Antimicrobial Resistance Monitoring System; TP-AMR, threshold prevalence of antimicrobial resistance

The threat of antimicrobial resistance (AMR) forces veterinarians and physicians to choose secondary or tertiary antimicrobial choices that may decrease the effectiveness and efficiency of antimicrobial therapy. This threat is especially important in terms of zoonotic bacteria, because a single pathogenic organism can endanger the health of both animal patients and the persons who contact these animals. *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Campylobacter jejuni* are zoonotic bacteria that are repeatedly isolated from NHP in biomedical research institutions,[17,23,26,43] and all 4 organisms can cause serious disease in the event of occupational exposure.[4,12,14,22,23,28] *S. flexneri* and *C. jejuni*, in particular, have been identified as serious threats to human health by the Centers for Disease Control and Prevention.[10] However, the occupational risk associated with these zoonotic bacteria in NHP is not well understood.

Antimicrobial therapy is used to treat infections in NHP patients and minimize the negative effects of disease on animal wellbeing. However, treatments administered to NHP serving as animal models of human disease in biomedical research can impair the validity of study results. For instance, antimicrobial therapy can have long-term effects in NHP by altering the gut microbiota.[30] Maximizing effective and efficient therapy is important to minimize unknown extraneous variables that can compromise study results. A more comprehensive understanding of the prevalence of AMR across biomedical institutions likely will provide veterinarians a reference point for making more informed treatment and policy decisions.

In addition, public health concerns exist regarding zoonotic bacteria in biomedical research. Tuberculosis, Q-fever, and salmonellosis are commonly studied zoonotic diseases and can cause mortality and morbidity among animal patients and personnel.[13,29,31,33,36,40,42] Therefore, ensuring the selection of an appropriate antimicrobial is important for effective therapy. However, without regularly monitoring AMR, veterinarians may unknowingly be applying unnecessary antimicrobial selective pressure and fueling the development or acquisition of AMR.[27]

This study provides comprehensive information on the prevalence of AMR among zoonotic bacteria in NHP. A similarly inclusive study was published in 1969,[17] but changes in AMR since then are expected, given the introduction of new antimicrobials and the potential emergence and dissemination of novel strains.

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Although several research teams have reported more recent data on AMR in zoonotic bacteria recovered from NHP,[20,21,26,43] none of these reports match the present study's combined temporal, institutional, and geographic scope in the United States. Given the paucity of available data on the AMR of zoonotic bacteria in NHP, the objectives of the present study were to: 1) estimate the prevalence of AMR among *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Campylobacter jejuni* isolates recovered from diagnostic laboratory samples from NHP between January 2012 and April 2015; 2) evaluate the current antimicrobial use strategies of veterinarians treating diseases caused by these bacteria; and 3) determine the likelihood of changes in antimicrobial use strategy among veterinarians with knowledge of AMR prevalence. This change in antimicrobial use strategy was quantified with participating veterinarians by identifying a threshold prevalence of AMR (TP-AMR) which, when exceeded by the true prevalence of AMR, would cause the veterinarians to change their strategies. We hypothesized that the prevalence of AMR among the 4 bacteria listed will exceed participating veterinarians' TP-AMR.

Materials and Methods

Overall study design. To test our hypothesis, we conducted a cross-sectional study of veterinarians within biomedical research institutions and a retrospective study of zoonotic bacteria recovered from diagnostic submissions to associated laboratories.

Selection criteria. Participants' inclusion required 1) the willingness of both the biomedical institution and 2) associated diagnostic laboratory to participate, and 3) the availability of a searchable database (paper or electronic) of results from antimicrobial susceptibility tests. If one or more criteria were not met, neither the biomedical research institution nor the diagnostic laboratory was included in the study population.

