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Extended-spectrum cephalosporin, carbapenem, and fluoroquinolone resistant gram-negative coliform bacteria present on equine environmental surfaces

Honors Research Thesis

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ABSTRACT:

Antimicrobial resistant bacteria are a rapidly growing concern in human and veterinary medicine. The rising prevalence of extended spectrum beta-lactamase (ESBL), AmpC beta-lactamase, carbapenemase (CRE), and flouroquinolone-resistant Enterobacteriaceae continually decreases the efficiency of vital antibiotics. Moreover, antibiotic resistant enteric bacteria can be transmitted between horses and people. Our objective was to evaluate the prevalence of antibiotic resistant bacteria on human contact surfaces in equine environments. Environmental surfaces in 20 Ohio equine barns were sampled using two electro-static-cloths (Swiffer®), yielding a total of 200 samples. Samples were screened for AmpC, ESBL, CRE, and fluoroquinolone phenotypic resistance using selective media. To select for cephalosporinase phenotypes, samples were incubated at 37°C in nutrient broth with 2 μg/mL cefotaxime. This broth was aseptically inoculated to MacConkey Agar with 8 μg/mL cefoxitin, 4 μg/mL cefepime, and 1 μg/mL meropenem to detect AmpC, ESBL, and CRE phenotypes, respectively. Additionally, samples were incubated in nutrient broth containing 16 μg/mL naladixic acid and then inoculated to MacConkey agar with 16 μg/mL naladixic acid and 2 μg/mL ciprofloxacin to detect fluoroquinolone resistance phenotypes. Genotypes were confirmed using standard PCR techniques. Of the 200 sampled surfaces, 39 (19.5%), 17 (8.5%), 12 (6.0%), and 7 (3.5%) harbored cefoxitin, naladixic acid, cefepime, and ciprofloxacin resistant bacteria, respectively. Drains and wash stalls were most commonly contaminated at 18 (90.0%) of the 20 barns, followed by handles of mucking equipment at 12 (60.0%) barns. These results suggest that equine environmental surfaces are contaminated with antibiotic resistant bacteria that can potentially be transmitted between human and horse populations. Furthermore, detecting these bacteria on common human contact surfaces suggests that the environment can serve as a reservoir for antibiotic resistance genes. Identifying interventions to lower the prevalence of antibiotic resistant bacteria in equine environments will protect both animal and public health.

INTRODUCTION:

Background

Bacteria resistant to multiple important antimicrobial drugs are one of the fastest growing concerns in both human and veterinary medicine, and the bacterial genes that regulate this resistance are continually evolving and diversifying. From an epidemiological standpoint, the detection and control of antibiotic resistance is crucial in maintaining the effectiveness of antibiotics against life-threatening pathogenic bacteria. The use of antibiotics provides selection pressure for the evolution and dissemination of antimicrobial resistant bacteria in both humans and animals, including horses. Settings that contain dense populations of animals, such as horse stables or barns, also facilitate the spread of resistant bacteria between animals due to their close proximity. The environment that these animals are housed in can retain the same resistant bacteria and aid in the dissemination process by serving as a reservoir for resistant bacteria or resistance genes.[1]

Antimicrobial resistance can be classified based on the class of antibiotics to which the resistance is conferred and the type of resistance mechanism. Two classes of antibiotics, cephalosporin and fluoroquinolone, include powerful broad-spectrum antimicrobials critical to both human and veterinary medicine.[2,3] Cephalosprorins, along with penicillins, are β-lactam antibiotics that target the transpeptidation reaction of peptidoglycan synthesis of the bacterial cell wall.[4] To combat this drug, bacteria can produce β-lactamases, a group of enzymes that catalyze the β-lactam ring and cause the drugs to become ineffective.[4] The first plasmid-encoded β-lactamase that expressed resistance to third generation cephalosporins, SHV-2, was identified in 1983.[4] Due to the genes' activity towards broad-spectrum cephalosproins, the corresponding family of resistance genes were termed Extended-spectrum β-lactamases.[4] On the genetic level, some virulence-associated mobile plasmids contain bla_{CMY}, a cephalomycinase (CMY) gene that conveys resistance to first, second, and third generation cephalosporin antibiotics including cefoxitin and ceftriaxone.[5]

