Prevalence of antimicrobial resistance and association with toxin genes in *Clostridium difficile* in commercial swine

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Objective—To estimate prevalence and determine association between antimicrobial resistance and toxin gene profile of *Clostridium difficile* in commercial pigs at the preharvest food-safety level.

Animals—68 sows and 251 young pigs from 5 farms in North Carolina and 3 in Ohio.

Procedures—Fecal samples were collected from sows (8/farm) and matched young pigs (32/farm) at farrowing. Cohorts were sampled again at nursery and finishing stages. *Clostridium difficile* isolates were tested for susceptibility to 6 antimicrobials. A PCR assay was used to detect genes coding for enterotoxin A (*tcdA*), cytotoxin B (*tcdB*), and binary toxin (*cdtB*).

Results—*C difficile* prevalence in young pigs at farrowing was 73% (n = 183) with significantly higher prevalence in Ohio (87.5%) than North Carolina (64%). *Clostridium difficile* was isolated from 32 (47%) sows with no significant difference between the 2 regions. A single pig had a positive test result at the nursery, and no isolate was recovered at the finishing farms. Resistance to ciprofloxacin was predominant in young pigs (91.3% of isolates) and sows (94%). The antimicrobial resistance profile ciprofloxacin-erythromycin-tetracycline was detected in 21.4% and 11.7% of isolates from young pigs and sows, respectively. Most isolates had positive results for *tcdA* (65%), *tcdB* (84%), and the binary toxin *cdtB* (77%) genes. Erythromycin resistance and tetracycline resistance were significantly associated with toxin gene profiles.

Conclusions and Clinical Relevance—The common occurrence of antimicrobial-resistant *C difficile* and the significant association of toxigenic strains with antimicrobial resistance could contribute to high morbidity in farms with farrowing pigs. (*Am J Vet Res* 2010;71: xxx–xxx)

Clostridium difficile is a common nosocomial infection and has been known for many decades to cause CDAD in patients. A recent increase in the deaths among hospital patients has been attributed to the pathogenic strain NAP1/027, which is hypervirulent and has resistance to fluoroquinolones.[1–4] *Clostridium difficile* is also an important pathogen in food animals and is responsible for causing colitis in neonatal pigs, enterocolitis in foals, typhocolitis in adult horses, and enteritis in calves.[5–8] Our understanding of the epidemiologic and microbiological features of *C difficile* in humans has tremendously improved over the past 2 decades. It has recently been suggested that pigs and other food animals might serve as a source of the pathogen for humans and cause community-acquired CDAD, with identical ribotypes and toxinotypes detected in food animals and humans.[2,9–14] It is also possible that humans are responsible for pathogen transmission to pigs; however, this has not been reported. Few studies have been conducted to determine the epidemiologic features and potential importance of toxigenic and antimicrobial-resistant strains of *C difficile* in pigs.

Abbreviations

CDAD	*Clostridium difficile*–associated diarrhea
CI	Confidence interval
MIC	Minimum inhibitory concentration
MIC_{50}	Minimum inhibitory concentration of 50% of isolates
MIC_{90}	Minimum inhibitory concentration of 90% of isolates
OR	Odds ratio

In pigs, *C difficile* is an important cause of neonatal enteritis, particularly from 1 to 7 days of age in pigs that develop CDAD characterized by colonic edema.[7,15] Loss of productivity is common, and affected pigs typically weigh 10% to 15% less at weaning than those in unaffected litters.[2] An important concern of swine pro-

Received May 18, 2009.
Accepted September 15, 2009.
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duction medicine is the development of resistance in pathogens against important classes of antimicrobials. This is particularly important in toxigenic *C difficile* strains that can damage the intestines and prolong treatment, leading to high morbidity and mortality rates. Although previous studies[2,6,7,11,15] have reported *C difficile* in swine, no study has been conducted to determine and compare its prevalence in the same pigs sampled at 3 stages of production and from 2 distinct geographic regions in the United States. In addition, to our knowledge, no reports concerning a statistical association between antimicrobial resistance and the virulence profile in the *C difficile* population have been published. Prompted by the lack of such data, the main objective of the study reported here was to determine the prevalence of *C difficile* in pigs at the preharvest food-safety level. We also aimed to assess the occurrence of MDR phenotypes and determine the statistical association between toxin genes and antimicrobial resistance of *C difficile* in commercial pigs at different stages of production in the United States.