Biomedical research institutions. We identified a source population of 21 biomedical research institutions within the United States. These institutions are among the largest and most active institutions that use NHP for research. In addition to considering size and research activity, we also included in the source population other institutions that use NHP and with whom we have had professional experience. We contacted each institution's veterinary director or attending veterinarian and asked for their participation in the study. Seven biomedical research institutions were willing to participate, and all 7 institutions maintained animals in accordance with the USDA Animal Welfare Act, Animal Welfare Regulations, and the *Guide for the Care and Use of Laboratory Animals*[19,47] and were fully accredited by AAALAC as of 2015. In addition, 5 of the 7 participating institutions' animal care programs maintained a Public Health Service Animal Welfare Assurance[32] during the study; among the 5 were institutions that provided us AMR data.

Diagnostic laboratories. Of the 7 participating biomedical research institutions, the veterinary director or attending veterinarian provided contact information for the diagnostic laboratory that performed routine antimicrobial susceptibility testing for bacterial pathogens recovered from clinical submissions. We then contacted the laboratory directors, requesting their participation in the study. Four labs agreed to participate, all of which had searchable databases of susceptibility test results.

Surveys. All surveys were approved by the Ohio State University Institutional Review Board. Surveys were distributed electronically by email or by using an online survey software (Qualtrics, Provo, UT). A pilot study involving 4 Diplomates of the American College of Laboratory Animal Medicine was conducted initially to evaluate the survey used in the present study. The survey was distributed to participating veterinarians, who were given 2 mo to complete the survey. The veterinarians' surveys provided data on the participants' antimicrobial use strategies. In addition, participating veterinarians identified their TP-AMR which, if exceeded by the true prevalence of AMR, would cause them to consider changing their antimicrobial use strategies.

A second and distinct survey was distributed to a microbiologist within each participating laboratory. The goal of the microbiologists' survey was to gather data on antimicrobial susceptibility test techniques used for the investigated bacteria, the type of database used (paper or electronic), and how the database was searchable.

Antimicrobial susceptibility test results. Participating diagnostic laboratories provided the investigators with antimicrobial susceptibility test results from samples with isolated *S. flexneri*, *Y. enterocolitica*, *Y. pseudotuberculosis*, or *C. jejuni* from January 2012 to April 2015. The sample source was not limited to clinical submissions alone.

Statistical analysis. A posthoc power analysis was conducted to identify lowest isolate-level prevalence of resistance detectable given the total number of isolates of each bacterial species in the final dataset.[15] It is reasonable to assume that the samples of isolates were taken from a considerably larger population, circulating among each institution's NHP colony, within the study's time frame. Therefore, we assumed that the investigated bacterial isolates were sampled from infinite populations. Data were analyzed by using JMP (version 11.0.0, SAS, Cary, NC) for descriptive statistics.

Results

Survey response. We sent the survey to 38 veterinarians among the 7 participating research institutions; 15 veterinarians (39.5%) provided the most useable data, with nearly complete to completed surveys. Three microbiologists received surveys, and all 3 were completed.

Biomedical research institutions. Each institution's veterinary staff ranged from 3 to 7 veterinarians. Three institutions reported having 350 to 1600 NHP, but the remaining 4 reported populations of 3800 to 8000 NHP in total. Participating institutions housed a variety of commonly used New World and Old World NHP species, including the red-bellied titi (*Callicebus moloch*), common marmoset (*Callithrix jacchus*), tufted capuchin (*Cebus paella*), sooty mangabey (*Cercocebus atys atys*), white-naped mangabey (*Cercocebus atys lunulatus*), collared mangabey (*Cercocebus torquatus*), African green monkey (*Chlorocebus aethiops*), cynomolgus macaque (*Macaca fascicularis*), rhesus macaque (*Macaca mulatta*), pig-tailed macaque (*Macaca nemestrina*), and hamadryas baboon (*Papio hamadryas*). Three institutions reported having both outdoor and indoor NHP colonies, whereas 2 others reported exclusively having outdoor colonies; the remaining 2 had exclusively indoor colonies.

