Antimicrobial Use and Resistance in Zoonotic Bacteria Recovered from Nonhuman Primates

Thesis

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Abstract

Antimicrobial resistance (AMR) has become a central topic as it is a growing threat in human and animal health. Major surveillance systems, such as the National Antimicrobial Resistance Monitoring System (NARMS), are now established to monitor AMR and provide physicians, veterinarians, and scientists with valuable information to make informed decisions on policy and therapeutic treatment. However, there is a lack of comprehensive literature on AMR among nonhuman primates (NHP). This study aims to provide data on current antimicrobial use strategies and on the prevalence of AMR in zoonotic bacteria recovered from NHPs within biomedical research institutions. We focused on four zoonotic enteric bacteria: *Shigella flexneri*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Campylobacter jejuni*. Fifteen veterinarians, seven biomedical research institutions, and four diagnostic laboratories participated, providing susceptibility test results across three years (1/2012 – 4/2015). 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 their associated primary antimicrobials. However, high proportions of AMR was observed to other antimicrobials. *S. flexneri* isolates were resistant to erythromycin (87.5%, 21/24), doxycycline (73.7%, 14/19), and tetracycline (38.2%, 157/411). *Y. enterocolitica* isolates were resistant to ampicillin (100%, 49/49) and
cefazolin (93.6%, 44/47). No *Y. pseudotuberculosis* isolate (0/58) was resistant to any tested antimicrobial. *C. jejuni* isolates were resistant to methicillin (99.5%, 569/572) and cephalothin (97.5%, 557/571). Notably, resistance patterns were not shared between this study’s NHP isolates and human isolates presented by NARMS. This study demonstrates that zoonotic bacteria recovered 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.
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Table of Contents

Abstract ........................................................................................................................ii
Acknowledgements .......................................................................................................iv
Vita ...............................................................................................................................v
List of Tables ...............................................................................................................vii
List of Figures ..............................................................................................................viii
Chapter 1: Introduction .................................................................................................1
Chapter 2: Literature Review .........................................................................................4
Chapter 3: Materials and Methods ...............................................................................19
Chapter 4: Results .........................................................................................................23
Chapter 5: Discussion ....................................................................................................36
References ....................................................................................................................42
Appendix A: Pilot Study - Veterinarian Antimicrobial Use Survey ...............................52
Appendix B: Initial Email to Veterinary Director .........................................................65
Appendix C: Initial Email to Director of Diagnostic Laboratory .................................67
Appendix D: Reminder Email to Veterinary Director ..................................................69
Appendix E: Reminder Email to Director of Diagnostic Laboratory ............................71
Appendix F: Initial Email to Participating Veterinarians ..............................................73
Appendix G: Consent Form for Participating Veterinarians ..........................................75
Table of Contents continued

Appendix H: Veterinary Staff Antimicrobial Use Survey ..................................................78
Appendix I: Initial Email to Participating Microbiologists ..............................................85
Appendix J: Consent Form for Participating Microbiologists ........................................87
Appendix K: Diagnostic Laboratory Survey ........................................................................90
List of Tables

Table 1. Antimicrobials tested in antimicrobial susceptibility tests for *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, or *Campylobacter jejuni* among participating diagnostic laboratories corresponding to participating Biomedical Research Institutions A, B, & C ................................................................. 24

Table 2. Prevalences of antimicrobial resistance within Biomedical Research Institutions A & B for *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Campylobacter jejuni*, recovered from nonhuman primates from January 2012 to April 2015 ........................................................................................................ 26

Table 3. Frequency of antimicrobial resistance patterns among *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Campylobacter jejuni* isolated from nonhuman primates in Biomedical Research Institutions A & B, within January 2012 and April 2015 ........................................................................................................ 30

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 nonhuman primates ................. 32

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 nonhuman primates ........................................... 33
List of Figures

Figure 1. Veterinarian reported threshold for the prevalence of antimicrobial resistance that would result in changing their primary antimicrobial choice for therapy, if exceeded by the true prevalence of antimicrobial resistance ........................................35
Chapter 1: Introduction

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 zoonotic bacteria because a single pathogen can threaten the health of both animal patients and individuals in contact with animal patients. *Shigella flexneri, Yersinia enterocolitica, Y. pseudotuberculosis,* and *Campylobacter jejuni,* are all zoonotic bacteria repeatedly isolated from nonhuman primates (NHP) in biomedical research institutions and can cause serious disease in the event of occupational exposure. *S. flexneri* and *C. jejuni,* in particular, are two bacteria identified as serious threats to human health by the Centers for Disease Control and Prevention (CDC). However, the occupational risk associated with the above zoonotic bacteria in NHPs is not well understood.

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

Additionally, significant public health concerns exist with zoonotic bacteria in biomedical research. Tuberculosis, Q-fever, and salmonellosis are zoonotic diseases commonly studied and can cause serious mortality and morbidity among animal patients and personnel.\textsuperscript{28,62,64,66,76,81,84} Thus, it is important to ensure appropriate antimicrobial selection for effective therapy. However, without regularly monitoring AMR, veterinarians may be unknowingly applying unnecessary antimicrobial selective pressure and fueling the development and/or acquisition of AMR.\textsuperscript{52}

The presented study provides the most comprehensive data on the prevalence of AMR among zoonotic bacteria in NHPs, in nearly five decades. Good et al.\textsuperscript{39} published a similarly comprehensive study, but since its publication in 1969, changes in AMR is expected with the introduction of new antimicrobials and the potential emergence and dissemination of novel strains. Although research teams have more recently published data on AMR in zoonotic bacteria recovered from NHPs,\textsuperscript{45,46,51,85} none share the present study’s temporal, institutional, and geographic scope in the United States. Given the paucity of available data on the AMR of zoonotic bacteria in NHPs, the objectives of the present study were to 1) estimate the prevalence of AMR among Shigella flexneri, Yersinia enterocolitica, Yersinia pseudotuberculosis, and Campylobacter jejuni.
recovered from diagnostic laboratory samples of NHPs between January 2012 and April 2015, 2) evaluate current antimicrobial use strategies veterinarians use to treat disease caused by the above bacteria, and 3) determine the likely change in antimicrobial use strategy for veterinarians with knowledge of AMR prevalence. The latter was quantified by asking participating veterinarians to identify a threshold prevalence of AMR (TP-AMR), where if exceeded by the true prevalence of AMR, would cause the veterinarians to change their antimicrobial use strategies. We hypothesized that the prevalence of AMR among the above bacteria will exceed participating veterinarians’ TP-AMRs.
Chapter 2: Literature Review

A. Enteric Bacteria and Zoonoses

Enteric bacteria have consistently threatened human health and have posed significant public health threats. John Snow, who is widely considered to be the father of epidemiology, identified and mitigated the now famous Broad Street Pump cholera epidemic of 1854.\(^{17}\) Since then, we have made tremendous advancements in molecular epidemiology, with the help of pulse-field gel electrophoresis, restriction fragment length polymorphisms, multi-locus sequence typing, and more. With these advancements, epidemiologists no longer merely identify and mitigate outbreaks caused by enteric bacteria, such as *Salmonella* and *Escherichia coli*, but they can also identify asymptomatic individuals and prevent outbreaks from occurring. And yet, enteric disease outbreaks remain central topics in public health.

In 2011, *Clostridium difficile* caused nearly half a million infections and approximately 29,000 deaths in the United States alone.\(^59\) Comparing 2014 with 2006-2008, incidence of campylobacteriosis, vibriosis, and salmonellosis (serotypes Javiana and Infantis) have significantly increased.\(^{25}\) Among the most dangerous foodborne pathogens, enteric bacteria cause three of the top five greatest number of illnesses, hospitalizations, and deaths;\(^{24}\) these include nontyphoidal *Salmonella, Clostridium perfringens,*
Campylobacter spp., E. coli (STEC) O157, Listeria monocytogenes.\textsuperscript{24} One noteworthy characteristic shared among the above enteric bacteria is that they are all zoonotic agents. Not only do they cause morbidity and mortality among animals, but people’s exposure to animals can also put them at risk of infection.

People frequently come into contact with many animals that naturally harbor Salmonella, E. coli, and Campylobacter, particularly farm animals.\textsuperscript{32,58,67} Because we heavily rely on these animals for food, they pose great risk to those handling the animals.\textsuperscript{30} People are commonly exposed to the zoonotic enteric bacteria, via the fecal-oral route.\textsuperscript{13,35,44} This is a clear route of transmission for farm workers who are at high risk of animal fecal exposure. However, occupational exposure is not the only route of exposure; a less obvious source of exposure is pets. Pet turtles have recently been linked to salmonellosis outbreaks.\textsuperscript{22} Additionally, with the increasing popularly of backyard poultry flocks, epidemiologists are seeing more salmonellosis outbreaks associated with mail-order hatcheries.\textsuperscript{8,42} Dry pet food has even been a source of human exposure to Salmonella.\textsuperscript{55} But of course, these are routes where people can become infected directly from animals or animal products. Enteric disease outbreaks have also been exacerbated due to horizontal transmission among people.\textsuperscript{74}

B. Pathogenesis and Epidemiology

Shigella, Yersinia, and Campylobacter are zoonotic enteric bacteria that can pose great public health risks.\textsuperscript{13,23,40} Among humans, Shigella spp. often cause bloody diarrhea,
fever, and abdominal pain.\textsuperscript{23} In serious cases, shigellosis can lead to reactive arthritis.\textsuperscript{23} \textit{Shigella spp.} is often horizontally transmitted between people fecal-oral through direct contact, or via contaminated surfaces, food, or water.\textsuperscript{23} With an extremely low infective dose, as few as 10 organisms can cause infection.\textsuperscript{13} There are approximately 500,000 shigellosis related diarrheal illnesses, 5,500 hospitalizations, and 40 deaths per year in the United States alone.\textsuperscript{23} Worldwide, \textit{Shigella spp.} leads to as high as 165 million illnesses and 600,000 deaths annually.\textsuperscript{13} \textit{Shigella spp.} is endemic in temperate and tropical areas, with \textit{S. flexneri} largely prevalent in developing countries.\textsuperscript{13} NHP are the only animals naturally infected with \textit{Shigella spp.}.\textsuperscript{13}

Among \textit{Yersinia spp.}, \textit{Y. enterocolitica} causes the greatest number of illnesses.\textsuperscript{40} Like shigellosis, clinical signs of yersiniosis include bloody diarrhea, abdominal pain, fever, and reactive arthrosis.\textsuperscript{40} Young infants, on the other hand, can also show signs of necrotizing enterocolitis.\textsuperscript{40} Humans usually contract \textit{Y. enterocolitica} via contaminated food, most frequently raw or undercooked pork, unpasteurized milk, and contaminated water.\textsuperscript{40} Yersiniosis is most common in northern Europe (mainly Scandinavia), Japan, and Canada, with seasonal peaks in winter.\textsuperscript{40}

