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In Vitro Activities of Cephalosporins and Quinolones against *Escherichia coli* Strains Isolated from Diarrheic Dairy Calves

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The in vitro activities of several cephalosporins and quinolones against 195 strains of *Escherichia coli* isolated from dairy calves affected by neonatal diarrhea were determined. One hundred thirty-seven of these strains produced one or more potential virulence factors (F5, F41, F17, cytotoxic necrotizing factor, verotoxin, and the *eae* gene), but the remaining 58 strains did not produce any of these factors. From 11 to 18% of the *E. coli* strains were resistant to cephalothin, nalidixic acid, enoxacin, and enrofloxacin. However, cefuroxime, cefotaxime, and cefquinome were highly effective against the *E. coli* isolates tested. Some significant differences ($P < 0.05$) in resistance to quinolones between the strains producing potential virulence factors and nonfimbriated, nontoxigenic, *eae*-negative strains were found. Thus, *eae*-positive, necrotoxigenic, and verotoxigenic (except for nalidixic acid) *E. coli* strains were significantly more sensitive to nalidixic acid, enoxacin, and enrofloxacin than nonfimbriated, nontoxigenic, *eae*-negative strains. Moreover, *eae*-positive strains were significantly more sensitive to enoxacin and enrofloxacin than F5-positive strains. Thus, the results of this study suggest that the bovine *E. coli* strains that produce some potential virulence factors are more sensitive to quinolones than those that do not express these factors.

Certain *Escherichia coli* strains are an important cause of diarrhea in calves (22). Thus, the role of enterotoxigenic *E. coli* (ETEC), which produces enterotoxins and which expresses fimbrial colonization factors (F5 and F41) in calves with neonatal diarrhea, has been well established. Moreover, other nonenterotoxigenic *E. coli* strains that produce other toxins (verotoxigenic *E. coli* [VTEC] and necrotoxigenic *E. coli* [NTEC] strains), that cause a characteristic histological lesion, and that possess the *eae* gene (attaching and effacing *E. coli* strains) or that express a fimbria called F17 have also been associated with neonatal diarrhea in calves (22). On the other hand, some *E. coli* strains isolated from cattle may cause diseases in humans (22).

Cephalosporins are beta-lactam antibiotics that have a wide range of antibacterial activities but that show considerable diversity in their properties. These antimicrobial agents have previously been found to be highly effective against *E. coli* isolated from animals (4, 7, 12). Some expanded-spectrum cephalosporins (i.e., ceftiofur and cefquinome) have been approved for use for the treatment of bovine respiratory disease and mastitis, but to our knowledge, cephalosporins are not approved for use for the treatment of diarrhea in calves.

The original quinolone drugs (nalidixic, oxolinic, and pipimedic acids) and the fluoroquinolones have been shown to have excellent in vitro activities against clinical *E. coli* isolates of human (25, 36) and animal (2, 11) origin, including ETEC strains (10, 17, 34). However, the number of reports of fluoroquinolone-resistant *E. coli* strains isolated from humans (3, 26) and animals (7, 31) seems to be on the increase. Among the fluoroquinolones used for the treatment of domestic animals in Spain, enrofloxacin is approved for use for the treatment of colibacillosis and diarrhea in calves.

The aims of this study were to evaluate the susceptibilities of *E. coli* strains isolated from diarrheic dairy calves to cephalosporins and quinolones and the relationships between potential virulence factors of *E. coli* and susceptibility to these antimicrobial agents.

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MATERIALS AND METHODS

***E. coli* strains.** The study was performed with 195 strains of *E. coli* isolated from 162 dairy calves (in 61 herds) with neonatal diarrhea. Fecal samples were obtained within 48 h of the onset of clinical signs from nontreated calves that were up to 3 months of age. The bacterial strains were isolated in our laboratory between 1993 and 1995. The farms on which the calves were located were in the central region of Spain. One hundred thirty-seven of the strains were selected because they produce one or more of the following potential virulence factors: 27 strains produced the F5 fimbrial antigen, 24 produced the F41 fimbria, 63 produced the F17 fimbrial antigen, 43 were NTEC strains, 20 were VTEC strains, and 29 possessed the *eae* gene (which encodes an outer membrane protein, intimin, necessary for intimate attachment to epithelial cells). Cytotoxic necrotizing factors and verotoxins were detected by cytotoxicity assays (5) and PCR (6, 29), the *eae* gene was detected by colony blot hybridization (23), and the F5, F41, and F17 fimbrial antigens were detected by slide agglutination (19). The remaining 58 strains, which did not produce any of the fimbrial antigens or toxins studied and which were *eae* negative, were selected for comparison.