Diagnostic laboratories. Three participating diagnostic laboratories reported the Kirby Bauer test as their primary susceptibility test for bacteria. In addition, 2 of these 3 laboratories indicated the minimum inhibitory concentration test as their secondary test but did not specify the technique. The last of these 3 laboratories in-

Table 1. Antimicrobials tested in susceptibility tests for *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, or *Campylobacter jejuni* among participating diagnostic laboratories corresponding to participating biomedical research institutions A, B, and C

	S. flexneri			*Y. enterocolitica*			*Y. pseudotuberculosis*			*C. jejuni*		
	A	B	C	A	B	C	A	B	C	A	B	C
Enrofloxacin	X	X	X	X	X	X	X	X	X	X		X
Ciprofloxacin	X	X			X			X		X		
Marbofloxacin	X			X			X			X		
Erythryomycin	X			X			X			X		
Azithromycin			X			X			X			X
Chloramphenicol	X	X		X	X		X	X		X		
Trimethoprim–sulfadiazine	X			X			X			X		
Trimethoprim–sulfamethoxazole		X	X		X	X		X	X			
Amikacin	X	X		X	X		X	X		X		
Gentamicin	X	X		X	X		X	X		X		
Neomycin	X		X			X	X		X			
Tobramycin				X								
Kanamycin	X						X					
Ampicillin	X	X		X	X		X	X		X		
Amoxicillin	X						X					
Methicillin										X		
Piperacillin				X			X					
Carbenicillin	X						X					
Amoxicillin–clavulanic acid	X			X			X			X		
Cephalothin	X			X			X			X		
Ceftriaxone			X			X			X			
Ceftiofur	X			X			X					
Cefazolin	X	X			X		X	X				
Cefpodoxime	X			X			X					
Cefovecin	X											
Tetracycline	X	X		X	X		X	X		X		
Doxycycline	X		X	X		X	X		X			X
Colistin	X						X					
Polymyxin B	X											
Clindamycin							X			X		

dicated that a secondary technique was not used with the investigated bacteria. A fourth laboratory, associated with institution D, did not provide any data; none of the investigated bacteria were isolated from institution D within the study's time frame.

Antimicrobials included in susceptibility tests are listed in Table 1.

Prevalence of AMR. ***Institution A.*** Isolates of *S. flexneri* were resistant most frequently to erythromycin (87.5%, 21 of 24 isolates), amoxicillin–clavulanic acid (60.0%, 15 of 25), and doxycycline (73.7%, 14 of 19) (Table 2). *Y. enterocolitica* isolates were resistant most frequently to erythromycin (100%, 2 of 2), amoxicillin–clavulanic acid (100%, 5 of 5), ampicillin (100%, 2 of 2), and doxycycline (100%, 2 of 2) (Table 2). No AMR was observed for *Y. pseudotuberculosis* (Table 2). Finally for *C. jejuni*, 99.5% (569 of 572) of isolates were resistant to methicillin and 97.5% (557 of 571) to cephalothin (Table 2). Although bone fide resistance of *C. jejuni* to ampicillin was rare, 98.1% (561 of 572) of isolates had decreased susceptibility (intermediate resistance; Table 2).

Institution B. Isolates of *S. flexneri* were resistant most frequently to tetracycline (38.3%, 157 of 410 isolates) (Table 2). *Y. enterocolitica* isolates were resistant most frequently to ampicillin (100%, 47 of 47) and cefazolin (93.6%, 44 of 47; Table 2). AMR among *Y. pseudotuberculosis* isolates from institution B was similar to that of institution A; no AMR was observed (Table 2). Susceptibility testing of *C. jejuni* was not performed in institution B (Table 2).

Institution C. Isolates of *S. flexneri* (n = 1), *Y. enterocolitica* (n = 1), and *Y. pseudotuberculosis* (n = 1) were all susceptible to enrofloxacin, azithromycin, ceftriaxone, doxycycline, neomycin, and trimethoprim–sulfamethoxazole. A nonspeciated *Campylobacter* (n = 1) isolate was susceptible to enrofloxacin and azithromycin.