\textit{Campylobacter spp.} often causes bloody diarrhea, fever, abdominal cramps, but in severe cases, temporary paralysis as well.\textsuperscript{23} Campylobacteriosis is famous as a foodborne disease, transmitted from animals to people via contaminated food, particularly raw or undercooked chicken and unpasteurized milk.\textsuperscript{23} It can also be horizontally transmitted via
fecal-oral between people, as well as contracted from direct contact with animals like
dogs and cats, but especially farm animals such as cows and chicken.\textsuperscript{38} \textit{Campylobacter}
\textit{spp.} cause approximately 1.3 million infections, 13,000 hospitalizations, and 120 deaths
per year in the United States.\textsuperscript{23} Worldwide, campylobacteriosis is the leading cause of
bacterial diarrhea cases, and has been reported from nearly every region around the
globe.\textsuperscript{38}

C. Antimicrobial Resistance

It is evident that \textit{Shigella}, \textit{Yersinia}, and \textit{Campylobacter spp.} can cause serious mortality
and morbidity, but even with recent advances in epidemiology and medicine,
antimicrobial resistance (AMR) is a constant threat to public health. This is due to
overuse and misuse of antimicrobials, both in human and veterinary medicine.\textsuperscript{23} Such
antimicrobial selective pressure fuels the development and acquisition of AMR.\textsuperscript{52}
Consequently, over two million individuals develop serious infections that are resistant to
at least one antimicrobial, each year in the United States.\textsuperscript{23} Of these two million, nearly
250,000 infections result in death.\textsuperscript{23} This collectively leads to as high as $20 billion in
excess healthcare costs, and as high as $35 billion a year lost from society due to lost
productivity.\textsuperscript{1} AMR clearly threatens the United States’ health outcomes and economy.

With the help of governmental public health organizations, it is now easy to track trends
in AMR year-to-year. The National Antimicrobial Resistance Monitoring System for
Enteric Bacteria (NARMS) is a major public health initiative, publishing AMR
surveillance data used among epidemiologists, physicians, and veterinarians. NARMS is a collaborative effort among state and local public health departments, the Centers for Disease Control and Prevention (CDC), the U.S. Food and Drug Administration (FDA), and the U.S. Department of Agriculture (USDA). NARMS tracks changes in AMR of enteric bacteria isolated from sick humans (CDC), retail meat (FDA), and food animals (USDA). NARMS data that is published helps public health professionals combat disease outbreaks, and helps physicians and veterinarians make informed decisions on policy and therapeutic treatment approaches.

According to both the CDC and NARMS, *Campylobacter* isolates among humans have shown increasing trends in AMR to ciprofloxacin and macrolides, which are first-line antimicrobials for treating campylobacteriosis. In 2013, approximately 23% of *Campylobacter* isolates were resistant to ciprofloxacin, and approximately 17% resistant to macrolides. *Salmonella* has similarly been increasingly more resistant to ciprofloxacin. *Shigella*, another zoonotic enteric bacteria, has also been more and more resistant to ciprofloxacin, as well as to trimethoprim-sulfamethoxazole. However, because these bacteria are zoonotic, it is important to also examine the prevalence of AMR among animals.

Extensive literature has been published on the prevalence of AMR among zoonotic enteric bacteria in farm animals. Such literature illustrates the occupational risk for farm workers and public health risks among consumers. However, extensive literature on
occupational exposure to antimicrobial resistant bacteria is not shared among laboratory animals used for research, particularly nonhuman primates (NHP). Like many farm animal species, NHP are also commonly infected with zoonotic enteric bacteria. These organisms include *Shigella flexneri*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Campylobacter jejuni*, which can also cause serious mortality and morbidity to humans.\[^9,27,31,47,57\]

D. Mechanisms of Antimicrobial Resistance

Multiple mechanisms of AMR exist to various classes of antimicrobials, but among enteric bacteria including *S. flexneri*, *Y. enterocolitica*, *Y. pseudotuberculosis*, and *C. jejuni*, researchers largely focus on resistance to quinolones, fluoroquinolones, and macrolides because they are critically important antimicrobials in human medicine.\[^97\]

Three key mechanisms of resistance have been identified for *S. flexneri*: 1) point mutations that alter DNA gyrase and topoisomerase IV, the targets of quinolones, 2) changes in efflux pumps and outer membrane permeability, and 3) plasmid-mediated quinolone resistance.\[^34,73,90\] Most mutations occur in a small region near the start of the *gyrA* gene, known as the quinolone resistance-determining region (QRDR).\[^71\] Azmi et al. demonstrated that among their fluoroquinolone-resistant *S. flexneri* isolates, all had two mutations in *gyrA* gene (Ser[^83]Leu, Asp[^87]Asn/Gly) and one mutation in the *parC* gene (Ser[^80]Ile).\[^7\] In contrast to developing AMR, the presence of the efflux-pump inhibitor carbonyl cyanide-m-chlorophenylhydrazone (CCCP) increases susceptibility to fluoroquinolones.\[^7\]

9
Similar to *S. flexneri*, mechanisms of resistance to quinolones among *Y. enterocolitica* O:3, a common strain causing yeryniosis in humans, are frequently caused by mutations along the *gyrA* gene. In Capilla et al.’s study, all of their quinolone-resistant *Y. enterocolitica* O:3 isolates presented at least one mutation in the *gyrA* gene, and no mutations were observed in the *parC* gene. An overexpression of efflux-mediated mechanisms have been noted to elicit quinolone resistance among *Y. enterocolitica* isolates as well.

Regarding *C. jejuni*, three mechanisms have been described: 1) modification of the antimicrobial, 2) modification of the antimicrobial target via methylation or mutation, and 3) efflux of the antimicrobial from the bacterium. The most common mechanism is the modification of the ribosomal target of macrolides via the A2075G point mutation in the 23S rRNA gene; this has especially been recognized to cause high-level erythromycin resistance among *C. jejuni* isolates. Efflux, in the other hand, seems to contribute to both intrinsic resistance, as well as low-level AMR to erythromycin among *Campylobacter spp.* Macrolide-resistant *C. jejuni* isolates usually have mutations on all three copies of the bacterium’s 23S rRNA gene.

Understanding the above mechanisms of resistance can help veterinarians combat antimicrobial resistance, and minimize occupational and public health risks, but occupational and public health risks are not always independent. Occupational exposure
can lead to a greater public health threat. Such was the case for the 1998-1999 Nipah Virus (*Henipavirus*) outbreak in Malaysia and Singapore, where occupational exposure to sick pigs led to a major epidemic. Because horizontal transmission among humans can exacerbate occupational risks into greater public health risks, like the Nipah Virus epidemic, it is important to evaluate the prevalence of antimicrobial resistance in zoonotic bacteria isolated from NHPs within biomedical research settings. It is also important to evaluate current antimicrobial use strategies utilized to treat infections caused by zoonotic bacteria in NHPs, because the antimicrobial selective pressure can increase occupational risk by fueling the development and/or acquisition of AMR.

E. Prevalence of Antimicrobial Resistance

Good et al. published a manuscript evaluating the prevalence of AMR among zoonotic enteric bacteria recovered from NHPs. This study examined *Salmonella anatum*, *S. stanley*, *Shigella dysenteriae*, *S. sonnei*, and *S. flexneri* (type 1, 2, 4, & 6). They demonstrated that AMR in *S. flexneri* isolates were very common, including multi-drug resistance. They also demonstrated clear differences in the prevalence of infection, as well as the prevalence of AMR, between newly imported NHPs and those colonized over time at the National Center for Primate Biology. Furthermore, it was evident that a prevalence of AMR among *S. flexneri* type 4 isolates to chloramphenicol could be explained by antimicrobial selective pressure. However, high prevalences of AMR were not always associated with antimicrobial use; high prevalences of AMR were observed to
antimicrobials that were rarely used for therapy.\textsuperscript{39} It is clear that the association between antimicrobial selective pressure and AMR is complex.

Ultimately, the Good et al. publication was the most comprehensive study investigating AMR in zoonotic enteric bacteria from NHPs. Not only did it evaluate multiple species and strains of enteric bacteria, but the study also included many species of NHPs used for biomedical research. Additionally, they investigated changes in infection and AMR since importation. However, several limitations exist. Some of the study’s results are no longer applicable to veterinarians today. Since its publication in 1968, many of the tested antimicrobials are no longer commonplace. Additionally, new antimicrobials have been introduced, and with that, antimicrobial selective pressure has changed. Especially in regard to current important antimicrobials to public health, third-generation cephalosporins and fluoroquinolones either were not in market, or were just beginning to gain popularity during that time. Thus, assessing the public health risks from Good et al.’s publication is challenging. Nonetheless, some institutions still import wild-caught NHPs, and so, Good et al.’s data may still be informative for those working with wild-caught NHPs.

Similar limitations exist with other publications investigating \textit{Shigella spp}. Galton et al. only examined resistance to sulfadiazine, and from two NHP species, chimpanzees (\textit{Pan troglodytes}) and spider monkeys (\textit{Ateles geoffroi}).\textsuperscript{37} And with its publication in 1948, minimal applications for current practice are likely. On the other hand, Arya et al.
examined resistance among isolates from only one NHP species, albeit the most common species in biomedical research, rhesus macaque (*Macaca mulatta*), but did examine resistance to more antimicrobials than Galton et al. Six antimicrobials (streptomycin, chloramphenicol, furazolidone, chlortetracycline, ampicillin and kanamycin) were included in their susceptibility tests, which is a relatively narrow susceptibility panel, however. Arya et al. also cultured potential asymptomatic carriers of *S. flexneri*, increasing the probability of determining the true prevalence of AMR, but the methods in which potential asymptomatic carriers were identified were not described. Similar to older studies described above, since rapid changes in AMR can occur, it is difficult to place high confidence in literature published >40 years ago.

Tribe and Fleming’s study provided valuable information by investigating both *S. flexneri* and *C. jejuni*, and among a very large population of approximately 10,000 imported cynomolgus macaques (*Macaca fascicularis*). During their four-year study, they found that *S. flexneri* infections were far more prevalent in the first month of quarantine, and remained less common throughout the rest of the study period. Contrary to this, *C. jejuni* remained commonplace and endemic throughout the study period, but treatment with macrolides erythromycin and tylosin were overall effective in treating campylobacteriosis. One-thousand six hundred and seventy additional asymptomatic animals were also cultured, providing readers with more representative data on the prevalence of AMR among their cynomolgus macaque colony. Tribe and Fleming
demonstrated, in particular, that the presence of zoonotic bacteria is commonplace, and consequently the occupational and public health risk remains consistent.