Materials and Methods

Sample collection and bacterial isolation—Pig fecal samples were collected from 8 farms, including 5 from North Carolina and 3 from Ohio. Thirty-two young pigs (1 to 7 days old) and 8 sows were sampled at every farm. Fecal samples were collected from 4 healthy young pigs/sow if available. The probability of a diseased young pig surviving to the time of slaughter is low; therefore, healthy-appearing young pigs were selected to determine the profile of *C difficile* in each pig as it moved from farrowing, nursery, and finishing farms to slaughter. Every pig was sampled 3 times; the first sample was taken at the farrowing unit from 8 to 10 days of birth, with subsequent sampling at nursery and finishing farms. The pigs were part of an all-in and all-out production flow and therefore were ear tagged for identification and subsequent sample collections during each production phase. Samples were randomly collected from more than 8 sows and associated young pigs at a few farms. This was done primarily to avoid a decrease in sample size because of pig deaths at the nursery and finishing farms, and these 4 pigs were included in the analysis only when used to replace missing samples caused by pig deaths. Fecal samples from the young pigs in the farrowing barns were collected with sterile loops. All samples from nursery and finishing-age pigs and sows in the farrowing barns were collected with gloved hands directly from the rectum and transported to the laboratory in a sterilized cup at 4°C. Pathogen isolation for the entire study was conducted in North Carolina, and samples originating in Ohio were shipped overnight under refrigerated conditions. The study was reviewed and approved by the North Carolina State University Institutional Animal Care and Use Committee.

Clostridium difficile was isolated by transferring 2 g of fecal sample in 10 mL of *C difficile* broth consisting of proteose peptone (4%), disodium hydrogen phosphate (0.5%), potassium dihydrogen phosphate (0.1%), magnesium sulfate (0.01%), sodium chloride (0.2%), and fructose (0.6%). Cycloserine, 0.1% sodium taurocholate, and cefoxitin were added and tubes were incubated under anaerobic conditions at 35°C for 7 days. Following incubation, the culture broths were centrifuged (7,800 × g for 5 minutes), treated with 96% ethanol for 50 minutes to select for spores, and plated on cycloserine cefoxitin fructose agar[a] with *C difficile* selective supplement[b] supplemented with 7% laked horse blood.[c] The presumptive *C difficile* isolates were biochemically tested via detection of L-proline aminopeptidase activity on discs.[d] The identity of the isolates was confirmed via PCR amplification of the specific marker housekeeping gene, *tpi*, which encodes for triose phosphate isomerase.[16]

Antimicrobial susceptibility testing—The MIC was determined for a panel of 6 antimicrobials by use of strips with exponential gradients of antimicrobial concentrations, which correspond to specific MIC dilutions.[e] Susceptibility testing was conducted on Muller Hinton plates supplemented with 5% sheep blood[f] following the manufacturer's instructions.[e] The antimicrobials tested, abbreviations, dilution range, and number of breakpoints used included ampicillin (Amp; dilution range, 0.016 to 256 µg/mL; 2 breakpoints), ciprofloxacin (Cip; dilution range, 0.002 to 32 µg/mL; 8 breakpoints), erythromycin (Ery; dilution range, 0.016 to 256 µg/mL; 2 breakpoints), metronidazole (Met; dilution range, 0.016 to 256 µg/mL; 16 breakpoints), tetracycline (Tet; dilution range, 0.016 to 256 µg/mL; 4 breakpoints), and vancomycin (Van; dilution range, 0.016 to 256 µg/mL; 4 breakpoints). Superscript letter R was used with an abbreviation to indicate resistance to that drug (eg, Van^R). The breakpoint used for the fluoroquinolone ciprofloxacin was 8 µg/mL.[17] The breakpoint values used in a previous study[18] were used for the remaining antimicrobials. The MIC_{50} and MIC_{90} were calculated.

Toxin gene detection—The DNA was extracted from the *C difficile* colonies by a resin-based DNA extraction kit following manufacturer's instructions.[g] Amplification of the housekeeping gene *tpi* and the toxin genes including *tcd*A, *tcdB*, and *cdt*B coding for TcdA (toxin A), TcdB (toxin B), and CDT (binary) toxins, respectively, was done by use of specific primers as described.[16,19] The PCR running conditions included an initial denaturing step at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 54°C for 1 minute, and an extension-elongation step of 72°C for 1 minute. This was followed by a final extension step at 72°C for 7 minutes. Amplified products were run on 2% agarose gel containing ethidium bromide in 0.5× Tris-acetate EDTA buffer.[h]

Statistical analysis—The frequency of antimicrobial resistance profiles and comparison of MIC values between isolates from different production phases and region were evaluated by use of the χ^2 test and Fisher exact 2-tailed test, when applicable, by use of a statistical software package.[i] Significant association of antimicrobial resistance and virulence gene profiles for the *C difficile* isolates was determined by use of the OR test with 95% CIs by use of commercially available software.[j] Values of $P \leq 0.05$ were considered significant.