Detecting AMR. Because of differences in total isolate numbers, the statistical power to detect AMR varied among the sample populations of isolates within participating institutions. Given the sample populations of bacterial species from each institution, the sample size was sufficient to have a 99% chance (α = 0.01) of detecting AMR when the prevalence of AMR was as low as 0.8% of the largest isolate population and as high as 14.2% of the smallest isolate population.

Isolate resistance patterns. Identical resistance patterns were recovered repeatedly within institutions (Table 3). In particular, 96% (551 of 574) of *C. jejuni* isolates from institution A expressed resistance to only cephalothin and methicillin, and 38.3% (157 of

Table 2. Estimated proportions of antimicrobial resistance within biomedical research institutions A and B for *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Campylobacter jejuni* recovered from NHP from January 2012 to April 2015

Institution	Enr	Ery	A–C	Amp	Car	Met	Cef	Cep	Tet	Dox	Gen	Chl
A												
S. flexneri	0%	88%	60%	50%	50%	—	—	0%	50%	74%	24%	50%
	0/30	21/24	15/25	3/6	3/6			0/6	3/6	14/19	7/29	3/6
Y. entericolitica	0%	100%	100%	100%	—	—	—	—	0%	100%	0%	0%
	0/5	2/2	5/5	2/2					0/3	2/2	0/5	0/3
Y. pseudotuberculosis	0%	—	—	0%	0%	—	0%	0%	0%	0%	0%	0%
	0/1			0/1	0/1		0/1	0/1	0/1	0/1	0/1	0/1
C. jejuni	0%	0%	0%	0.3%	—	99.5%	—	98%	0%	—	0%	1%
	0/574	0/574	0/572	0/572[a]		569/572		557/571	0/572		0/574	7/571
B												
S. flexneri	0%	0%	—	0.2%	—	—	0%	—	38%	—	0%	0.7%
	0/410	0/410		1/410			0/410		157/410		0/410	0/410
Y. entericolitica	0%	0%	—	100%	—	—	94%	—	0%	—	0%	0%
	0/47	0/47		47/47			44/47		0/47		0/47	0/47
Y. pseudotuberculosis	0%	0%		0%	—	—	0%	—	0%	—	0%	0%
	0/57	0/55		0/55			0/55		0/55		0/55	0/57
C. jejuni	—	—	—	—	—	—	—	—	—	—	—	—

A–C, amoxicillin–clavulanic acid; Amp, ampicillin; Car, carbencillin; Cef, cefazolin; Cep, cephalothin; Chl, chloramphenicol; Dox, doxycycline; Enr, enrofloxacin; Ery, erythromycin; Gen, gentamicin; Met, methicillin; Tet, tetracycline
[a]98.1% (561 of 572) of samples tested intermediate to ampicillin.

410) of *S. flexneri* isolates from institution B expressed resistance to tetracycline alone (Table 3). Shared resistance patterns might indicate shared resistance genes or the persistence and transmission of clonal strains within institutions.[37] In addition, *S. flexneri* isolates from institution A showed substantial diversity in resistance patterns (Table 3).

Therapeutic antimicrobial selection among NHP veterinarians. It is often appropriate to initiate empirical antimicrobial therapy prior to receiving microbiologic test results when treating diarrhea-associated clinical signs.[49] Therefore, we developed the survey to identify participating veterinarians' primary antimicrobials for treating clinical signs of diarrhea and gingivitis, which we included because of *S. flexneri*'s ability to cause periodontal disease.[3] Most participating veterinarians reported enrofloxacin (40%, 6 of 15) or tylosin (40%, 6 of 15) as their primary antimicrobial for treating NHP with clinical signs of diarrhea. In addition, 74% (10 of 14) reported enrofloxacin as their primary choice for treating NHP with clinical signs of gingivitis.

Once bacterial agents from submitted samples have been isolated and identified, most of the participating veterinarians reported that they request susceptibility tests. Specifically, 73% (11 of 15) always request susceptibility tests for isolated *S. flexneri* strains, 67% (10 of 15) when *Y. enterocolitica* and *Y. pseudotuberculosis* are isolated, and 53% (8 of 15) for isolated *C. jejuni* (Table 4).