However, there are two clear limitations. Firstly, Tribe and Fleming only examined wild-caught imported NHPs. Thus, there may be fewer applications to biomedical research institutions today, where facility-bred NHPs are commonly used for research, but this limitation does not devalue the presented data because the use of wild-caught NHPs for research is not extinct. Secondly, only five antimicrobials (ampicillin, chloramphenicol, neomycin, tetracycline, and Sulphonamide-trimethoprim) were tested for resistance.91

Lederer et al.’s letter to an editor of the International Journal of Infectious Diseases was insightful because it demonstrated that occupational exposure to zoonotic bacteria from NHPs does occur, and can lead to serious morbidity. Pulsed-field gel electrophoresis (PFGE) revealed clonal strains between S. flexneri isolated from an infected animal-keeper and two infected orangutans (Pongo pygmaeus) that the keeper contacted.57 With clear differences to twenty-four other human S. flenxeri Sv 2a isolates, and with onset of symptoms within 24 hours of potential occupational exposure,57 it seems highly probable that occupational exposure to the infected orangutans was the source of infection.

Kennedy et al. also described human cases of shigellosis acquired via occupational exposure to laboratory NHPs. Laboratory analysis via tube agglutination, a more reliable method than slide agglutination, confirmed that three employee shigellosis cases matched serotypes of S. flexneri isolated from NHPs the employees handled.48 Both publications
illustrate the importance of biosecurity and personal protective equipment (PPE). The lack of extensive PPE may have contributed to the serious cases of shigellosis described.

Minimal data has been published on AMR among *Y. enterocolitica* and *Y. pseudotuberculosis*, a stark contrast compared to literature on *S. flexneri*. Most of the literature discusses the pathogenicity and virulence of yersiniosis among NHPs, without discussing the public health risks associated with AMR.\textsuperscript{14,45,46,72,83,85,86} Although Mair did not evaluate AMR nor yersiniosis among NHPs, the data on yersiniosis among wildlife is still informative to biomedical research institutions, especially those with outdoor colonies. Because yersiniosis is widespread in wildlife, particularly birds,\textsuperscript{61} potential introduction into outdoor NHP colonies should not be ignored. *Yersinia* infections in wildlife may contribute to occupational and public health risks associated with NHPs. Vore et al. specifically examined the prevalence of *Y. enterocolitica* and *Y. pseudotuberculosis* among NHP colonies, but did not isolate any of the bacteria. The authors suggested that their data may indicate a low prevalence of *Yersinia spp.* as a whole.\textsuperscript{95} However, with three colonies ranging from 40 to 61 NHPs,\textsuperscript{95} the small sizes within each colony may explain their observed low prevalences. Thus, the data may not be as applicable to larger biomedical research institutions in the United States.

Although little data on the prevalence of AMR among *Yersinia spp.* isolated from NHPs has been published, more data from other animal sources have been published. High prevalences of AMR was observed among *Y. enterocolitica* isolated from 404 pigs across
47 farms in Latvia, especially to ampicillin, erythromycin, cephalothin, and sulfamethoxazole, with all 71 isolates expressing resistance to all four antimicrobials. Low levels of AMR were observed overall to Y. pseudotuberculosis, with the exception of erythromycin (100%, 5/5) and sulfamethoxazole (100%, 5/5). Yersinia spp., thus, presents both occupational and foodborne risks. Bonardi et al. also found similar results in Italy. Of 1152 samples (451 fecal samples, 451 carcass swabs and 250 tonsils) from 451 finishing pigs, high levels of resistance were observed to cephalothin (92%, 109/119) and ampicillin (89%, 106/119). Even though these two studies did not evaluate AMR among NHPs, they evidently demonstrate that occupational and public health risks associated with antimicrobial resistant exist.

Koga et al. published incredibly useful data on the prevalence of AMR among C. jejuni isolated from cynomolgus macaques. They examined isolates from two hundred and thirty-eight macaques, between 2009 and 2012, that were bred in a conventional and antimicrobial-free production system. They tested isolates against a broad panel of thirteen antimicrobials (amoxicillin, amoxicillin-clavulanic acid, tetracycline, chloramphenicol, gentamicin, amikacin, tobramycin, streptomycin, neomycin, kanamycin, spectinomycin, erythromycin, and ciprofloxacin) using minimum inhibitory concentration (MIC) breakpoints established by the Clinical and Laboratory Standards Institute (CLSI) or NARMS. Neomycin was an exception since neither CLSI nor NARMS breakpoints were established. In this case, the breakpoint for E. coli was used (32 µg/mL). This study’s results were especially revealing because it connected
susceptibility test results with each patient’s country, province, and breeding facility of origin.\textsuperscript{51} Such data is useful since it is not uncommon for NHP colonies in the United States to contain lineages tracing back to multiple countries and breeding facilities. However, only a small sample of \textit{C. jejuni} isolates were tested for AMR (n=17).\textsuperscript{51} In spite of this, an eradication strategy of \textit{Campylobacter spp.} was clearly described, which can be transferred to other biomedical research institutions.

With the exception of Koga et al., there appears to be a significant literature gap on the prevalence of AMR among \textit{C. jejuni} isolated from NHPs used for research. Tribe and Fleming, discussed previously, provided useful information on the prevalence of AMR among \textit{S. flexneri}, but no susceptibility test was performed for \textit{Campylobacter} isolates.\textsuperscript{91} The authors had \textit{C. jejuni} (n=484) isolates available, but with the lack of susceptibility tests, they unfortunately did not present comprehensive data on the occupational and public risks associated with AMR in zoonotic bacteria.\textsuperscript{91} Tenover et al. did examine \textit{C. jejuni} resistance, finding 34.7\% (25/72) of the isolates were tetracycline-resistant, but they only tested for resistance to tetracycline. Additionally, Tenover et al. were unable to provide conclusive epidemiologic data on the spread of 38-megadalton plasmid (pFKT1000) among tetracycline-susceptible isolate clones.\textsuperscript{87} Other publications did examine the pathogenesis and treatment of campylobacteriosis among NHPs, but did not evaluate the prevalence of AMR as a whole and its associated public health risks.\textsuperscript{26,29,78,83}
F. Future Needs

Current literature provides valuable data on zoonotic bacteria, but few research teams have comprehensively studied AMR among zoonotic enteric bacteria. Although some have investigated this topic, as described above, these studies were conducted decades prior to the presented study. Thus, it was clear that new data on the prevalence of AMR among zoonotic bacteria from NHPs was necessary, in order to understand current occupational and public health risks. With this new data, scientists, physicians, and veterinarians alike are able to better protect employees of biomedical research, as well as the general public as a whole.
Chapter 3: 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 the following inclusion criteria: 1) willingness of both the biomedical institution and 2) associated diagnostic laboratory participation, and 3) searchable database (paper or electronic) of antimicrobial susceptibility test results. If one or more criteria were not met, neither the biomedical research institution nor the diagnostic laboratory were included in the study population.

Biomedical research institutions
The investigators identified a source population of twenty-one biomedical research institutions within the United States. These institutions are among the largest and most active institutions that use NHPs for research. In addition to size and research activity, other institutions using NHPs that the authors have had professional experience, were also included into the source population. 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 seven institutions maintained animals in accordance with the United States Department of Agriculture Animal Welfare Act, Animal Welfare Regulations, and the *Guide for the Care and Use of Laboratory Animals*, and were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) as of 2015. Additionally, five of the seven participating institutions’ animal care programs maintained a Public Health Service Animal Welfare Assurance, during the study; among the five were institutions providing the investigators with diagnostic data.

**Diagnostic laboratories**

Of the seven 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. The investigators contacted the laboratory directors and asked for their participation in the study. Four labs were willing to participate, all with searchable databases of susceptibility test results.

**Surveys**

All surveys were approved by the Ohio State University Institutional Review Board. Surveys were distributed electronically via email or Qualtrics. (Qualtrics,
http://www.qualtrics.com, Provo, UT, United States of America), an online survey software. A pilot study was initially conducted with four board-certified laboratory animal veterinarians to evaluate the survey that would ultimately be distributed to veterinarians participating in the present study. After moderate edits, the resulting survey was distributed to participating veterinarians. Participants were given two months to complete the survey, with one reminder email sent one month after each survey was distributed. The veterinarians’ survey provided the investigators with data on participants’ antimicrobial use strategies. Participating veterinarians also identified their TP-AMR, where 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*, and/or *C. jejuni*, from January 2012 to April 2015. The sample source was not limited to clinical submissions alone.
**Statistical analysis**

A post-hoc 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. It is reasonable to assume that the samples of isolates were taken from a significantly larger number, circulating among each institution’s NHP colony, within the study’s time frame. Thus, it was assumed that the investigated isolates were sampled from infinite isolate populations. Alpha was set at 0.01. Data were analyzed using JMP (version 11.0.0, SAS, Cary, North Carolina) for descriptive statistics.

The following is post-hoc power analysis formula:

\[ q = e^{\left[ \frac{\ln \alpha}{n} \right]} \]

**Legend**

\( n \)=isolate sample size
\( \alpha \)=0.01
\( q \)=minimum prevalence of antimicrobial resistance detectable
Chapter 4: Results

Survey response

Thirty-eight veterinarians among the seven participating research institutions were sent the survey, and of those, 15 (39.5%) completed or nearly completed the survey and provided the most usable data. 3 microbiologists were sent surveys, and all 3 were completed.

Biomedical research institutions

Each institution’s veterinary staff ranged from three to seven veterinarians. Three institutions reported having smaller numbers of NHPs ranging from 350-1,600, but four others reported larger numbers ranging from 3,800-8,000 NHPs in total. Participating institutions housed a variety of commonly used New World and Old World NHP species, including the following: 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*) also known as Grivet, Cynomolgus Macaque (*Macaca fascicularis*), Rhesus Macaque (*Macaca mulatta*), Southern Pig-Tailed Macaque (*Macaca nemestrina*), and Hamadryas Baboon (*Papio hamadryas*). Three institutions reported having both outdoor and indoor NHP
colonies, while two others reported exclusively having outdoor colonies and another two having exclusively indoor colonies. All seven participating institutions met psychological well-being requirements under section §3.81a of the USDA Animal Welfare Act. Four of the seven participating institutions that provided us with diagnostic data of AMR, collectively housed NHP colonies that consisted upwards of 38.3% (18,350/47,930) of all NHPs used for research in the United States in 2014.

Diagnostic laboratories

Three participating diagnostic laboratories reported the Kirby Bauer test as their primary antimicrobial susceptibility test. Two of the three also indicated they measured the minimum inhibitory concentration (MIC) as their secondary test, but did not specify the technique. One of the three indicated that a secondary technique is not utilized 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 can be found in Table 1.