Bacterial resistance genes that confer heightened resistance to cefotaxime, another third generation cephalosporin, encoded by the plasmid-associated *bla*$_{CTX-M}$ β-lactamase gene are termed cefotaximases (CTX-M).[4] CTX-producing bacteria also contain heightened resistance to fourth generation cephalosporins, such as cefepime, that are a more recently introduced generation of antimicrobial.[J] The bacterial family *Enterobacteriaceae* may commonly acquire both the *bla*$_{CMY}$ and the *bla*$_{CTX-M}$ gene on mobile plasmids.[4,5] Additionally, Enrofloxacin (Baytril®) was the first fluoroquinolone introduced for use in veterinary medicine.[6] The quinolone and fluoroquinolone classes of antibiotics prevent bacterial DNA replication by inactivating two type II topoisomerase enyzmes: DNA gyrase and topoisomerase IV.[7] Resistance to these classes of antibiotics involves mutations to the *gyr*A and *gyr*B genes of DNA gyrase or the *par*C gene of topoisomerase IV.[7] Mutations to gyrB have been associated with low-level quinolone resistance whereas mutations in the gyrA gene are associated with high-level resistance.[7] The second type II topoisomerase enzyme, topoisomerase IV, consists of two subunits: ParC and ParE.[7] Mutations in both the *par*C gene, paired with *gyr*A mutations, are associated with high-level fluoroquinolone resistance.[7]

The use of antimicrobial drugs in veterinary medicine, both in prophylactic or active treatment, can select for antimicrobial resistance in animal populations.[B] Isolates from cattle, poultry, swine, and retail meats were obtained from 5 states from 1998-2000 and produced 54 *Escherechia coli* and 21 *Salmonella enterica* with the *bla*$_{CMY}$ gene.[5] Additionally, *bla*$_{CMY}$ genes could undergo conjugation and transfer between bacterial species[5], such as from commensal *Escherichia coli* to pathogenic *Salmonella*. Furthermore, direct antibiotic use was not correlated with the presence of the *bla*$_{CMY}$ gene, as some isolates containing the resistance gene were recovered from animals that had no previous history of cephamycin or extended-spectrum cephalosporin use.[5] In 2011, 1000 purchased packages of retail beef and pork from grocery stores located in two US states harbored 81 (8.1%) and 5 (0.5%) *E. coli* and *Salmonella* isolates encoding *bla*$_{CMY}$ genes, respectively.[8] Another study in 2011 associated ceftiofur use, a third generation of cephalosporin,

in swine finishing barns with increased incidence of ceftriaxone resistance and the presence of *bla*$_{CMY}$ genes.[9] Conversely, another study in 2011, *bla*$_{CMY}$ genes were recovered from 79% of the fecal samples (n=1,495) recovered from swine finishing barns in Ohio, Kansas, Illinois, Michigan, and Minnesota.[10] Regarding *bla*$_{CTX}$, 24 (1.6%) of these swine fecal samples harbored the gene, although only in Ohio, Michigan, and Illinois.[10] In dairy cattle in Ohio, 747 fecal samples were obtained from 25 dairy farms and produced 711 (94.8%) isolates harboring the *bla*$_{CMY}$ gene from all 25 herds and 70 (9.4%) isolates harboring the *bla*$_{CTX}$ gene from 5 herds.[11] From a pathogen standpoint, 12 (0.6%) of 2,034 clinical *Salmonella* isolates in US livestock populations collected from 2010-2011 harbored the *bla*$_{CTX}$ gene on a mobile plasmids.[12] ESBL-producing *Salmonella* were also identified in diagnostic submissions from horses located in Texas.[12] All five equine-associated isolates likely originated from a common source because of their same state of origin and close genetic makeup (>97%).[12]