Results

***C difficile* prevalence**—Two hundred fifty-one young pigs and 68 sows were sampled from 5 farms in North Carolina (155 young pigs and 44 sows) and 3 farms in Ohio (96 young pigs and 24 sows). In North Carolina, 13 sows only had 3 young pigs/sow available for sampling. In addition, we could not locate 8 pigs representing 2 finishing farms in North Carolina during sampling. It was assumed that these pigs died during their stay at the nursery farm or finishing farm or during transport. The overall *C difficile* prevalence in young pigs was 73% (n = 183) with significantly ($P < 0.001$) higher prevalence in Ohio (87.5% [n = 84]) than in North Carolina (64% [99]). The overall *C difficile* prevalence in sows was 47% (n = 32) with no significant geographic difference between North Carolina (50%) and Ohio (41.7%). All the *C difficile*–positive pigs were detected at the farrowing stage, except a single nursery-age pig from North Carolina. No deaths were observed when pigs were sampled at the nursery farms, and none of the pigs tested positive at the finishing farms.

Antimicrobial susceptibility—*Clostridium difficile* isolates had resistance to 4 of the 6 antimicrobials tested (**Table 1**). Overall, the frequency of Cip^R was significantly greater, compared with other antimicrobials, irrespective of the source or the region, with 91.3% frequency among isolates from young pigs and 94% from sows. All the *C difficile* isolates in this study were susceptible to metronidazole (MIC_{50}-to-MIC_{90} range, 0.13 to 0.25 µg/mL) and vancomycin (MIC_{50}-to-MIC_{90} range, 0.5 to > 0.5 µg/mL), 2 drugs of choice for treatment of *C difficile* in human medicine. Only 6 isolates, including 5 from young pigs (Ohio, n = 4; North Carolina, 1) and a single isolate from a sow (North Carolina), were susceptible to all drugs tested in the study. Resistance to ampicillin was detected in 2.7% (n = 5) of isolates from young pigs only. The single isolate from the nursery farm was susceptible to all the antimicrobials except ciprofloxacin. Antimicrobial resistance to tetracycline was detected in isolates from sows (31.3%) and young pigs (46%) and to erythromycin in sows (34.4%) and young pigs (38.3%).

A significantly ($P < 0.001$) higher number of isolates from young pigs (66.7%) and sows (90%) in Ohio had resistance to the macrolide, erythromycin, a class of antimicrobial commonly used in swine production systems, than in North Carolina (14% in young pigs and 9% in sows). The MIC_{90} for the *C difficile* isolates from both sources to erythromycin was > 256 µg/mL (the highest concentration tested). The MIC_{90} to tetracycline varied from 64 µg/mL in isolates from sows to 16 µg/mL in isolates from young pigs. The MIC_{50} and $MIC_{/90}$ for isolates to ciprofloxacin were > 32 µg/mL for isolates from the 2 sources.

Phenotyping based on antimicrobial resistance profile—Nine distinct antimicrobial resistance profiles were detected. Ciprofloxacin resistance was represented in 5 profiles. The antimicrobial resistance profile Cip^R-Ery^R-Tet^R was the predominant pattern and was represented by 19.5% (n = 42) of the isolates. Specific resistance phenotypes were found to be associated with the region of sample collection. For example, the Cip^R-Tet^R pattern was found in isolates from young pigs (n = 6) and sows (34) in North Carolina, but none of the isolates from Ohio had this pattern. This clear distinction of resistance phenotypes in the 2 geographic regions was also observed for the antimicrobial resistance profile Cip^R-Ery^R-Tet^R, with significantly ($P < 0.001$) higher frequency among young pigs (35.7%) and sows (40%) in Ohio. Eleven young pigs from 5 sows had matching antimicrobial resistance profiles.