Table 1. Antimicrobials tested in antimicrobial susceptibility tests for *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, or *Campylobacter jejuni* among participating diagnostic laboratories corresponding to participating Biomedical Research Institutions A, B, & C. (continued)
<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th><em>S. flexneri</em></th>
<th><em>Y. enterocolitica</em></th>
<th><em>Y. pseudotuberculosis</em></th>
<th><em>C. jejuni</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Trimethoprim/</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>sulfadiazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim/</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>sulfamethoxazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Neomycin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanamycin</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methicillin</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperacillin</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin/</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>(Augmentin)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cefovecin</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Colistin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Prevalence of antimicrobial resistance

**Institution A**

Isolates of *S. flexneri* were most frequently resistant to erythromycin (87.5%, 21/24), amoxicillin/clavulanic acid (63.2%, 12/19), and doxycycline (73.7%, 14/19) (Table 2). *Y. enterocolitica* isolates were most frequently resistant to erythromycin (100%, 2/2), amoxicillin/clavulanic acid (100%, 5/5), ampicillin (100%, 2/2) and doxycycline (100%, 2/2) (Table 2). No AMR was observed for *Y. pseudotuberculosis* (Table 2). Finally for *C. jejuni*, 99.5% (569/572) of isolates were resistant to methicillin and 97.5% (557/571) to cephalothin (Table 2). Although *C. jejuni* resistance to ampicillin was not observed, 98.1% (561/572) of isolates had reduced susceptibility (intermediate resistance) (Table 2).

**Table 2.** Prevalences of antimicrobial resistance within Biomedical Research Institutions A & B for *Shigella flexneri, Yersinia enterocolitica, Yersinia pseudotuberculosis*, and *Campylobacter jejuni*, recovered from nonhuman primates from January 2012 to April 2015. (continued)
<table>
<thead>
<tr>
<th>Institution</th>
<th>Bacterial Species</th>
<th>Enr</th>
<th>Ery</th>
<th>Cla</th>
<th>Amp</th>
<th>Car</th>
<th>Met</th>
<th>Cef</th>
<th>Cep</th>
<th>Tet</th>
<th>Dox</th>
<th>Gen</th>
<th>Chl</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><em>Shigella flexneri</em></td>
<td>0%</td>
<td>88%</td>
<td>60%</td>
<td>50%</td>
<td>50%</td>
<td>-</td>
<td>0%</td>
<td>50%</td>
<td>74%</td>
<td>24%</td>
<td>24%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/30</td>
<td>21/24</td>
<td>15/25</td>
<td>3/6</td>
<td>3/6</td>
<td></td>
<td>0/6</td>
<td>3/6</td>
<td>14/19</td>
<td>7/29</td>
<td>3/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Yersinia Enterocolitica</em></td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/5</td>
<td>2/2</td>
<td>5/5</td>
<td>2/2</td>
<td></td>
<td></td>
<td>0/3</td>
<td>2/2</td>
<td>0/5</td>
<td>0/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Yersinia pseudotuberculosis</em></td>
<td>0%</td>
<td>-</td>
<td>-</td>
<td>0%</td>
<td>0%</td>
<td>-</td>
<td>0%</td>
<td>0%</td>
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<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td></td>
<td></td>
<td>0/1</td>
<td></td>
<td></td>
<td>0%</td>
<td></td>
<td></td>
<td>0/1</td>
<td>0%</td>
<td></td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0.3%</td>
<td>-</td>
<td>99.5%</td>
<td>-</td>
<td>98%</td>
<td>0%</td>
<td>-</td>
<td>0%</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/574</td>
<td>0/574</td>
<td>0/572</td>
<td>2/572*</td>
<td>56/572</td>
<td>557/571</td>
<td>0/572</td>
<td>0/574</td>
<td>7/571</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td><em>Shigella flexneri</em></td>
<td>0%</td>
<td>0%</td>
<td>-</td>
<td>0%</td>
<td>-</td>
<td>-</td>
<td>0%</td>
<td>38%</td>
<td>-</td>
<td>0%</td>
<td>0.7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/411</td>
<td>0/411</td>
<td>0/410</td>
<td></td>
<td></td>
<td></td>
<td>0/411</td>
<td>157/411</td>
<td>0/411</td>
<td>3/411</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Yersinia Enterocolitica</em></td>
<td>0%</td>
<td>0%</td>
<td>-</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>94%</td>
<td>-</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/47</td>
<td>0/47</td>
<td>47/47</td>
<td></td>
<td></td>
<td></td>
<td>44/47</td>
<td>0/47</td>
<td>0/47</td>
<td>0/47</td>
<td>0/47</td>
<td>0/47</td>
</tr>
<tr>
<td></td>
<td><em>Yersinia pseudotuberculosis</em></td>
<td>0%</td>
<td>0%</td>
<td>-</td>
<td>0%</td>
<td>-</td>
<td>-</td>
<td>0%</td>
<td>-</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td></td>
<td></td>
<td>0/57</td>
<td>0/55</td>
<td>0/55</td>
<td></td>
<td></td>
<td></td>
<td>0/55</td>
<td>0/55</td>
<td>0/55</td>
<td>0/55</td>
<td>0/55</td>
<td>0/55</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. *98.1% (561/572) tested intermediate to ampicillin.* Continued
Table 2 Legend. (continued)

Enr = Enrofloxacin
Ery = Erythromycin
Cla = Clavamox
Amp = Ampicillin
Car = Carbencillin
Met = Methicillin
Cef = Cefazolin
Cep = Cephalothin
Tet = Tetracycline
Dox = Doxycycline
Gen = Gentamicin
Chl = Chloramphenicol

Institution B

Isolates of *S. flexneri* were most frequently resistant to tetracycline (38%, 157/411) (Table 2). *Y. enterocolitica* isolates were most frequently resistant to ampicillin (100%, 47/47) and cefazolin (93.6%, 44/47) (Table 2). AMR among *Y. pseudotuberculosis* isolates from Institution B was the same as Institution A; no AMR was observed (Table 2). Susceptibility tests were not performed in Institution B for *C. jejuni* (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. Non-speciated *Campylobacter* (n=1) was susceptible to enrofloxacin and azithromycin.

**Detecting antimicrobial resistance**

We had sufficient power to detect AMR, if AMR was present in 0.8%-14.2% within the sample populations of bacterial species from each institution (α=0.01). Multiple minimum prevalences of AMR detectable existed because there were multiple isolate sample sizes of each bacterium from both Institutions A and B.

**Isolate resistance patterns**

Identical resistance patterns were repeatedly recovered within institutions (Table 3). In particular, 96% (551/574) of *C. jejuni* isolates from Institution A expressed resistance to only cephalothin and methicillin, and 38% (157/411) of *S. flexneri* isolates from Institution B expressed resistance to tetracycline alone (Table 3). Shared resistance patterns may indicate shared resistance genes, as well as persistence and transmission of clonal strains within institutions.** Additionally, substantial diversity in resistance patterns was observed among *S. flexneri* isolates from Institution A (Table 3).
<table>
<thead>
<tr>
<th>Institution</th>
<th>Bacterium</th>
<th>Frequency</th>
<th>Resistance Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><strong>Shigella flexneri</strong> (n=30)</td>
<td>7%</td>
<td>EryDoxClaT/Sd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3%</td>
<td>EryGenT/Sd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3%</td>
<td>EryGenDoxCla</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>EryGenCla</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3%</td>
<td>EryGenDox</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3%</td>
<td>EryGen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>EryDoxCla</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13%</td>
<td>EryDox</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17%</td>
<td>Ery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>DoxCla</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>AmpChlTetCarCla</td>
</tr>
<tr>
<td>B</td>
<td><strong>Shigella flexneri</strong> (n=410)</td>
<td>0.70%</td>
<td>AmpTetChl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5%</td>
<td>AmpTet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37%</td>
<td>Tet</td>
</tr>
<tr>
<td>Yersinia enterocolitica (n=5)</td>
<td>40%</td>
<td>EryDoxCla</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20%</td>
<td>Cla</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40%</td>
<td>AmpCla</td>
</tr>
<tr>
<td>Campylobacter jejuni (n=574)</td>
<td>0.20%</td>
<td>MetCepT/S</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.30%</td>
<td>AmpMetCep</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96%</td>
<td>MetCep</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50%</td>
<td>Cep</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20%</td>
<td>ChlMetCli</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>ChlMet</td>
</tr>
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<td></td>
<td>1.20%</td>
<td>MetCli</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20%</td>
<td>Met</td>
</tr>
<tr>
<td>Yersinia enterocolitica (n=48)</td>
<td>92%</td>
<td>AmpCef</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8%</td>
<td>Amp</td>
</tr>
</tbody>
</table>

**Table 3.** Frequency of antimicrobial resistance patterns among *Shigella flexneri*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Campylobacter jejuni* isolated from nonhuman primates in Biomedical Research Institutions A & B, within January 2012 and April 2015. (continued)
Therapeutic antimicrobial selection among NHP veterinarians

In order to relieve patient discomfort, it is often appropriate to initiate antimicrobial therapy empirically prior to microbiological test results. Therefore, the survey was developed to identify participating veterinarians’ primary antimicrobials for treating clinical signs of diarrhea and gingivitis. The latter was included because of the ability of
*S. flexneri* to cause periodontal disease. Most participating veterinarians reported enrofloxacin (40%, 6/15) or tylosin (40%, 6/15) as their primary antimicrobial for treating NHPs with clinical signs of diarrhea. Seventy-four percent (10/14) reported enrofloxacin as their primary choice for treating NHPs 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. Seventy-three percent (11/15) always request susceptibility tests for isolated *S. flexneri*, 67% (10/15) for isolated *Y. enterocolitica* and *Y. pseudotuberculosis*, and 53% (8/15) for isolated *C. jejuni* (Table 4).

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Always</th>
<th>Most of the time</th>
<th>Sometimes</th>
<th>Rarely</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella flexneri</em></td>
<td>73% (11/15)</td>
<td>13% (2/15)</td>
<td>-</td>
<td>13% (2/15)</td>
<td>-</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>67% (10/15)</td>
<td>20% (3/15)</td>
<td>-</td>
<td>13% (2/15)</td>
<td>-</td>
</tr>
<tr>
<td><em>Yersinia pseudotuberculosis</em></td>
<td>67% (10/15)</td>
<td>20% (3/15)</td>
<td>-</td>
<td>7% (1/15)</td>
<td>7% (1/15)</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>53% (8/15)</td>
<td>13% (2/15)</td>
<td>13% (2/15)</td>
<td>7% (1/15)</td>
<td>13% (2/15)</td>
</tr>
</tbody>
</table>

**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 nonhuman primates.
In addition to identifying primary antimicrobials for treating clinical signs prior to microbial results, the survey was also designed to identify 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%, 13/15), *Y. enterocolitica* (79%, 11/14), and *Y. pseudotuberculosis* (69%, 9/13) (Table 5). In contrast, enrofloxacin was not a common choice among participants for *C. jejuni* (13%, 2/15) (Table 5). Instead, azithromycin (40%, 6/15) and tylosin (40%, 6/15) were the most popular antimicrobial therapies for suspected diarrhea cases caused by *C. jejuni* (Table 5).