Antibiotic resistant *E. coli* have been found in equine fecal samples from clinical settings in multiple studies.[13,14,15] In one study, 450 of 650 fecal samples taken from hospitalized horses contained bacteria that were resistant to one or more antibiotics.[16] In one clinical study, 60% of the bacterial isolates from critically ill foals were resistant to extended spectrum cephalosporins.[17] Further, ESBLs were present in the fecal samples of 55 of the 103 horses collected from in a referral equine hospital in England.[15] Multi-drug resistant *Salmonella* were also responsible for the temporary closing of a large animal referral hospital, during which time the case fatality rate was 31.5%.[18] Furthermore, antibiotic resistance genes have been observed in the intestinal flora of healthy horses located in communal facilities in addition to sick horses in hospitals.[19] From a single horseback riding center in the Czech Republic, the feces of 4 (9.0%) of the horses contained ESBL-producing *E. coli*.[20]

Because of increasing rates of antibiotic resistance, carbapenem antibiotics have become one of the last truly effective classes of antibiotics, but carbapenem-resistant Enterobacteriaceae

(CRE) are now becoming an emerging threat to modern medicine.[21] In addition to their use in human medicine, carbapenems are sometimes used to treat equine bacterial infections that show resistance to most other antibiotics.[22] Little to no information is known about the prevalence of CREs in horses or their environments, although carbapenemase-resistant *Acinetobacter* were isolated from the feces of two hospitalized horses in Belgium.[23] There is also limited information on equine commensal bacteria that are resistant to fluoroquinolone antimicrobials, although one study found fluoroquinolone resistant bacteria in hospitalized horses in southeastern England.[14] Fluoroquinolones and cephalosporins are commonly used in equine medicine to treat bacterial meningitis because of the drugs' ability to penetrate the blood brain barrier.[24] Therefore, antibiotic resistance can cause severe complications in treating clinical cases of bacterial infection. Although extensive research has recorded the prevalence of antibiotic resistant bacteria in horses,[13,14,15,16] how the environment of equine centers facilitates the spread of antibiotic resistance is unknown.

Justification

Our objective was to estimate the prevalence of gram-negative coliform bacteria resistant to extended-spectrum cephalosporins, flouroquinolones, and carbapenems on surfaces in equine environments. Third and fourth generation cephalosporins are considered critically important antimicrobials according to the World Health Organization.[25] The corresponding cephalosporinase-producing genes are commonly present in fecal flora of animal populations, according to similar studies.[1,14,15,16] Therefore, a greater prevalence of cephalosporin resistance was expected compared to carbapenem or flouroquinolone resistance on equine environmental surfaces. On the other hand, the percentage of cephalosporin resistance found on equine environmental surfaces was expected to be lower than that found in equine fecal samples because coliform bacteria proliferate more readily in the gut of animals compared to the exterior environment.

Horse owners, riders, and trainers typically follow standard hygienic procedures after handling, such as washing hands, tack, and equipment. This lowers the chance of a person introducing foreign bacteria into their body. However, the same safety precautions may not be observed after using equipment or interacting with the environment, such as opening doors or stalls. These surfaces could be contaminated with the same antibiotic resistant microbes present in the intestinal flora of horses and should be studied to determine the prevalence of antibiotic resistance in the environment. Such environments, therefore, could act as a reservoir for the transmission for various resistance genes.

The potential zoonotic transmission of antibiotic resistant bacteria, or the ability to spread between humans and animals, contributes to the challenge in medicine of maintaining the effectiveness of antibiotics against microbes.[25] For this reason, the environmental contamination and spread of antimicrobial resistant bacteria is a concern for animal and public health and should be evaluated. This is especially true because the antibiotics that this study utilizes are used in both human and animal medicine[25], suggesting that the resistance genes would be effective in both populations. Identifying which animal environments are more prone to harboring antibiotic resistance will aid in creating more effective measures taken to stop the spread of antibiotic resistant bacteria amongst individual animals or across species.