In addition to identifying primary antimicrobials for treating clinical signs prior to microbial results, the survey was designed to reveal participating veterinarians' primary antimicrobials for treating patients with known etiologies. Participating veterinarians reported enrofloxacin as their primary antimicrobial for treating suspected diarrhea cases caused by *S. flexneri* (87%, 13 of 15), *Y. enterocolitica* (79%, 11 of 14), and *Y. pseudotuberculosis* (69%, 9 of 13; Table 5). In contrast, enrofloxacin was not a common choice among participants for *C. jejuni* (13%, 2 of 15; Table 5). Instead, azithromycin (40%, 6 of 15) and tylosin (40%, 6 of 15) were the most popular antimicrobial therapies for suspected cases of diarrhea caused by *C. jejuni* (Table 5).

Changes in primary antimicrobials chosen for therapy. Participating veterinarians identified a threshold prevalence of AMR in a population of isolates which, when exceeded, would cause them to change their primary antimicrobial for therapy (Table 5). This value was intended to represent a threshold, or prevalence, at which each veterinarian felt it reasonable to change his or her primary antimicrobials, on the basis of the prevalence of AMR; it did not represent a breakpoint of antimicrobial effectiveness. We hypothesized that the prevalence of AMR (Table 2) would exceed participating veterinarians' TP-AMR. Figure 1 illustrates the distribution of the self-identified TP-AMR among participating veterinarians for the 4 investigated bacteria; these values ranged from 0% to 80%, with medians of 15% to 20%. Comparing the data in Figure 1 with those in Table 2 revealed that the prevalence of AMR to many antimicrobials did exceed veterinarians' TP-AMR. For example, 99.5% (569 of 572) of institution A's *C.*

Table 3. Prevalence of antimicrobial resistance patterns among *Shigella flexneri, Yersinia enterocolitica, Yersinia pseudotuberculosis*, and *Campylobacter jejuni* isolates from NHP at biomedical research institutions A and B from January 2012 to April 2015

Institution	Prevalence	Resistance pattern
A		
S. flexneri (*n* = 30)	7%	Ery, Dox, Cla, T–Sd
	3%	Ery, Gen, T–Sd
	3%	Ery, Gen, Dox, A–C
	10%	Ery, Gen, A–C
	3%	Ery, Gen, Dox
	3%	Ery, Gen
	10%	Ery, Dox, A–C
	13%	Ery, Dox
	17%	Ery
	10%	Dox, A–C
	10%	Amp, Chl, Tet, Car, A–C
Y. enterocolitica (*n* = 5)	40%	Ery, Dox, A–C
	20%	A–C
	40%	Amp, A–C
C. jejuni (*n* = 574)	0.20%	Met, Cep, T–S
	0.30%	Amp, Met, Cep
	96%	Met, Cep
	0.50%	Cep
	0.20%	Chl, Met, Cli
	10%	Chl, Met
	1.20%	Met, Cli
	0.20%	Met
B		
S. flexneri (*n* = 410)	0.70%	Amp, Tet, Chl
	5%	Amp, Tet
	38%	Tet
Y. enterocolitica (*n* = 48)	92%	Amp, Cef
	8%	Amp

A–C, amoxicillin–clavulanic acid; Amp, ampicillin; Car, carbencillin; Cef, cefazolin; Cep, cephalothin; Chl, chloramphenicol; Dox, doxycycline; Enr, enrofloxacin; Ery, erythromycin; Gen, gentamicin; Met, methicillin; Tet, tetracycline; T-S, trimethoprim–sulfametheoxazole; T–Sd, trimethoprim– sulfadiazine