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th><em>Shigella flexneri</em></th>
<th><em>Yersinia enterocolitica</em></th>
<th><em>Yersinia pseudotuberculosis</em></th>
<th><em>Campylobacter jejuni</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>87% (13/15)</td>
<td>79% (11/14)</td>
<td>69% (9/13)</td>
<td>13% (2/15)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>7% (1/15)</td>
<td>-</td>
<td>-</td>
<td>40% (6/15)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>-</td>
<td>14% (2/14)</td>
<td>8% (1/13)</td>
<td>-</td>
</tr>
<tr>
<td>Tylosin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40% (6/15)</td>
</tr>
</tbody>
</table>

**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 nonhuman primates.
Changes in primary antimicrobials chosen for therapy

Participating veterinarians identified a threshold prevalence of antimicrobial resistance in a population of isolates where if exceeded, would cause them to change their primary antimicrobial chosen for therapy (Table 5). This was intended to represent a threshold for changes in decision making, and not a breakpoint of antimicrobial effectiveness. We hypothesized that the prevalence of AMR (Table 2) would exceed participating veterinarians’ TP-AMRs. Figure 1 illustrates the distribution of the self-identified TP-AMRs among participating veterinarians for the four investigated bacteria, ranging from 0-80% with means 15-20%. Comparing Figure 1 to Table 2, the prevalence of AMR did exceed veterinarians’ TP-AMRs on many occasions. For instance, AMR was observed among 99.5% (569/572) of Institution A’s C. jejuni isolates to methicillin, and 98% (557/572) to cephalothin, both exceeding the largest TP-AMR reported. However, when focusing 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, and C. jejuni, including an absence of AMR to enrofloxacin. This is emphasized by the fact that we had sufficient power to detect AMR, if AMR was present in as low as 0.08% of the sample populations. The two most popular antimicrobials to treat clinical cases of C. jejuni, azithromycin and tylosin, were not included in susceptibility tests for Institutions A and B; however, erythromycin, another macrolide, was included. No AMR was observed among all investigated bacteria to participating veterinarians’ primary antimicrobials.
Figure 1. Veterinarian reported threshold for the prevalence of antimicrobial resistance that would result in changing their primary antimicrobial choice for therapy, if exceeded by the true prevalence of antimicrobial resistance.
Chapter 5: Discussion

The objective of the present study is to estimate the prevalence of AMR in zoonotic bacteria from diagnostic NHP samples and evaluate current antimicrobial use practices among NHP veterinarians against the zoonotic bacteria. No comprehensive multi-institutional studies on the prevalence of AMR among zoonotic bacteria from NHPs have been published. This study provides veterinarians and scientists with critical data to make informed decisions on policy and therapeutic treatment. *S. flexneri, Y. enterocolitica, Y. pseudotuberculosis,* and *C. jejuni* all commonly cause diarrhea among NHPs,²,³,¹⁴,¹⁵,³⁹,⁷⁸,⁸⁶,⁹² and in rarer cases, *S. flexneri* can also cause gingivitis.⁵ Enrofloxacin was a popular antimicrobial choice among participating veterinarians. It was commonly the primary antimicrobial for treating clinical cases, prior to susceptibility results, of diarrhea with isolated *S. flexneri* (87%, 13/15), and clinical cases of gingivitis (74%, 10/14). Enrofloxacin is clinically important against shigellosis because it is effective and safe.⁵⁰ Overall, this study’s results does illustrate a high prevalence of resistance to specific antimicrobials among *S. flexneri, Y. enterocolitica, Y. pseudotuberculosis,* and *C. jejuni,* consistent with previous studies.¹¹,³⁷,³⁹,⁵¹,⁸⁸,⁹² However, our results reveal no evidence of resistance to enrofloxacin, even with consistent antimicrobial selective pressure. Furthermore, AMR was not observed among *C. jejuni* isolates to erythromycin. 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 three. Cross-resistance between erythromycin and azithromycin\(^4^9\) and erythromycin and tylosin\(^1^6\) is evident from previous studies.

No isolate was resistant to third generation cephalosporins (ceftiofur, ceftriaxone, cefpodoxime) and fluoroquinolones (enrofloxacin, ciprofloxacin, and marbofloxacin). This is especially informative for physicians and epidemiologists because third generation cephalosporins and fluoroquinolones are important antimicrobial classes in public health. However, participating laboratories infrequently included the above antimicrobials 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 with susceptibility testing, both within and across institutions. Because the investigated bacteria can have greater public health impacts, it is worthwhile for veterinarians to consistently include third generation cephalosporins and fluoroquinolones in susceptibility tests. Ceftiofur crystalline free acid (CCFA) is an example that may provide veterinarians with a secondary option, in the event of AMR to enrofloxacin; CCFA has recently been investigated for use in rhesus macaques and may have positive animal welfare and management impacts as long-term single-dose therapy.\(^7^9\)
Comparing resistance patterns between NHP and human isolates may suggest relationships among strains, but it is difficult to evaluate without further genotyping. Among our NHP data, there were great differences in resistance pattern diversity between participating institutions. Intra-institutional differences may be explained by novel introductions of animals or bacterial strains, intra-institutional 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. For instance in the NARMS report, 37.5% (24/64) of S. flexneri isolates showed AmpChlStrSulTet resistance pattern (resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/sulfisoxazole, tetracycline) and 51.6% (33/64) showed AmpT/S resistance pattern (resistance to ampicillin, trimethoprim-sulfamethoxazole). However, none of the observed NHP resistance patterns (0/440) shared those published by NARMS. Comparisons between NHP and human data on Y. enterocolitica and Y. pseudotuberculosis are challenging because comprehensive surveillance data of AMR among the two Yersinia spp. was not found among literature searches. Similarly, NARMS does not publish specific data on C. jejuni resistance patterns. Although isolates can be genetically identical and still express different resistance patterns, the lack of shared patterns and the lack of recovery of resistance patterns that are globally disseminated may indicate limited transmission between NHPs and humans. Nonetheless, resistance genes can be horizontally transferred between NHP and human populations isolates via plasmids and transposons, 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, as shared resistance patterns may indicate shared bacterial strains.\(^{41}\)

The CDC reports that both *Shigella spp.* and *Campylobacter spp.* are becoming increasingly resistant to ciprofloxacin and azithromycin.\(^{23}\) However, our study reveals no evidence of AMR among *S. flexneri* and *C. jejuni* to ciprofloxacin, enrofloxacin, and azithromycin. Because enrofloxacin is metabolized into ciprofloxacin,\(^{50,68}\) isolates susceptible to enrofloxacin are generally susceptible to ciprofloxacin.\(^{68}\) Moreover, the CDC reports that a high prevalence of *Shigella spp.* are also resistant to first-line antimicrobials ampicillin and trimethoprim-sulfamethoxazole.\(^{23}\) However, only 1.2% (5/410) of Institution B’s *S. flexneri* isolates were resistant to ampicillin, again emphasizing the greater likelihood of distinct populations of bacteria between NHPs and humans.

Although this was a multi-institutional study, larger studies that include more institutions would nevertheless be useful to more precisely estimate the prevalence of AMR of zoonotic bacteria from NHPs. More institutions may emphasize similar heterogeneity of AMR across institutions that was observed. Such heterogeneity could be driven by strict biosecurity, including quarantining new NHPs prior to the introduction to establish colonies, as well as potentially minimal importation of wild-caught NHPs.
Some participating institutions provided few susceptibility test results. This could be because of 1) few clinical cases of diarrhea, 2) samples were infrequently submitted for microbial identification, 3) the investigated bacteria were infrequently isolated, and/or 4) susceptibility tests were infrequently requested by veterinarians. The first and third are unlikely based on the institutions’ size and housing arrangements, as well as support from previous studies.\(^2,3,14,15,39,78,86,92\) Even so, it is clear that clinical decisions when involving zoonotic bacteria, whether they are antimicrobial choices or susceptibility testing, affect not only the NHP patient but potentially personnel as well. Our data does illustrates that veterinarians have overall been successfully prescribing effective antimicrobials, but it also illustrates inconsistent susceptibility testing. Based on data published by NARMS, AMR can and has abruptly increased dramatically,\(^21\) which is possible with the introduction of novel strains. With this said, consistent and routine susceptibility testing is recommended among both veterinarians and diagnostic laboratories. A strong collaborative approach between veterinarians and diagnostic laboratories can inform empirical antimicrobial choices, or revise current practices based on test results, consequently reducing AMR risk.

With some variations in response rates, several potential limitations are evident. Response bias is a possibility, because the responses of participating veterinarians may differ from non-participating veterinarians. Additionally, potential data from non-participating biomedical research institutions may be systematically different from those that participated. Thus, it is important to highlight that because the prevalence of AMR
was only estimated for three biomedical research institutions, the results cannot be extrapolated to every institution using NHPs. Antimicrobial therapy should ultimately depend on the patient’s susceptibility test result. However, because antimicrobials are frequently initiated 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 literature exists that demonstrates, as comprehensively as the present study, the prevalence of AMR among zoonotic bacteria from NHPs in biomedical research.

In summary, the present study demonstrates low levels of AMR among S. flexneri, Y. enterocolitica, and Y. pseudotuberculosis to enrofloxacin. The present study also illustrates low levels of AMR among C. jejuni to erythromycin, azithromycin, and tylosin. Because limited transmission between NHP and human isolates is likely, first-line antimicrobials are likely to be effective options in the event of occupational exposure. Although discrepancies among susceptibility testing exists, simple collaboration between veterinary staff and diagnostic laboratories can foster consistent and routine practices that reduce AMR potential. Such collaboration will ultimately help veterinarians more confidently ensure effective antimicrobial therapy among patients, and will also help minimize occupational risk.
References

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44. **Iwamoto M.** Salmonellosis (Nontyphoidal). CDC Health Information for


54. Lai EPC, Iqbal Z, Avis TJ. Combating Antimicrobial Resistance in Foodborne


86. Taffs LF, Dunn G. An outbreak of Yersinia pseudotuberculosis infection in a small indoor breeding colony of red-bellied (Saguinus labiatus) tamarins. Laboratory Animals. 1983;17;:311–320.


Appendix A: Pilot Study - Veterinarian Antimicrobial Use Survey
Thank you for participating in our study. Your contribution is critical as it will directly benefit the health of your veterinary/husbandry staff, laboratory personnel, and of course, your animals. Essentially, your contribution will help minimize occupational risk. Working with your associated diagnostic laboratory, we will calculate the prevalence of antimicrobial resistance within 4 potentially threatening zoonotic bacteria, from 2012-2014. Our ultimate goal is to help you treat your animals as effectively and efficiently as possible. This is only possible with your help. Please complete this survey, and as thoroughly as possible where appropriate. This study's results will provide you with necessary information to maintain or change your current veterinary practices, ultimately to ensure the health of your animals, staff, and laboratory personnel. The survey should take about 20 minutes.