Bacteria can pass resistance genes to other species of bacteria via horizontal gene transfer on mobile plasmids.[5] Commensal bacteria, even being non-pathogenic, can harbor resistance genes and thus act as a resistance reservoir for other bacteria including human pathogens, such as *Salmonella*.[5] People can share intestinal flora with companion animals, such as horses, and thus acquire these resistant bacteria by a direct or indirect contact with horses or their environment. Knowing the extent of antibiotic resistance present in equine environments will allow prompt steps to be taken to prevent further dissemination of resistance genes among horses or across species to infect humans. For instance, a study in Greece estimated a 35% mortality rate of human patients

with bacterial infections attributed to resistant cases,[26] which was higher than expected in patients with susceptible infections. Such evidence supports the fact that resistance genes are dangerous in human populations and bacterial transfer could possibly cause an antibiotic resistant infection. Pet owners may be more likely to pick up resistant bacteria shed by their companion animals due to regular interaction between the two species, thus increasing the risk of a resistant infection.

METHODS:

Sampling was conducted at privately owned horse facilities including boarding and riding stables. Samples were collected from a total of 20 equine facilities, with 10 environmental surfaces sampled from each facility, providing a total of 200 samples. This sample size was estimated based on an expected population size of 50-100 farms in the region of central Ohio and the ability to detect an estimated 10-30% antibiotic resistance prevalence rate with a 5-10% confidence interval.[27] Samples were obtained from 10 various flat surfaces at each farm, including stalls, tack rooms, halters & lead ropes, feed rooms, storage rooms, pen gates, drains, cross ties &/or tie posts, mucking equipment, and water spigots. When sampling, only the areas most commonly touched by people were selected (ex: the handles of a mucking rake). The environment was sampled using electro-static-cloths (Swiffer®) following previously validated environmental sampling procedures[28]. Two samples were collected simultaneously from a single surface, one used to screen for AmpC, ESBL, and carbapenemase producing bacteria and the second used to screen for fluoroquinolone resistant bacteria. One sample from each surface was enriched in a nutrient broth containing 2 μg/mL cefotaxime. After incubation, this broth was inoculated onto MacConkey Agar with 8 μg/mL of cefoxitin, 4 μg/mL of cefepime, and 1 μg/mL of meropenem, in order to identify AmpC, ESBL and CRE phenotypes. The other sample from each surface was enriched in a nutrient broth containing 16 μg/mL of naladixic acid. After incubation, the nutrient broth was inoculated onto MacConkey Agar with 16 μg/mL of naladixic acid and 2 μg/mL ciprofloxacin. In order to

identify genotypes, isolates with the appropriate resistance phenotypes underwent genotyping using standard PCR techniques and gene sequencing.

RESULTS:

Of the 200 environmental samples collected from August to December, 2016, coliform isolates resistant phenotypes to all antibiotics were identified except for resistance to meropenem. (Table 1) The antibiotic that produced the most resistant isolates at 39 (19.5%) was cefoxitin. Naladixic acid produced the second-most resistant isolates at 17 (8.5%) isolates. Cefepime and ciprofloxacin followed at 12 (6.0%) and 7 (3.5%) isolates, respectively. More isolates were resistant to cefoxitin and naladixic acid than to their newer counterparts, cefepime and ciprofloxacin. Because cefoxitin has been in circulation longer than cefepime, the corresponding ESBL gene, bla_{CMY}, has disseminated more among animal populations. The prevalence of resistance to cefoxitin compared to cefepime in this study supports this deduction. The same inference applies when comparing a quinolone such as naladixic acid to a fluoroquinolone such as ciprofloxacin. Again, the corresponding number of resistant isolates identified in this study support the same hypothesis, although to less of a degree.