jejuni isolates showed AMR to methicillin, and 98% (557 of 572) were resistant to cephalothin; both of these prevalences exceed the largest TP-AMR reported. However, when we focused on the veterinarians' primary antimicrobial choices, the prevalence of AMR between January 2012 and April 2015 did not exceed any reported TP-AMR for *S. flexneri*, *Y. enterocolitica*, *Y. pseudotuberculosis*, or *C. jejuni*, including an absence of AMR to enrofloxacin. This finding is emphasized by the fact that we had sufficient power to detect AMR, if AMR was present in as low as 0.8% of the sample populations. Azithromycin and tylosin, the 2 most popular antimicrobials for treating clinical cases of *C. jejuni*, were not included in susceptibility tests for institutions A and B, but erythromycin, another macrolide, was included. None of the investigated bacteria showed AMR to participating veterinarians' primary antimicrobials.

Discussion

The objectives of the present study were 1) to estimate the prevalence of AMR in zoonotic bacteria from diagnostic NHP samples and 2) to evaluate current antimicrobial use practices against the zoonotic bacteria among NHP veterinarians. No comprehensive multiinstitutional studies on the prevalence of AMR among zoonotic bacteria from NHP have been published previously. Therefore, our current study provides veterinarians and scientists with critical data for making informed decisions regarding policy and therapeutic treatment. *S. flexneri, Y. enterocolitica*, *Y. pseudotuberculosis*, and *C. jejuni* all commonly cause diarrhea among NHP;[1,2,6,7,17,38,44,46] in addition, *S. flexneri* sometimes causes gingivitis.[3] Enrofloxacin was a popular antimicrobial choice among participating veterinarians and was the primary antimicrobial for treating, prior to susceptibility results, clinical cases of diarrhea with isolated *S. flexneri* (87%, 13 of 15), and clinical cases of gingivitis (74%, 10 of 14). Because it is effective and safe, enrofloxacin is clinically important against shigellosis.[25] Overall, the current study illustrates a high prevalence of resistance to specific antimicrobials among *S. flexneri*, *Y. enterocolitica*, *Y. pseudotuberculosis*, and *C. jejuni*, consistent with previous studies.[5,16,17,26,45,46] However, our results reveal no evidence of resistance to enrofloxacin, even with consistent antimicrobial selective pressure. Furthermore, AMR to erythromycin was not observed among *C. jejuni* isolates. Although participating veterinarians frequently use tylosin and azithromycin as primary antimicrobials to treat clinical cases caused by *C. jejuni*, excluding tylosin and azithromycin and including erythromycin in susceptibility tests will be equally as informative as including all 3 drugs. Cross-resistance between erythromycin and azithromycin[24] and erythromycin and tylosin[8] is evident from previous studies.

No isolate was resistant to any tested third-generation cephalosporin (ceftiofur, ceftriaxone, cefpodoxime) or fluoroquinolone (enrofloxacin, ciprofloxacin, and marbofloxacin). This finding is especially informative for physicians and epidemiologists because the World Health Organization identifies third-generation cephalosporins and fluoroquinolones (in addition to macrolides: tylosin, azithromycin, and erythromycin) as among the highest priority critically important antimicrobial classes in public health.[49] However, participating laboratories infrequently included most of these third-generation cephalosporins and fluoroquinolones into their susceptibility tests; enrofloxacin was the only exception. Furthermore, the range of antimicrobials included in susceptibility tests varied substantially between participating diagnostic laboratories. There seems to be little consistency regarding susceptibility testing, both within and across institutions. Because the investigated bacteria can have marked public health effects, it is worthwhile for veterinarians to consistently include third-generation cephalosporins and fluoroquinolones in susceptibility tests. Ceftiofur crystalline free acid may provide veterinarians with a secondary option in the event of AMR to enrofloxacin; Ceftiofur crystalline free acid has recently been investigated for use in rhesus macaques and, as a long-term single-dose therapy, may have positive animal wellbeing and management effects.[39]

Comparing resistance patterns between NHP and human isolates may suggest relationships among strains, but they are dif-

Table 4. Proportion of participating veterinarians that request susceptibility tests when *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, or *Campylobacter jejuni* is isolated from a clinical case of diarrhea in NHP