Toxin gene profile—The PCR testing of the 215 *C difficile* isolates for the presence of important toxin genes revealed that toxigenic strains commonly occurred among the isolates. Genes for toxins A (65% of isolates) and B (84%) and the binary toxin coding genes (77%) were detected. Further analysis revealed 4 toxin gene profile combinations of the 3 toxin encoding genes *tcdA*, *tcdB*, and *cdtB* (**Table 2**). The predominant toxin gene profile coding for $A^+B^+CDT^+$ was found in 59% (n = 127) of the isolates. Forty-two (19.5%) isolates had the $A^-B^+CDT^+$ profile, whereas 33 (15.3%) isolates tested negative for all toxins tested. A significantly ($P < 0.001$) higher number of *C difficile* isolates from young pigs in Ohio farms (n = 28) had that profile. Twenty-two young pigs representing 13 sows had matching toxin profiles. The pansusceptible *C difficile* isolate from a sow and a single young pig from North Carolina had the $A^-B^+CDT^+$ toxin profile. The remaining 4 pansusceptible isolates from young pigs in Ohio had the $A^+B^+CDT^+$ profile.

Table 1—Frequency of antimicrobial resistance at different MICs in *Clostridium difficile* isolated from 183 young pigs and 32 sows.

Antimicrobial (dilution range [µg/mL])	Breakpoint (µg/mL)	Source	MIC range (µg/mL)	MIC_{90} (µg/mL)	Resistance	No. (%)*
Ampicillin (0.016–256)	2	Sow	0.032–1.5	0.5	0.75	0
		Young	0.0016–2	0.75	1	5 (2.7)
Ciprofloxacin (0.002–32)	8	Sow	0.38–> 32	> 32	> 32	30 (94)
		Young	0.047–> 32	> 32	> 32	167 (91.3)
Erythromycin (0.016–256)	2	Sow	0.047–> 256	0.75	> 256	11 (34.4)
		Young	0.032–> 256	1	> 256	70 (38.3)
Metronidazole (0.016–256)	16	Sow	0.016–0.38	0.13	0.47	0
		Young	0.016–2	0.13	0.25	0
Tetracycline (0.016–256)	4	Sow	0.023–64	2	64	10 (31.3)
		Young	0.016–48	4	16	84 (46)
Vancomycin (0.016–256)	4	Sow	0.19–1	0.5	0.75	0
		Young	0.13–2	0.5	0.75	0

*Indicates number (%) of *C difficile* isolates with resistance to an antimicrobial.

Table 2—Toxin gene profiles (number [%] or proportion) in *C difficile* isolated from the same pigs as in Table 1.

Source	Region	$A^+B^+CDT^+$	$A^-B^+CDT^+$	$A^-B^-CDT^-$	$A^+B^+CDT^-$
Young	North Carolina	59 (59.6)	25 (25.3)	2 (2)	10 (10)
	Ohio	46 (54.8)	7 (8.3)	28 (33.3)	3 (3.5)
Sow	North Carolina	16 (72.7)	5 (22.7)	0	0
	Ohio	6/10	1/10	3/10	0
Total		**127 (59)**	**42 (19.5)**	**33 (15.3)**	**13 (6)**

A = Toxin encoded by gene TcdA. B = Toxin encoded by gene TcdB. C = Toxin encoded by gene CDT.

Two isolates from young pigs and 1 from a sow in North Carolina had the $A^-B^+CDT^-$ toxin profile; 1 isolate from a young pig in North Carolina had the $A^-B^-CDT^+$ profile.

Table 3—Distribution (number [%] or proportion) of *C difficile* isolates with antimicrobial resistance to tetracycline or erythromycin by virulence genes, source, and geographic region.

Source	Region	Antimicrobial	*tcdB*	*cdtB*
Young pigs	North Carolina	Tet 50 (50.5)	47 (94)	39 (78)
	(n = 99)	Ery 14 (14)	13/14	2/14
	Ohio	Tet 34 (40.5)	6 (17.6)	6 (17.6)
	(n = 84)	Ery 56 (66.7)	29 (51.7)	29 (51.7)
Sows	North Carolina	Tet 6 (27.3)	6/6	6/6
	(n = 22)	Ery 2 (9)	2/2	2/2
	Ohio	Tet 4/10	1/4	1/4
	(n = 10)	Ery 9/10	6/9	6/9

Tet = Tetracycline. Ery = Erythromycin.