With the data separated by the 10 sampled surfaces from each individual barn, trends in antibiotic resistance contamination were observed. (Table 2) Due to the limit of 10 surfaces sampled at each facility, a maximum of 10 isolates could have been cultured from each barn as resistant to a specific antibiotic. The 75 total resistant isolates collected were represented over 14 (70.0%) of the 20 barns. Thus, 6 barns expressed no bacterial isolates resistant to any of the four tested antibiotics. Some barns, such as Barns 4 and 14, contained a relatively large proportion of resistant isolates. When divided by environmental sample, the more commonly contaminated surfaces were identified. (Table 3) Based on the sampling procedure, the maximum number of isolates that could have been recovered from each surface was 20 due to 20 barns being included in

the sample. The most frequently contaminated surfaces included drains, mucking equipment, and stalls. Surfaces least contaminated were feed rooms, cross-ties and/or tie posts, and pen gates. Every environmental surface sample produced at least one resistant isolate over the 20 barns sampled. Upon genotyping the isolates expressing resistance to cefepime, a fourth generation cephalosporin, the specific gene within the Group 1 class of the $bla_{\text{CTX-M}}$ gene could be identified. All 15 cefepime resistant isolates contained the bla_{CTX} gene, with 2 (13.3%) of those isolates harboring $bla_{\text{CTX-M-15}}$ and 13 (86.7%) harboring $bla_{\text{CTX-M-1}}$.

DISCUSSION:

The use of cephalosporin and fluoroquinolone antibiotics in equine medicine occurs when treating patients with significant infections, especially respiratory tract infections, or when administering prophylactic treatment prior to surgery.[25] Such antibiotic use selects for corresponding resistance genes in intestinal bacteria.[13] In horses, ceftiofur – a third-generation cephalosporin – can be used to treat difficult bacterial infections, mainly of the respiratory tract.[2,13] Because both bla_{CMY} and $bla_{\text{CTX-M}}$ ESBL genes confer resistance to third-generation cephalosporins, the use of ceftiofur in equine populations increases the propagation of both genes and their subsequent resistance profiles. Although an antibiotic such as ceftiofur is often administered in a hospital setting, previous studies have shown that horses are capable of shedding resistant bacteria upon returning to their barns after hospitalization.[14,15] Our identification of both cefoxitin and cefepime resistance in non-medical equine facilities (Table 1) supports this analysis. These data suggest that surfaces regularly contaminated with fecal matter (drains, mucking equipment, and stalls) harbor antibiotic resistant bacteria that can be dispersed to other areas of the facility. However, 188 (94.0%) surface samples lacked resistance to cefepime and 161 (80.5%) of surface samples lacked resistance to cefoxitin. Thus, if bacterial isolates carrying $bla_{\text{CTX-M}}$ or bla_{CMY} are present on more surfaces in equine barns or in a larger prevalence, they were either present in such

small amounts as to not be detected by the methods of our study, or they did not survive or proliferate efficiently outside of the gastrointestinal tract of the animals. The 15 recovered cefepime resistant bacteria underwent further genotypic characterization and identified a mix of $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ β-lactamase gene with $bla_{\text{CTX-M-1}}$ being more frequent. In the US, the $bla_{\text{CTX-M-1}}$ gene has previously been isolated from livestock species whereas the $bla_{\text{CTX-M-15}}$ gene has been identified from human and companion animal samples, as well as the environment.[29] Due to the mix of $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ genes, whether the isolates originated from livestock or human sources could not be conclusively determined, although $bla_{\text{CTX-M-1}}$ appears more common.

More surfaces remained uncontaminated by quinolone and fluoroquinolone resistant bacteria: 183 surfaces at 91.5% and 193 surfaces at 96.5% respectively (Table 1). The lower prevalence of quinolone and fluoroquinolone resistant bacteria compared to ESBL-producing bacteria is likely due to the limited use of fluoroquinolone antibiotics in equine medicine.[6] The selection pressure by one antibiotic on resistance genes could also select for unrelated resistance genes if both genes are housed on the same conjugative plasmid. Therefore, the use of ceftiofur or another antibiotic used in equine medicine could translate to the proliferation of quinolone or fluoroquinolone resistance in this way. It should be noted that the methodology of this study heavily selects for any resistant bacteria present in a sample. For that reason, we are unable to determine the amount of resistant bacteria on individual surfaces; we are only able to state if there is resistance present. In addition, it is not known at what threshold abundance resistant bacteria reach a critical level that imposes a health risk to the horses or their handlers.