Note on Confidentiality: We understand the sensitivity of the data and the necessity for confidentiality. Only members working on this study will have access to the data. Your results will not be identified to your institution, whatsoever. This is to ensure your safety and confidentiality.

Again, thank you for participating.

Sincerely,

Jeffrey Kim & Greg Habing MS, DVM, PhD

How many clinical veterinarians are part of your staff?

How many non-human primates (NHP) are currently in your facilities?

List the NHP species in your facilities.

Do you have an outdoor or indoor NHP colony?

☐ Outdoor only
☐ Indoor only
☐ Both
If you have an indoor NHP colony, do NHPs ever have access to one another?

- Yes
- No
- We do not have an indoor colony

The following questions are regarding antibiotic susceptibility tests, which is defined as tests to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections.

For each of the following organisms, how often is antibiotic susceptibility testing requested when they are isolated from a clinical case of diarrhea?

<table>
<thead>
<tr>
<th></th>
<th>Always</th>
<th>Most of the time</th>
<th>Sometimes</th>
<th>Rarely</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shigella flexneri</strong></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td><strong>Yersinia enterocolitica</strong></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td><strong>Yersinia pseudotuberculosis</strong></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td><strong>Campylobacter jejuni</strong></td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>
Rank your top 3 antimicrobial preferences for treating NHPs with the diarrhea and dehydration (prior to receiving culture and sensitivity results)

<table>
<thead>
<tr>
<th></th>
<th>Amoxicillin</th>
<th>Amoxicillin</th>
<th>Amoxicillin</th>
<th>Cloxacillin</th>
<th>Tylosin</th>
<th>Chloramphenicol</th>
<th>Sulfoxazole</th>
<th>1st Generation Cephalosporins (cephaprin)</th>
<th>2nd Generation Cephalosporins</th>
<th>3rd Generation Cephalosporins (e.g. ceftriocoxone)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Choice</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Secondary Choice</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Tertiary Choice</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

Click to write the question text
- ○ Click to write Choice 1
- ○ Click to write Choice 2
- ○ Click to write Choice 3
If you selected "other" in the previous question, please specify your antimicrobial of choice.

Primary Choice
Secondary Choice
Tertiary Choice

Rank your top 3 antibiotic preferences for treating NHPs with gingivitis (prior to receiving any culture/sensitivity results)

<table>
<thead>
<tr>
<th>Primary Choice</th>
<th>Secondary Choice</th>
<th>Tertiary Choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>Amoxicillin</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Clavulanic Acid</td>
<td>Enrofloxacine</td>
</tr>
<tr>
<td>Tylosin</td>
<td>Chlormephanicol</td>
<td>Sulmethoxazole</td>
</tr>
<tr>
<td>1st Generation Cephalosporins (cefaclor)</td>
<td>2nd Generation Cephalosporins (ceftoxime)</td>
<td>3rd Generation Cephalosporins (e.g. ceftiofur, ceftriaxone)</td>
</tr>
<tr>
<td>3rd Generation Cephalosporins (e.g. ceftiofur, ceftriaxone)</td>
<td>1st Generation Cephalosporins (cefaclor)</td>
<td>2nd Generation Cephalosporins (ceftoxime)</td>
</tr>
<tr>
<td>2nd Generation Cephalosporins (ceftoxime)</td>
<td>3rd Generation Cephalosporins (e.g. ceftiofur, ceftriaxone)</td>
<td>1st Generation Cephalosporins (cefaclor)</td>
</tr>
<tr>
<td>3rd Generation Cephalosporins (e.g. ceftiofur, ceftriaxone)</td>
<td>2nd Generation Cephalosporins (ceftoxime)</td>
<td>1st Generation Cephalosporins (cefaclor)</td>
</tr>
</tbody>
</table>

56
If you selected "other" in the previous question, please specify your antimicrobial of choice.

   Primary Choice  
   Secondary Choice  
   Tertiary Choice

Initial antimicrobial choice (prior to receiving culture/sensitivity) for NHPs with diarrhea, dehydration, and gingivitis is based on: (click all that apply)

☑ Historical efficacy within your institution  
☑ Historical efficacy based your personal experience (throughout your career)  
☑ Historical culture and susceptibility results within your institution  
☑ Historical culture and susceptibility results within your personal experience (throughout your career)  
☑ Published literature/protocols  
☐ Other ____________________

Click to write the question text  
       Click to write Item 1  
       Click to write Item 2  
       Click to write Item 3
Suppose *Shigella flexneri* is isolated from a sick NHP with diarrhea and dehydration. Assuming the animal requires antimicrobial therapy, rank the following antimicrobials for your initial choice of antimicrobial therapy (prior to receiving antimicrobial susceptibility reports)

<table>
<thead>
<tr>
<th></th>
<th>Amoxicillin</th>
<th>Ampicillin</th>
<th>Amoxicillin Clavulanic Acid</th>
<th>Enrofloxacin</th>
<th>Tylosin</th>
<th>Chloramphenicol</th>
<th>Sulfamethoxazole</th>
<th>1st Generation Cephalosporins (cephaprin)</th>
<th>2nd Generation Cephalosporins</th>
<th>3rd Generation Cephalosporins (e.g. ceftriaxone)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Secondary</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Tertiary</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

If you selected "other" in the previous question, please specify your antimicrobial of choice.

Primary Choice
Secondary Choice
Tertiary Choice
Treatment with the above antimicrobials for *Shigella flexneri* was based on: (click all that apply)

- [ ] Historical efficacy within your institution
- [ ] Historical efficacy within your personal experience (throughout your career)
- [ ] Historical susceptibility results within your institution
- [ ] Historical culture and susceptibility results within your personal experience (throughout your career)
- [ ] Published literature/protocols
- [ ] Other ____________________

Suppose *Yersinia enterocolitica* is isolated from a sick NHP with diarrhea and dehydration. Rank the following antimicrobials for your initial choice of antimicrobial therapy (prior to receiving antimicrobial susceptibility reports)

<table>
<thead>
<tr>
<th></th>
<th>1st Generation Cephalosporins (cephaprin)</th>
<th>2nd Generation Cephalosporins</th>
<th>3rd Generation Cephalosporins (e.g. ceftriaxone)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Choice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary Choice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary Choice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Amoxicillin
- Ampicillin
- Amoxicillin Clavulanic Acid
- Enrofloxacine
- Tylosin
- Chloramphenicol
- Sulfamethoxazole
- 1st Generation Cephalosporins (cephaprin)
- 2nd Generation Cephalosporins
- 3rd Generation Cephalosporins (e.g. ceftriaxone)
If you selected "other" in the previous question, please specify your antimicrobial of choice.

   Primary Choice
   Secondary Choice
   Tertiary Choice

Treatment with the above antimicrobials for *Yersinia enterocolitica* was based on: (click all that apply)

- [ ] Historical efficacy within your institution
- [ ] Historical efficacy within your personal experience (throughout your career)
- [ ] Historical susceptibility results within your institution
- [ ] Historical culture and susceptibility results within your personal experience (throughout your career)
- [ ] Published literature/protocols
- [ ] Other ____________________
Suppose *Yersinia pseudotuberculosis* is isolated from a sick NHP with diarrhea and dehydration. Rank the following antimicrobials for your initial choice of antimicrobial therapy (prior to receiving antimicrobial susceptibility reports)

<table>
<thead>
<tr>
<th></th>
<th>Amoxicillin</th>
<th>Ampicillin</th>
<th>Amoxicillin Clavulanic Acid</th>
<th>Enrofloxacine</th>
<th>Tylosin</th>
<th>Chloramphenicol</th>
<th>Sulfamethoxazole</th>
<th>1st Generation Cephalosporins (cephaprin)</th>
<th>2nd Generation Cephalosporins</th>
<th>3rd Generation Cephalosporins (e.g. ceftriaxone)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Choice</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Secondary Choice</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Tertiary Choice</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

If you selected "other" in the previous question, please specify your antimicrobial of choice.

Primary Choice
Secondary Choice
Tertiary Choice
Treatment with the above antimicrobials for *Yersinia pseudotuberculosis* was based on: (click all that apply)

- Historical efficacy within your institution
- Historical efficacy within your personal experience (throughout your career)
- Historical susceptibility results within your institution
- Historical culture and susceptibility results within your personal experience (throughout your career)
- Published literature/protocols
- Other ____________________

Suppose *Campylobacter jejuni* is isolated from a sick NHP with diarrhea and dehydration. Rank the following antimicrobials for your initial choice of antimicrobial therapy (prior to receiving antimicrobial susceptibility reports)

<table>
<thead>
<tr>
<th></th>
<th>1st Generation Cephalosporins (cephaprin)</th>
<th>2nd Generation Cephalosporins</th>
<th>3rd Generation Cephalosporins (e.g. ceftiofur, ceftriaxone)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Choice</strong></td>
<td>Amoxicillin (Amoxicillin)</td>
<td>Amoxicillin</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td><strong>Secondary Choice</strong></td>
<td>Ampicillin (Ampicillin)</td>
<td>Enrofloxacin (Enrofloxacin)</td>
<td>Tylosin (Tylosin)</td>
</tr>
<tr>
<td><strong>Tertiary Choice</strong></td>
<td>Chloramphenicol (Chloramphenicol)</td>
<td>Sulfa methoxazole (Sulfa methoxazole)</td>
<td>First Generation Cephalosporins (1st Generation Cephalosporins (cephaprin))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

62
If you selected "other" in the previous question, please specify your antimicrobial of choice.

Primary Choice
Secondary Choice
Tertiary Choice

Treatment with the above antimicrobials for *Campylobacter jejuni* was based on: (click all that apply)

- ✔️ Historical efficacy within your institution
- ✔️ Historical efficacy within your personal experience (throughout your career)
- ✔️ Historical susceptibility results within your institution
- ✔️ Historical culture and susceptibility results within your personal experience (throughout your career)
- ✔️ Published literature/protocols
- ✔️ Other ____________________

A change in the primary antimicrobial against *Shigella flexneri* would be considered if the average prevalence of resistance to this antimicrobial in your institution exceeded:

_____ Prevalence %

A change in the primary antimicrobial against *Yersinia enterocolitica* would be considered if the average prevalence of resistance to this antimicrobial in your institution exceeded:

_____ Prevalence %

A change in the primary antimicrobial against *Yersinia pseudotuberculosis* would be considered if the average prevalence of resistance to this antimicrobial in your institution exceeded:

_____ Prevalence %
A change in the primary antimicrobial against *Campylobacter jejuni* would be considered if the average prevalence of resistance to this antimicrobial in your institution exceeded:

_____ Prevalence %
Appendix B: Initial Email to Veterinary Director
Dear (veterinary director),

The Ohio State University is conducting a study on antimicrobial resistance, and on veterinarians’ opinions and practices on antimicrobial treatment of zoonotic diseases among non-human primates. The study has two components:

By working with your diagnostic laboratory, we hope to estimate your institution’s prevalence of antimicrobial resistance among *Shigella flexneri*, *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, and *Campylobacter jejuni* between 2012 and 2014. **At the end of this study, we will provide your staff with a report on the prevalence of antimicrobial resistance within your institution from 2012-2014.** This important data will help your staff make more informed decisions, ultimately to more effectively and efficiently treat your sick animals. Because all of these bacteria are also zoonotic, this will help you protect your staff and laboratory personnel as well.