Association between antimicrobial resistance and virulence gene profile—The association between antimicrobial resistance (Tet^R and Ery^R) and virulence determinants (*tcdB* and *cdtB*) in *C difficile* isolates recovered from young pigs was determined (**Table 3**). This association was tested at 2 levels—the source and geographic location of the sample. The low number of isolates recovered from sows precluded testing this association in sows. A strong association was detected between Tet^R *C difficile* isolated from young pigs with the virulence genes *tcdB* (OR, 9) and *cdtB* (OR, 4). A strong association was detected between Ery^R isolates from young pigs and the *tcdB* genes (OR, 3). However, no significant association was detected between Ery^R and the *cdtB* toxin gene (OR, 1.7; 95% CI, 0.7 to 4.2) for the 2 sources. When isolates were stratified by geographic region, North Carolina isolates had a stronger association between Tet^R and *tcdB* (OR, 73; 95% CI, 17 to 298) or *cdtB* (OR, 16.4; 95% CI, 5.5 to 49) genes. However, the CI for the *tcdB* and the *cdtB* genes was wide. The same pattern was observed in the Ery^R isolates from young pigs in North Carolina with significant association with *tcdB* (OR, 12.5; 95% CI, 1.9 to 78) genes. However, the Ery^R isolates from young pigs in Ohio were significantly more associated with the *cdtB* genes (OR, 6.4; 95% CI, 5.5 to 49).

Discussion

The high prevalence (73%) of *C difficile* in young pigs in this study was not surprising because CDAD is a known cause of neonatal enteritis.[7,15,20] Previous studies[21,k,l] have detected *C difficile* prevalence in young pigs from 25.9% to 49.5%, and the findings in this study are in agreement with those reports. Sows in this study had a prevalence of 47%, which was higher than reported for sows and boars in a previous study (3.8%).[k] It is important to mention that none of the pigs tested in this study had diarrhea or signs of any illness. The young pigs in this study appeared healthy, yet the prevalence of *C difficile* in these pigs was high. However, during stress or illness caused by another pathogen, it is possible that *C difficile* might contribute to the diseased state of the pig. Other than a single pig that had positive results at the nursery stage, none of the pigs had positive results at the nursery and finishing stages of production. A previous study[k] has also reported that *C difficile* is primarily clustered in young pigs, and the prevalence in nursery and finishing age pigs is significantly lower. An important reason for lower prevalence among older pigs could be reduced susceptibility to the pathogen. Exposure to *C difficile* elicits an immune response, which could be more pronounced in adults.[22,23] However, more studies are needed to determine the reasons for this finding. The difference in prevalence in young pigs between the 2 regions could be the diverse potential sources of *C difficile* or a random error caused by the limited sample size of this study.

There is little published information regarding the antimicrobial resistance profile of *C difficile* isolated from pigs. Antimicrobial resistance to ciprofloxacin was observed at the highest concentration tested (32 µg/mL) in most of the isolates from young pigs (91.3%) and sows (94%). The results are in accordance with other studies[3,24] that have reported high frequency of resistance to ciprofloxacin in *C difficile* isolates from different sources, including pigs and humans. It should be noted that this class of antimicrobials had not been used in swine production in the United States for any purpose at the time of the study. Recently, the FDA approved therapeutic treatment for respiratory infections in swine, but all the samples in this study were collected prior to that approval. Fluoroquinolone resistance in *C difficile* is possibly attributable to the extensive use of this class of antimicrobial in hospitals, which might lead to nonsynonymous mutations in the gyrase region. These acquired mutations in the *C difficile gyr*A and *gyr*B regions are stable and have resulted in the clonal expansion of the resistant strains. Pigs may have acquired these antimicrobial-resistant strains from humans, but this was not investigated in the present study.

There was no indication of high MICs for metronidazole or vancomycin, the 2 drugs most commonly used for treating infections in humans. These results are in accordance with previous reports.[3,18,24,25] In the present study, resistance was detected to erythromycin in isolates from young pigs (38.3%) and sows (34.4%) with a clear distinction based on the geographic origin of isolates. The reason for this difference was not apparent because farms in both regions used erythromycin in the feed for growth promotion purposes. It is possible that other factors including environment, type of flooring, and production flow might be associated with the dissemination of these *C difficile* strains in specific geographic locations. The bimodal distribution (2 distinct clusterings of MIC values) of erythromycin susceptibility, as indicated by the MIC_{50} and MIC_{90}, among isolates from young pigs and sows in this study,

has been reported in isolates from pigs and humans.[24,26] This distribution has been attributed to the possible random circulation of resistance coding determinants in the population and the use of tylosin as a growth promotor.[26] The detection of specific resistance profiles associated with geographic location of sampling in the present study was interesting. This may indicate that specific *C difficile* strains are circulating in specific locations. But it is also important to note that we did not observe a single antimicrobial resistance or toxin gene profile that was restricted to either North Carolina or Ohio. However, the differences between the 2 states could be attributable to random error because the study was conducted in a limited number of farms and may not have external validity for making generalized inferences. The detection of resistance to important antimicrobials is concerning and should be studied in more detail by use of representative samples.