The absence of resistant bacteria in 6 of the 20 barns (Table 2) would suggest that bacteria resistant to antibiotics are not of a significant clinical or epidemiological concern in general equine facilities in central Ohio. Of all of the resistant isolates identified, 23 (30.7%) were located in 2 of the 20 barns, likely due to clonal dissemination of a single resistant bacterium throughout the facility. More specifically, barns with a large number of isolates could contain bacterial clones

originating from a single host source that were then dispersed over various surfaces throughout the facility due to the dispersion of fecal matter.

A single resistant bacterium in a barn may seem insignificant, but the resistance genes contained within it could potentially be transferred to pathogenic bacteria present in the same environment. Furthermore, if commensal bacteria also receive the resistance genes via conjugation, their proliferation in the hindgut further proliferates that gene in the microflora population. Thus, should an opportunistic pathogen arise, a dangerous antibiotic resistant infection may arise. Such was the case when a large veterinary teaching hospital was closed for an extended duration due to an outbreak of MDR Salmonella.[18] The bacteria identified in this study, being primarily commensal, are shed from the gastrointestinal tract via fecal matter. A facility that houses horses, a species with a large daily output of fecal material, is difficult to maintain in terms of sanitation. As such, horses that harbor antibiotic resistant bacteria may spread the bacteria throughout the barn as their fecal matter is spread to equipment, surfaces, people, and/or other animals.

Another epidemiological issue is the transfer of resistant bacteria from animal to animal or from animal to human. Areas such as the feed room, storage room, or tack room were contaminated with resistant microorganisms (Table 3), and because horses are not usually allowed in such areas, the bacteria was likely transferred there via humans as mechanical vectors. Many of the surfaces sampled (stalls, halters & lead ropes, pen gates, etc.) are a common surface shared by both a human and a horse. This presents an opportunity for fecal matter to be deposited by the horse and then transferred by the human. However, it is also possible that the resistant bacteria originated from the human population of a barn and then transferred to the equine population. Of the farms sampled, other livestock animal species such as chickens, cattle, and goats within close proximity to the horses were observed at 4 facilities whereas companion animal species, such as barn cats or dogs, were observed at all 20 farms. Trailers, cleaning equipment, and feeding locations are often shared between different species located on the same farm, and many of the animals share the

same pasture or facility areas. As such, the resistance genes could also have been picked up from equipment used for other species located at the same facility. Upon sequencing the cefepime resistant isolates to identify the $bla_{\text{CTX-M}}$ gene present in each bacterium, a mix of $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ carrying isolates were found (Table 4). Microorganisms with $bla_{\text{CTX-M-1}}$ have been found in bacteria isolated from livestock species whereas those with $bla_{\text{CTX-M-15}}$ have been found in bacteria isolated from humans. Although $bla_{\text{CTX-M-1}}$ was identified in more of the isolates compared to $bla_{\text{CTX-M-15}}$ in this study, no conclusive inference could be made as to whether the cefepime resistant isolates originated from human, equine, or other animal sources.

These results suggest that equine environmental surfaces are commonly contaminated with resistant bacteria that can potentially be transmitted between human and horse populations, although the exact transmission pattern is unknown. Identifying resistant bacteria on communal human contact surfaces suggests that the equine environment can serve as a reservoir for dangerous antibiotic resistance genes. Recognizing interventions to lower the prevalence of antibiotic resistant bacteria in equine environments will protect both animal and public health in the future. In order to determine the measurable severity of antibiotic resistance in equine populations, further studies will be necessary to quantify the amount of resistant bacteria present in the horses themselves or their environments.