	Always	Most of the time	Sometimes	Rarely	Never
S. flexneri	73% (11/15)	13% (2/15)	—	13% (2/15)	—
Y. enterocolitica	67% (10/15)	20% (3/15)	—	13% (2/15)	—
Y. pseudotuberculosis	67% (10/15)	20% (3/15)	—	7% (1/15)	7% (1/15)
C. jejuni	53% (8/15)	13% (2/15)	13% (2/15)	7% (1/15)	13%(2/15)

Table 5. Proportion of participating veterinarians' primary antimicrobials for therapy in clinical cases of *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, or *Campylobacter jejuni* isolated from NHP

	S. flexneri	*Y. enterocolitica*	*Y. pseudotuberculosis*	*C. jejuni*
Enrofloxacin	87% (13/15)	79% (11/14)	69% (9/13)	13% (2/15)
Azithromycin	7% (1/15)	—	—	40% (6/15)
Gentamicin	—	14% (2/14)	8% (1/13)	—
Tylosin	—	—	—	40% (6/15)

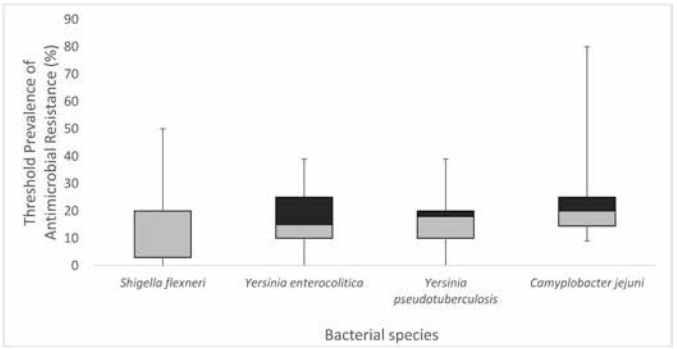

Figure 1. Veterinarian-reported thresholds for the prevalence of AMR that, when exceeded by the true AMR, would prompt veterinarians to change their primary antimicrobial of choice for therapy. Data are presented in quartile increments: the bottom whisker includes 0% to 25% of the reported thresholds; gray box, 25% to 50%; black box, 50% to 75%; and top whisker, 75% to 100% of reported TP-AMR. The border between the gray and black boxes indicates the median.

ficult to evaluate without further genotyping. Among our NHP data, there were large differences in resistance pattern diversity between participating institutions. Intrainstitutional differences may be explained by novel introductions of animals or bacterial strains, intrainstitutional genetic evolution, or differences in antimicrobial selection pressure. Regardless of the diversity, it is noteworthy that no resistance patterns among *S. flexneri* isolates were shared between this study and those published in the NARMS 2013 human isolates final report.[10] For example, in the NARMS report, 37.5% (24 of 64) of *S. flexneri* isolates showed a pattern of resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole–sulfisoxazole, and tetracycline, and 51.6% (33 of 64) were resistant to both ampicillin and trimethoprim–sulfamethoxazole.[10] However, none of the observed NHP resistance patterns (0 of 440) mimicked those published by NARMS. Comparisons between NHP and human data on *Y. enterocolitica* and *Y. pseudotuberculosis* are challenging because literature searches did not yield comprehensive surveillance data of AMR in the 2 *Yersinia* species. Similarly, NARMS does not publish specific data on *C. jejuni* resistance patterns.[10] Although isolates can be genetically identical and still express different resistance patterns,[35] the lack of shared patterns and the lack of recovery of resistance patterns that are globally disseminated may indicate limited transmission between NHP and humans. Nonetheless, resistance genes can be transferred horizontally between NHP and human populations isolates by means of plasmids and transposons,[9,18,41] and NHP veterinarians can use consistent AMR monitoring as a tool to reduce occupational risk. Known resistance patterns can help physicians treat sick staff, in the event of zoonotic transmission, given that shared resistance patterns may indicate shared bacterial strains.[18]

