Antibiotic resistance among enterotoxigenic *Escherichia coli* from piglets and calves with diarrhea

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Abstract — In vitro resistance to 8 antimicrobials among enterotoxigenic *Escherichia coli* from piglets and calves over a 13-year period was evaluated. Least resistance occurred against ceftiofur for all, followed by apramycin and gentamicin for porcine and florfenicol for bovine isolates. No significant differences were found between the first 8 and last 5 years.

Résumé — Résistance aux antibiotiques de *Escherichia coli* entérotoxigène provenant de porcelets et de veaux souffrant de diarrhée. La résistance in vitro de 8 agents antimicrobiens d'*Escherichia coli* provenant de porcelets et de veaux fut évaluée durant une période de 13 ans. La moindre résistance a été fournie par le ceftiofur pour tous les animaux, suivie par l'apramycine et la gentamicine pour les porcelets et par le florfénicol pour les isolats bovins. Aucune différence significative n'a été trouvée entre les 8 premières années et les 5 dernières années.

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Diarrhea due to enterotoxigenic *Escherichia coli* (ETEC) is one of the most frequent diseases in young piglets and calves. Despite vaccination programs and management measures, treatment with antibiotics may be required in some cases. Although antimicrobial susceptibility testing is recommended, information on drug resistance trends in a geographic area is helpful to veterinarians in drug selection for empirical therapy (1). The drugs suggested against colibacillosis in pigs include apramycin, ceftiofur sodium, gentamicin, neomycin, potentiated sulfa drugs, and enrofloxacin, gentamicin by injection recommended for the scouring piglet (2,3). Currently, drugs recommended for systemic use against septicemia associated with neonatal colibacillosis due to ETEC in calves include trimethoprim-sulfonamide (TMS) combinations and florfenicol. Gentamicin and ampicillin sulbactam are other potential drugs, but residues in kidneys and high costs are limitations of the latter (4,5).

In the 1980s and early 1990s, a high percentage of strains were likely to be susceptible to TMS combinations (1,6), but a study in 1989 on isolates from Prince Edward Island (PEI) (7) revealed that up to 50% of ETEC strains from piglets and calves were resistant to TMS. In order to generate objective data to implement prudent use of veterinary antimicrobials in food animals, we conducted a retrospective analysis of ETEC isolates from clinical cases of diarrhea in piglets and calves on PEI during the 13-year period ending in 2002.

We also reviewed the records of the Bacteriology Diagnostic Laboratory of the Atlantic Veterinary College to determine the drug susceptibility of all ETEC isolates recovered from the feces or intestines of diarrheic pigs and calves during the period 1990 to 2002. Throughout the study period, it was the practice not to conduct drug susceptibility tests on multiple isolates from the same group of animals. Also, the laboratory specimen submission guidelines required that samples for culture be taken before antimicrobial treatment. The *E. coli* isolates were classified as ETEC on the basis of their agglutination obtained with antisera pools (source: Dr. J.M. Fairbrother, Université de Montréal, St. Hyacinthe, Quebec) against the common diarrhea-causing serogroups and fimbrial adhesions of bovine or porcine origin.

Pool 1 and K:99 antisera were used for isolates from piglets and calves 0 to 2 wk of age; additionally, K:88 antiserum was used for isolates from piglets. Pool 1 detected O8: K"S16", O8: K25, O9: K28, O9; K30, O9: K35, O9: K103, O9: K"79-416," O20: K101, O64: K"V142," and O8: K+.

Pool 2 and K: 88 antisera were used for pigs $>$ 2 to $\leq$ 10 wk of age. Pool 2 detected O138: K81, O139: K82, O141: K85ab, O141: K85ac, O45ac: K"E65," O157: K"V17," O115: K"V165," O8: K"X105," O?: K48, and O149: K91.