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Table 1: Total number and proportion of isolates resistant to specific antibiotics from 200 environmental surfaces located on 20 privately owned equine barns in central Ohio.

Antibiotic	**No. of Positive Isolates**	**Proportion (n=200)**
Cefoxitin	39	19.5%
Cefepime	12	6.0%
Meropenem	0	0.0%
Naladixic Acid	17	8.5%
Ciprofloxacin	7	3.5%

Table 2: Number (proportion) of Gram-negative coliform bacteria resistant to cephalosporin, quinolone, and fluoroquinolone antibiotics recovered from environmental surfaces at 20 private equine barns in central Ohio.

Barn	Cefoxitin	Cefepime	Naladixic Acid	Ciprofloxacin
1	3 (0.3)	0	0	0
2	4 (0.4)	0	0	0
3	2 (0.2)	3 (0.3)	2 (0.2)	0
4	4 (0.4)	0	5 (0.5)	1 (0.1)
5	3 (0.3)	0	0	0
6	0	0	0	0
7	1 (0.1)	2 (0.2)	0	0
8	4 (0.4)	0	1 (0.1)	1 (0.1)
9	3 (0.3)	1 (0.1)	1 (0.1)	0
10	3 (0.3)	2 (0.2)	1 (0.1)	0
11	0	0	0	0
12	1 (0.1)	2 (0.2)	0	0
13	1 (0.1)	0	0	0
14	2 (0.2)	2 (0.2)	5 (0.5)	4 (0.4)
15	0	0	0	0
16	3 (0.3)	0	2 (0.2)	1 (0.1)
17	0	0	0	0
18	0	0	0	0
19	5 (0.5)	0	0	0
20	0	0	0	0

Table 3: Number (proportion) of Gram-negative coliform bacteria resistant to cephalosporin, quinolone, and fluoroquinolone antibiotics recovered from 10 environmental surfaces at each of 20 privately-owned equine barns in central Ohio.

Surface	Cefoxitin	Cefepime	Naladixic Acid	Ciprofloxacin
1. Stall	5 (0.25)	1 (0.05)	4 (0.20)	1 (0.5)
2. Tack room	3 (0.15)	0	1 (0.05)	0
3. Halters & lead ropes	5 (0.25)	1 (0.05)	3 (0.15)	1 (0.05)
4. Feed room	2 (0.10)	0	0	0
5. Storage room	5 (0.25)	2 (0.10)	1 (0.05)	1 (0.05)
6. Pen gates	1 (0.05)	0	0	0
7. Drains	11 (0.55)	2 (0.10)	4 (0.20)	1 (0.05)
8. Cross-ties &/or tie post	1 (0.05)	1 (0.05)	0	0
9. Mucking equipment	5 (0.25)	3 (0.15)	2 (0.10)	2 (0.10)
10. Water Spigot	1 (0.50)	2 (0.10)	2 (0.10)	1 (0.05)

Table 4: Sequencing results of 15 unique CTX Group 1 positive *E. coli* isolated from environmental surfaces at 6 privately owned equine barns located in central Ohio.

Isolate No.	Barn No.	Surface Source	CTX-M-Group
1	3	Storage room A*	CTX-M-1
2	3	Storage room B*	CTX-M-1
3	3	Drains A*	CTX-M-1
4	3	Drains B*	CTX-M-1
5	3	Drains C*	CTX-M-1
6	3	Mucking equipment	CTX-M-1
7	7	Drains	CTX-M-1
8	7	Mucking equipment	CTX-M-1
9	9	Storage room	CTX-M-1
10	10	Mucking equipment	CTX-M-1
11	10	Water spigot	CTX-M-1
12	12	Stall	CTX-M-1
13	12	Cross ties	CTX-M-1
14	14	Halters & lead ropes	CTX-M-15
15	14	Water spigot	CTX-M-15

*Colonies with different morphologies on MacConkey agar that were isolated from the same surface sample.