The Centers for Disease Control and Prevention reports that both *Shigella* spp. and *Campylobacter* spp. are becoming increasingly resistant to ciprofloxacin and azithromycin.[11] However, our study reveals no evidence of AMR to ciprofloxacin, enrofloxacin, and azithromycin among *S. flexneri* and *C. jejuni* isolates. Because enrofloxacin is metabolized into ciprofloxacin,[25,34] isolates susceptible to enrofloxacin are generally susceptible to ciprofloxacin.[34] Moreover, the Centers for Disease Control and Prevention reports that a high prevalence of *Shigella* spp. are also resistant to the first-line antimicrobials ampicillin and trimethoprim–sulfamethoxazole.[11] However, only 1.2% (5 of 410) of institution B's *S. flexneri* isolates were resistant to ampicillin and 0% (0 of 410) were resistant to trimethoprim-sulfamethoxazole, again emphasizing the greater likelihood of distinct populations of the investigated bacteria between NHP and humans.

Although the current study involved several institutions, larger study populations that include even more institutions would nevertheless be useful to precisely estimate the prevalence of AMR of zoonotic bacteria from NHP. Some participating institutions provided few susceptibility test results. This situation might be due to: 1) few clinical cases of diarrhea, 2) infrequent submission of samples for microbial identification, 3) infrequent isolation of the investigated bacteria, or 4) infrequent requests for susceptibility tests by veterinarians. Few cases of diarrhea and infrequent isolation of investigated bacteria are unlikely given the institutions' sizes and housing arrangements and the supporting data from previous studies.[1,2,6,7,17,38,44,46] Even so, when zoonotic bacteria are involved, clinical decisions—whether regarding antimicrobial choices or susceptibility testing—affect not only NHP patients but potentially personnel as well. Our data illustrate that, overall, veterinarians have successfully been prescribing effective antimicrobials, but our findings also reveal inconsistent susceptibility testing. According to data published by NARMS, AMR can and

has abruptly increased dramatically,[10] and this change is possible due to the introduction of novel strains. With this said, we recommend that both veterinarians and diagnostic laboratories consistently and routinely request and perform susceptibility testing. A strong collaborative approach between veterinarians and diagnostic laboratories can inform empirical antimicrobial choices or revise current practices in light of test results, consequently reducing AMR risk.

Given the variations in response rates, several potential limitations are evident. Response bias is a possibility, because the responses of participating veterinarians may differ from those of nonparticipating veterinarians. In addition, the data from nonparticipating biomedical research institutions might systematically differ from those of institutions that participated. Therefore, because we estimated the prevalence of AMR for only 3 biomedical research institutions, the results cannot be extrapolated to every institution that uses NHP. We believe that antimicrobial therapy ultimately should depend on the patient's susceptibility test result. However, because antimicrobials are initiated frequently at the onset of clinical signs and prior to test results, our results provide a reference point, especially illustrating which antimicrobials should be avoided. Regardless, no recent publication exists that demonstrates the prevalence of AMR among zoonotic bacteria from NHP in biomedical research as comprehensively as does the current study. According to the most recent USDA Annual Report of Animal Usage for the 2014 fiscal year, our current study includes approximately 23.3% (24,650 of 105,665) of all NHP in biomedical research institutions in United States.[48]

In summary, the present study reveals low levels of AMR to enrofloxacin among *S. flexneri*, *Y. enterocolitica*, and *Y. pseudotuberculosis* isolates. In addition, we note low levels of AMR to erythromycin, azithromycin, and tylosin among *C. jejuni*. Because of the observed dissimilarity of zoonotic bacterial isolates between NHP and humans, first-line antimicrobials are likely to be effective options in the event of occupational exposure. Although discrepancies between susceptibility tests exist, simple collaboration between veterinary staff and diagnostic laboratories can foster consistent and routine practices that reduce AMR potential. Such collaboration will ultimately help veterinarians to ensure effective antimicrobial therapy among patients and will help to minimize occupational risk.

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