The toxin profile of the isolates characterized in this study was interesting. The $TcdA^+B^+CDT^+$ profile was the predominant gene profile and was found in 59% of the isolates. Detection of toxins A and B in the feces has been used as a standard for diagnosis of *C difficile* infection in pigs and humans.[6,27] However, the detection of toxin-negative *C difficile* strains from the feces of pigs in the present study (15.3%) was an important finding and indicates the importance of pathogen isolation and not simply relying on toxin detection. This also has important implications if a particular *C difficile* strain is toxin negative but resistant to multiple antimicrobials.

To the authors' knowledge, the present study is the first to report the $TcdA^-B^+CDT^+$ toxin profile in *C difficile* isolates from pigs. *Clostridium difficile* strains with that toxin profile have been reported to cause outbreaks and clinical cases in humans and have been isolated with increasing frequency from infants and elderly humans.[28–32] The prevalence of binary toxin coding genes was high, with 77% (n = 166) of the isolates testing positive for the *cdtB* gene. Previous reports[1,33] indicate a higher prevalence of this toxin in young pigs, ranging from 78.4% to 83%. Recently, binary toxin has been increasingly present in strains responsible for community-acquired CDAD in humans.[34,35] The role of binary toxin in the pathogenesis of *C difficile* infection and its role in conjunction with TcdA and TcdB toxins need further investigation. In the present study, the isolation of *C difficile* isolates with similar antimicrobial resistance and toxin gene profiles from the sows and young pigs may imply direct transmission of *C difficile* from sows to the young pigs. It is possible that the reverse is true; young pigs might acquire the pathogen from the barn floor and infect the sows. Because the barn floor was not tested for the presence of *C difficile*, its possible role in pathogen transmission to either the sows or the young pigs was not determined. No *C difficile* organisms were isolated from adult pigs at nursery and finishing levels even though the sows had positive test results in the farrowing barns. A possible explanation for this observation is that the sows were continuously exposed to the pathogen in the farrowing environment, where they spend a lot of time. This could also explain why the young pigs had positive results immediately after birth.

The main objective of determining a significant association between antimicrobial resistance and virulence profile was to identify and devise strategies to differentiate, target, and control the pathogenic species of *C difficile* on farrowing farms, thereby reducing young pig morbidity. Significant associations were detected between the Tet^R and the Ery^R isolates with virulence markers that were dependent on the geographic origin of the isolates. There are conflicting reports in the literature regarding this association between antimicrobial resistance and pathogen virulence. Although no study has reported on this possible association in *C difficile*, a recent study[36] found a positive association between resistance and virulence in *Escherichia coli* isolated from healthy pigs. In contrast, a study[37] conducted with *Enterococcus faecalis* isolated from retail foods revealed both positive and negative associations between antimicrobial resistance and virulence determinants. It is possible that antimicrobial resistance has the potential for selecting virulence in bacteria, which may result in high mortality rates in animals. Other investigators have also reported a similar association in *E coli* with the concern that antimicrobial use may contribute to persistence of virulent strains.[38] It is important to mention that association between virulence and antimicrobial resistance could be dependent on the bacterial population, strain, source, and other important factors that must be taken into account before making valid interpretations.

a. Cycloserine-cefoxitin-fructose agar, Oxoid, Baskingstoke, Hampshire, England.
b. *Clostridium difficile* selective supplement, Oxoid, Baskingstoke, Hampshire, England.
c. Laked horse blood, Hemostat Laboratories, Dixon, Calif.
d. PRO Disc, Remel, Lenexa, Kan.
e. Epsilometric test, AB Biodisk, Solna, Sweden.
f. 5% sheep blood, BD Diagnostics, Franklin Lakes, NJ.
g. Chelex, InstaGene Matrix, Biorad Laboratories, Hercules, Calif.
h. Tris-Acetate EDTA, Fisher Scientific, Fair Lawn, NJ.
i. Minitab Inc, State College, Pa.
j. Egret, version 2.0.1, Cytel Software Corp, Cambridge, Mass.
k. Harvey RB, Norman KN, Scott HM, et al. Prevalence of *Clostridium difficile* in an integrated swine operation (abstr), in *Proceedings*. 9th Bienn Cong Anaerobe Soc Am 2008;5. Available at: www.anaerobe.org/2008/ASA 2008 Session 14.pdf. Accessed Aug 11, 2010.
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