If you are willing to participate, we will send a survey to each of your staff veterinarians. In order to do this, we kindly ask for the email addresses of each of your veterinarians. No one uninvolved with this study will have access to this information. Additionally, all individuals, including your institution as a whole, will not be identifiable in any publications resultant from our study.

If you do not work with non-human primates, please check here [ ] and reply to this email. No further action is needed.

If you have any questions, do not hesitate to contact us.

Sincerely,

Greg Habing MS, DVM, PhD
Assistant Professor
Veterinary Preventive Medicine
1920 Coffey Rd
Columbus, OH 43210
(614) 292-7205

Jeffrey Kim
MPH – Veterinary Public Health Candidate
kim.4703@osu.edu
(614) 292-5884

(614) 575-5884
Appendix C: Initial Email to Director of Diagnostic Laboratory
Dear (diagnostic lab director),

The Ohio State University is conducting a study to describe the antimicrobial resistance of zoonotic bacteria in research institutions. You are receiving this email because your laboratory receives samples from (institution name here).

**Purpose of the study:** Our goal is to aggregate data on the *in-vitro* susceptibility of non-human primate pathogens based on diagnostic laboratory records. Further analysis of this type of data will help us provide veterinarians with necessary information to help them treat their animals more effectively and efficiently. Your participation is very important to this research project.

We are recruiting laboratories with searchable electronic records that received samples from (institution name here) between 2012 and 2014. If you are willing to help with this project, here is how.

**How to participate:** Forward to us contact information of a staff microbiologist or technician who can complete a brief survey and assist with data collection from an electronic database. We kindly ask for antimicrobial susceptibility test results of four zoonotic pathogens. And by working collaboratively with this individual, we will provide a summary report of the antimicrobial resistance to participating research institutions.

If you have any questions, please contact us anytime.

Sincerely,

Greg Habing MS, DVM, Phd  
Assistant Professor  
Veterinary Preventive Medicine  
1920 Coffey Rd  
Columbus, OH 43210  
(614) 292-7205

Jeffrey Kim  
MPH – Veterinary Public Health Candidate  
kim.4703@osu.edu  
(661) 575-5884
Appendix D: Reminder Email to Veterinary Director
Dear (veterinary director),

A few weeks ago, you received an email from The Ohio State University regarding a new study on antimicrobial resistance, and on veterinarians’ opinions and practices on antimicrobial treatment of zoonotic diseases among non-human primates. The study has two components:

By working with your diagnostic laboratory, we hope to estimate your institution’s prevalence of antimicrobial resistance among *Shigella flexneri, Yersinia pseudotuberculosis, Yersinia enterocolitica*, and *Campylobacter jejuni* between 2012 and 2014. **At the end of this study, we will provide your staff with a report on the prevalence of antimicrobial resistance within your institution from 2012-2014.** This important data will help your staff make more informed decisions, ultimately to more effectively and efficiently treat your sick animals. Because all of these bacteria are also zoonotic, this will help you protect your staff and laboratory personnel as well.

If you are willing to participate, we will send a survey to each of your staff veterinarians. In order to do this, we kindly ask for the email addresses of each of your veterinarians. No one uninvolved with this study will have access to this information. Additionally, all individuals, including your institution as a whole, will not be identifiable in any publications resultant from our study.

If you do not work with non-human primates, please check here [ ] and reply to this email. No further action is needed.

If you have any questions, do not hesitate to contact us.

Sincerely,

Greg Habing MS, DVM, Phd
Assistant Professor
Veterinary Preventive Medicine
1920 Coffey Rd
Columbus, OH 43210
(614) 292-7205

Jeffrey Kim
MPH – Veterinary Public Health Candidate
kim.4703@osu.edu
(661) 575-5884
Appendix E: Reminder Email to Director of Diagnostic Laboratory
Dear (diagnostic lab director),

A few weeks ago, you received an email from me regarding a new study that will describe the antimicrobial resistance of zoonotic bacteria in research institutions. You are receiving this email because your laboratory receives samples from (institution name here).

**Purpose of the study:** Our goal is to aggregate data on the in-vitro susceptibility of non-human primate pathogens based on diagnostic laboratory records. Further analysis of this type of data will help us provide veterinarians with necessary information to help them treat their animals more effectively and efficiently. Your participation is very important to this research project.

We are recruiting laboratories with searchable electronic records that received samples from (institution name here) between 2012 and 2014. If you are willing to help with this project, here is how.

**How to participate:** Forward to us contact information for a staff microbiologist or technician who can complete a brief survey and assist with data collection from an electronic database. We will work collaboratively with this individual, and provide a summary report of the antimicrobial resistance to participating research institutions.

If you have any questions, do not hesitate to contact us.

Sincerely,

Greg Habing MS, DVM, Phd
Assistant Professor
Veterinary Preventive Medicine
1920 Coffey Rd
Columbus, OH 43210
(614) 292-7205

Jeffrey Kim
MPH – Veterinary Public Health Candidate
kim.4703@osu.edu
(661) 575-5884
Appendix F: Initial Email to Participating Veterinarians
Dear (staff veterinarian),

The Ohio State University is conducting a new study on antimicrobial resistance and antimicrobial use strategies of zoonotic diseases among non-human primates.

We will estimate your institution’s prevalence of antimicrobial resistance among *Shigella flexneri*, *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, and *Campylobacter jejuni* between 2012 and 2014. **At the end of this study, we will provide you with a report on the prevalence of antimicrobial resistance within your institution from 2012-2014.** With this estimated prevalence, it will help you make more informed decisions, ultimately to more effectively and efficiently treat your sick animals. Because all of these bacteria are also zoonotic, this will help you protect your staff and laboratory personnel as well.

If you are willing to participate, please complete a brief survey (link found below). All data collected during this study will be kept confidential. Furthermore, you and your institution as a whole, will not be identifiable in any publications resultant from our study.

*(Link to survey)*

Thank you for participating. Your response to the survey is important to generate knowledge that will benefit your institution and others throughout the country.

Sincerely,

Greg Habing MS, DVM, Phd  
Assistant Professor  
Veterinary Preventive Medicine  
1920 Coffey Rd  
Columbus, OH 43210  
(614) 292-7205

Jeffrey Kim  
MPH – Veterinary Public Health Candidate  
kim.4703@osu.edu  
(661) 575-5884
Appendix G: Consent Form for Participating Veterinarians
Consent Form for Study Participation

Study: Current Antimicrobial Practices Against Four Zoonotic Bacteria in Non-Human Primates

Principal investigator: Dr. Greg Habing

1. PURPOSE OF RESEARCH

You are being asked to participate in a research project by The Ohio State University. From this study, the investigators hope to describe the knowledge and attitudes about antimicrobial treatment strategies against zoonotic bacteria at research institutions. Additionally, we will use diagnostic laboratory records to describe the in-vitro efficacy of antimicrobials against four zoonotic bacteria at several major biomedical research institutions in the US that use non-human primates.

2. HOW TO PARTICIPATE

Complete the survey by answering the questions. Completion should require less than 20 minutes of your time. You may complete the survey online, sent via email. Once completed, the results will be automatically sent to us.

3. POTENTIAL BENEFITS

You will receive a summarized report on the antimicrobial resistance of four major zoonotic pathogens recovered at your research institution. This report will be based on an evaluation of the diagnostic laboratory records. This study is designed to help you and other institutions make informed decisions in changing or maintaining current veterinary practices, to ultimately ensure the health of your animals, staff, and laboratory personnel.

4. POTENTIAL RISKS

There are no appreciable risks from participating in the research.

Your decision to participate will not affect any current or future relationship with the Ohio State University.
5. PRIVACY AND CONFIDENTIALITY

The information gathered will be kept confidential. Only members of the study will have access to the confidential information. We will work to make sure that no one sees your online responses without approval. But, because we are using the Internet, there is a chance that someone could access your online responses without permission. In some cases, this information could be used to identify you. Nonetheless, the investigators have experience with the online system (Qualtrics) and are confident that it is unlikely persons not involved in the study will access your responses.

The Ohio State University may publish, or authorize the publication in academic journals, the findings acquired from the study for the benefit of the laboratory animal science field and other interested groups, but will ensure that your participation is not revealed.

6. YOUR RIGHTS TO PARTICIPATE, SAY NO, OR WITHDRAW

Your participation in this project and completing the survey is VOLUNTARY. There is no penalty for not completing and returning the survey. You may choose to skip any question that you do not want to answer. You can withdraw from the study at any time prior to mailing the survey back. For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251

7. COSTS FOR BEING IN THE STUDY

There will be no cost to you for participating in this survey.

By completing and returning the survey you give consent to participate in this research.

If you have any questions regarding the research, please contact:

Dr. Greg Habing
Assistant Professor
Veterinary Preventive Medicine
1920 Coffey Rd
Columbus, OH 43210
(614) 292-7205
Appendix H: Veterinary Staff Antimicrobial Use Survey
Thank you for participating in our study. Your contribution is critical as it will directly benefit the health of your veterinary/husbandry staff, laboratory personnel, and of course, your animals. Essentially, your contribution will help minimize occupational risk. Working with your associated diagnostic laboratory, we will calculate the prevalence of antimicrobial resistance within 4 potentially threatening zoonotic bacteria, from 2012-2014. Our ultimate goal is to help you treat your animals as effectively and efficiently as possible. This is only possible with your help. Please complete this survey, and as thoroughly as possible where appropriate. This study's results will provide you with necessary information to maintain or change your current veterinary practices, ultimately to ensure the health of your animals, staff, and laboratory personnel. The survey should take about 20 minutes.

Note on Confidentiality: We understand the sensitivity of the data and the necessity for confidentiality. Only members working on this study will have access to the data. Your results will not be identified to your institution, whatsoever. This is to ensure your safety and confidentiality.

Again, thank you for participating.

Sincerely,

Jeffrey Kim & Greg Habing MS, DVM, PhD

---

How many clinical veterinarians are part of your staff?

How many non-human primates (NHP) are currently in your facilities?