Antimicrobial drug susceptibility testing was done by the Kirby-Bauer disk diffusion method (8) on Iso-Sensitest agar (Oxoid Canada, Nepean, Ontario), which compares well with Mueller-Hinton agar for testing *Enterobacteriaceae* (9). *Escherichia coli* strain ATCC 25922 (American Type Culture Collection, Manassas, Virginia, USA), which gave reproducible growth inhibition zones, was used as a susceptible control throughout. During the 13-year study period, 319 to 669 porcine isolates were tested against the following antibiotics: apramycin, ceftiofur, gentamicin, neomycin, oxytetracycline, spectinomycin, and TMS, and 131 to 463 bovine isolates were tested against ceftiofur, florfenicol, gentamicin, neomycin, oxytetracycline, spectinomycin, and TMS (all antibiotic disks were obtained from Oxoid Canada except for ceftiofur, and florfenicol, which

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Table 1. Antimicrobial drug resistance of enterotoxigenic *Escherichia coli* from pigs and calves with diarrhea from 1990 to 2002

	% Resistant (number tested)			
	Porcine		Bovine	
Drug	1990–1997	1998–2002	1990–1997	1998–2002
Apramycin	9 (55)	11 (264)	ND	ND
Ceftiofur	0 (325)	2 (264)	4 (244)	8 (131)
Florfenicol	ND	ND	ND	11 (131)
Gentamicin	11 (404)	13 (264)	6 (323)	0 (4)
Neomycin	7 (404)	27 (264)	64 (323)	ND
Oxytetracycline	82 (326)	81 (264)	81 (244)	75 (130)
Spectinomycin	67 (320)	62 (264)	44 (237)	ND
Trimethoprim-sulfa	35 (405)	32 (264)	46 (328)	48 (135)

ND — not determined

were from Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA). The zone diameter interpretive criteria used to classify an isolate as susceptible, intermediate, or resistant were in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) for bacteria isolated from animals (10). Because of nonavailability of specific criteria for ceftiofur and florfenicol against *E. coli*, the zone size chart for gram-negative respiratory pathogens was used to classify a strain as resistant. Analysis of variance (ANOVA) was used to determine the effect of time period on drug resistance.

The percentages of resistance for porcine isolates in ascending order during the first 8-year period (Table 1) were ceftiofur 0%, apramycin 9%, and gentamicin 11%. The rate of resistance to TMS was 35%. A similar trend was also noted for the last 5 y (1998 to 2002); the rates of resistance against ceftiofur, apramycin, gentamicin, and TMS were 2%, 11%, 13%, and 32%, respectively. A low resistance rate to ceftiofur has been documented among *E. coli* isolates from young pigs in Quebec during the last several years (11,12). An earlier study on 88 ETEC isolates from neonatal pigs for the period of 1986 to 1988 showed a resistance of 36% against TMS (7), a rate almost similar to that obtained in the present study. Neomycin, an antibiotic used for oral treatment of porcine diarrhea due to *E. coli* (5), was as effective as TMS against ETEC in vitro. The majority of isolates ($\geq$ 62%) were resistant to spectinomycin, a drug that has therapeutic application in pigs (2). A high rate of resistance to oxytetracycline ($\geq$ 81%), as found in this study, has previously been reported in Quebec (11).

The percentages of resistance of the bovine isolates in ascending order in the first 8 y were ceftiofur 4%, gentamicin 6%, spectinomycin 44%, TMS 46%, neomycin 64%, and oxytetracycline 81%. For the last 5-year period, least resistance was seen against ceftiofur, followed by florfenicol, TMS, and tetracycline, the resistance rates being 8%, 11%, 48%, and 75%, respectively. Statistical tests utilizing ANOVA showed no significant changes (P-value $\leq$ 0.05) in resistance between the first 8 y and the last 5 y for ceftiofur, gentamicin, oxytetracycline, and TMS for isolates of both porcine and bovine origin. It is interesting to note that the resistance rate for TMS was 50% for isolates from PEI during 1986 to 1988 (7), indicating that there had been no apparent increase in resistance to this drug among *E. coli* from cases of diarrhea in calves in this geographic area.

In summary, among the 8 antimicrobial drugs examined in vitro in this study, least resistance ($\leq$ 8%) was seen against ceftiofur among the ETEC isolates tested. The next 2 drugs with least resistance were apramycin and gentamicin for porcine strains, and gentamicin and florfenicol for bovine strains. This information should be considered along with drug concentration at infection site, as a function of route of administration, in order to make knowledge-based drug selection. CVJ

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