List the NHP species in your facilities.

Do you have an outdoor or indoor NHP colony?

- Outdoor only
- Indoor only
- Both
If you have an indoor NHP colony, do NHPs ever have access to one another?

- Yes
- No
- We do not have an indoor colony

Please name your research institution.

The following questions are regarding antibiotic susceptibility tests, which is defined as tests to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections.

For each of the following organisms, how often is antimicrobial susceptibility testing requested when they are isolated from a clinical case of diarrhea?

<table>
<thead>
<tr>
<th>Organism</th>
<th>Always</th>
<th>Most of the time</th>
<th>Sometimes</th>
<th>Rarely</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella flexneri</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Yersinia pseudotuberculosis</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

List your top 3 antimicrobial preferences for treating NHPs with diarrhea (prior to receiving culture/sensitivity results)

- Primary Choice
- Secondary Choice
- Tertiary Choice
List your top 3 antimicrobial preferences for treating NHPs with gingivitis (prior to receiving culture/sensitivity results)

Primary Choice  
Secondary Choice  
Tertiary Choice

Initial antimicrobial choice (prior to receiving culture/sensitivity) for NHPs with diarrhea and gingivitis is based on: (click all that apply)

☐ Historical efficacy within your institution  
☐ Historical efficacy based your personal experience (throughout your career)  
☐ Historical culture and susceptibility results within your institution  
☐ Historical culture and susceptibility results within your personal experience (throughout your career)  
☐ Published literature/protocols  
☐ Other ____________________

Suppose *Shigella flexneri* is isolated from a sick NHP with diarrhea. Assuming the animal requires antimicrobial therapy, list your top 3 antimicrobials of choice choice for antimicrobial therapy (prior to receiving antimicrobial susceptibility reports)

Primary Choice  
Secondary Choice  
Tertiary Choice

Treatment with the above antimicrobials for *Shigella flexneri* was based on: (click all that apply)

☐ Historical efficacy within your institution  
☐ Historical efficacy within your personal experience (throughout your career)  
☐ Historical susceptibility results within your institution  
☐ Historical culture and susceptibility results within your personal experience (throughout your career)  
☐ Published literature/protocols  
☐ Other ____________________
What do you believe is the true proportion of *Shigella flexneri* cases that is resistant to your primary antimicrobial stated above, from your institution between 2012 and 2014?  
_____ Prevalence %

Suppose *Yersinia enterocolitica* is isolated from a sick NHP with diarrhea. Assuming the animal requires antimicrobial therapy, list your top 3 antimicrobials of choice for antimicrobial therapy (prior to receiving antimicrobial susceptibility reports)

- Primary Choice
- Secondary Choice
- Tertiary Choice

Treatment with the above antimicrobials for *Yersinia enterocolitica* was based on: (click all that apply)

- [ ] Historical efficacy within your institution
- [ ] Historical efficacy within your personal experience (throughout your career)
- [ ] Historical susceptibility results within your institution
- [ ] Historical culture and susceptibility results within your personal experience (throughout your career)
- [ ] Published literature/protocols
- [ ] Other ____________________

What do you believe is the true proportion of *Yersinia enterocolitica* cases that is resistant to your primary antimicrobial stated above, from your institution between 2012 and 2014?  
_____ Prevalence %

Suppose *Yersinia pseudotuberculosis* is isolated from a sick NHP with diarrhea. Assuming the animal requires antimicrobial therapy, list your top 3 antimicrobials of choice for antimicrobial therapy (prior to receiving antimicrobial susceptibility reports)

- Primary Choice
- Secondary Choice
- Tertiary Choice
Treatment with the above antimicrobials for *Yersinia pseudotuberculosis* was based on: (click all that apply)

- Historical efficacy within your institution
- Historical efficacy within your personal experience (throughout your career)
- Historical susceptibility results within your institution
- Historical culture and susceptibility results within your personal experience (throughout your career)
- Published literature/protocols
- Other ____________________

What do you believe is the true proportion of *Yersinia pseudotuberculosis* cases that is resistant to your primary antimicrobial stated above, from your institution between 2012 and 2014?

_____ Prevalence %

Suppose *Campylobacter jejuni* is isolated from a sick NHP with diarrhea. Assuming the animal requires antimicrobial therapy, list your top 3 antimicrobials of choice for antimicrobial therapy (prior to receiving antimicrobial susceptibility reports)

Primary Choice
Secondary Choice
Tertiary Choice

Treatment with the above antimicrobials for *Campylobacter jejuni* was based on: (click all that apply)

- Historical efficacy within your institution
- Historical efficacy within your personal experience (throughout your career)
- Historical susceptibility results within your institution
- Historical culture and susceptibility results within your personal experience (throughout your career)
- Published literature/protocols
- Other ____________________
What do you believe is the true proportion of *Campylobacter jejuni* cases that is resistant to your primary antimicrobial stated above, from your institution between 2012 and 2014?
______ Prevalence %

A change in the primary antimicrobial against *Shigella flexneri* would be considered if the average prevalence of resistance to this antimicrobial in your institution exceeded:
______ Prevalence %

A change in the primary antimicrobial against *Yersinia enterocolitica* would be considered if the average prevalence of resistance to this antimicrobial in your institution exceeded:
______ Prevalence %

A change in the primary antimicrobial against *Yersinia pseudotuberculosis* would be considered if the average prevalence of resistance to this antimicrobial in your institution exceeded:
______ Prevalence %

A change in the primary antimicrobial against *Campylobacter jejuni* would be considered if the average prevalence of resistance to this antimicrobial in your institution exceeded:
______ Prevalence %
Appendix I: Initial Email to Participating Microbiologists
Dear ____________.

The Ohio State University is conducting a study that will allow you and ___________ institution __________ to collaboratively treat sick non-human primates more effectively and efficiently. This study’s results will provide several major institutions with important data, which will allow them to make informed decisions to change or maintain current veterinary practices. Ultimately, we want to help the institutions ensure the health of their animals, staff, and laboratory personnel. However, we cannot do this without your help. **We ask that you will participate in a research survey on antimicrobial resistance in non-human primates.**

The survey contains only 5 questions. The survey can be found below.

*(Link to survey)*

Thank you for participating. Your response to the survey is important to generate knowledge that will benefit biomedical research institutions throughout the country.

Sincerely,

Greg Habing MS, DVM, Phd
Assistant Professor
Veterinary Preventive Medicine
1920 Coffey Rd
Columbus, OH 43210
(614) 292-7205

Jeffrey Kim
MPH – Veterinary Public Health Candidate
kim.4703@osu.edu
(661) 575-5884
Appendix J: Consent Form for Participating Microbiologists
Consent Form for Study Participation

Study: Current Antimicrobial Practices Against Four Zoonotic Bacteria in Non-Human Primates

Principal investigator: Dr. Greg Habing

1. PURPOSE OF RESEARCH

You are being asked to participate in a research project by The Ohio State University. From this study, the investigators hope to describe the in-vitro efficacy of current veterinary practices against four zoonotic bacteria, at several major biomedical research institutions in the U.S. that use non-human primates.

2. HOW TO PARTICIPATE

First, complete the survey by answering the questions. Completion should require about 5 minutes of your time. You may complete the survey online, sent via email. Once completed, the results are automatically sent to us.

3. POTENTIAL BENEFITS

This study is designed to help participating biomedical research institutions make informed decisions in changing or maintaining current veterinary practices, to ultimately help ensure the health of their animals, staff, and laboratory personnel. Participating research institutions will receive summary reports for the prevalence of antimicrobial resistance within their institutions.

4. POTENTIAL RISKS

There are no appreciable risks from participating in the research.

Your decision to participate will not affect any current or future relationship with the Ohio State University.

5. PRIVACY AND CONFIDENTIALITY

The information gathered will be kept confidential. Only members of the study will have access to the confidential information. We will work to make sure that no one sees your online responses without approval. But, because we are using the Internet, there is a chance that someone could access your online responses
without permission. In some cases, this information could be used to identify you. Nonetheless, the investigators have experience with the online system (Qualtrics) and are confident that it is unlikely persons not involved in the study will access your responses.

The Ohio State University may publish, or authorize the publication in academic journals, the findings acquired from the study for the benefit of the laboratory animal science field and other interested groups, but will ensure that your participation is not revealed.

6. YOUR RIGHTS TO PARTICIPATE, SAY NO, OR WITHDRAW

Your participation in this project and completing the survey is VOLUNTARY. There is no penalty for not completing and returning the survey. You may choose to skip any question that you do not want to answer. You can withdraw from the study at any time prior to mailing the survey back. For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251.

7. COSTS FOR BEING IN THE STUDY

There will be no cost to you for participating in this survey.

By completing and returning the survey you give consent to participate in this research.

If you have any questions regarding the research, please contact:

Dr. Greg Habing
Assistant Professor
Veterinary Preventive Medicine
1920 Coffey Rd
Columbus, OH 43210
(614) 292-7205
Appendix K: Diagnostic Laboratory Survey
Thank you for participating in our study. Your contribution is critical to this important study, and with it, we gladly offer authorship within our manuscript. With your help, we will calculate the prevalence of antimicrobial resistance within 4 potentially threatening zoonotic bacteria between 2012 and 2014. Our ultimate goal is to ensure that participating biomedical research institutions' primary antibiotics are effective against our bacteria in-vitro. But this is only possible with your help. Please complete this survey, and as thoroughly as possible where appropriate. Additionally and more importantly, we will need you to provide antimicrobial susceptibility test results, those associated with participating institutions, between 2012 and 2014 of *Shigella flexneri, Yersinia enterocolitica, Yersinia pseudotuberculosis*, and *Campylobacter jejuni*. This study's results will allow participating institutions to make informed decisions to maintain or change their current veterinary practices, ultimately to ensure the health of their animals, staff, and laboratory personnel. The survey should take about 10 minutes.

Note on Confidentiality: We understand the sensitivity of the data and the necessity for confidentiality. Only members working on this study will have access to the data. Your results will not be identified to your institution, whatsoever.

Again, thank you for participating.

Sincerely,

Jeffrey Kim & Greg Habing MS, DVM, PhD

Do you perform antimicrobial susceptibility tests?

- Yes
- No
How many microbiologists perform antimicrobial susceptibility tests?

What is your primary and secondary technique for measuring in-vitro antimicrobial susceptibility testing with the following pathogens (For example, broth microdilution with Sensititre)?

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Primary Technique</th>
<th>Secondary Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yersinia enterocolitica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yersinia pseudotuberculosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Your electronic record-keeping database is searchable by: (click all that apply)

- Bacterial species
- Animal species
- Clinical signs
- Client

Who enters records into your electronic database? (click all that apply)

- Secretary/Receptionist
- Microbiologists
- Interns/Volunteers
- Laboratory Manager
- Other ____________________