Antimicrobial agents. The following antimicrobial agents were studied and were provided by the manufacturers: cephalothin (Antibióticos, Madrid, Spain), cefuroxime (Glaxo Wellcome, Tres Cantos, Madrid, Spain), cefotaxime (Hoechst Farma, Sant Feliu de Llobregat, Barcelona, Spain), cefquinome (Hoechst Roussel Vet, San Fernando de Henares, Madrid, Spain), nalidixic acid (Hipra, Amer, Girona, Spain), oxolinic acid (Hipra), enoxacin (Almirall, Barcelona, Spain), enrofloxacin (Química Farmaceútica Bayer, Barcelona, Spain), and danofloxacin (Pfizer, Madrid, Spain). The antimicrobial agents were dissolved and diluted as recommended by the manufacturers. Fresh dilutions of all compounds were prepared daily.

Antimicrobial susceptibility testing. In vitro susceptibility tests were performed by the agar dilution method, according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (28), with Mueller-Hinton agar (Difco). The plates were incubated at 37°C for 24 h, and the MIC was the lowest concentration of antimicrobial agent that suppressed visible bacterial growth. Reference strain *E. coli* ATCC 25922 was included as an internal control in all parts of the study. The range of interpretative categories of susceptibility for cephalothin, cefuroxime, cefotaxime, nalidixic acid, enoxacin, and enrofloxacin were those recommended by NCCLS (27, 28). None of the listed breakpoints are specific to the treatment of calves with diarrhea caused by *E. coli*. For the remaining antimicrobial agents (cefquinome, oxolinic acid, and danofloxacin), the NCCLS guidelines do not contain recommended breakpoints.

Statistical analysis. Significant differences in the frequencies of resistance to the tested antimicrobial agents with recommended breakpoints in the NCCLS

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TABLE 1. In vitro susceptibilities of 195 *E. coli* strains isolated from diarrheic calves to cephalosporins and quinolones

Antimicrobial agent[a]	MIC (μg/ml)[b]			No. (%) resistant strains
	Range	50%	90%	
Cephalothin (≥32)	1–>512	8	32	32 (16.4)
Cefuroxime (≥32)	0.125–64	4	8	4 (2.1)
Cefotaxime (≥64)	≤0.0625–2	≤0.0625	0.125	0 (0)
Cefquinome	≤0.0625–2	≤0.0625	0.125	
Nalidixic acid (≥32)	0.5–>512	4	>512	35 (17.9)
Oxolinic acid	≤0.0625–>512	0.250	64	
Enoxacin (≥8)	≤0.0625–256	0.125	16	24 (12.3)
Enrofloxacin (≥2)	≤0.0625–64	≤0.0625	4	23 (11.8)
Danofloxacin	≤0.0625–64	≤0.0625	4	

[a] Numbers in parentheses are the MIC breakpoints (in micrograms per milliliter) indicating susceptibility according to the recommendations of NCCLS (27, 28). None of the listed breakpoints are specific for the treatment of calves with diarrhea caused by *E. coli*. For antimicrobial agents without breakpoints, the NCCLS guidelines do not contain recommended breakpoints, and thus the percentages of resistant strains were not calculated.

[b] 50% and 90%, MICs at which 50 and 90% of the tested strains are inhibited, respectively.

guidelines among the strains producing each potential virulence factor and nonfimbriated, nontoxigenic, *eae*-negative strains were determined by the chi-square test. A P value of <0.05 was considered significant.

RESULTS

The results of the in vitro susceptibilities to cephalosporins and quinolones of the 195 strains of *E. coli* studied are presented in Table 1. The range of MICs and the MICs at which 50 and 90% of the tested strains are inhibited for each of the nine antibiotics, as well as the percentage of resistant *E. coli* strains (only for antimicrobial agents with recommended breakpoints in the NCCLS guidelines), were determined (Table 1).

From 11 to 18% of the *E. coli* strains studied were resistant to cephalothin, nalidixic acid, enoxacin, and enrofloxacin on the basis of the NCCLS breakpoints for human and animal clinical isolates. However, cefuroxime, cefotaxime, and cefquinome were highly effective against the *E. coli* isolates tested.

When the frequencies of resistance among the strains producing each potential virulence factor and the nonfimbriated, nontoxigenic, *eae*-negative strains were compared, the following significant differences in resistance to quinolones were found: (i) F17-positive, *eae*-positive, and NTEC strains were significantly more sensitive to nalidixic acid than nonfimbriated, nontoxigenic, *eae*-negative strains; (ii) NTEC strains were significantly more sensitive to nalidixic acid than F5-positive and F17-positive strains; (iii) *eae*-positive, NTEC, and VTEC strains were significantly more sensitive to enoxacin and enrofloxacin than nonfimbriated, nontoxigenic, *eae*-negative strains; and (iv) *eae*-positive strains were significantly more sensitive to enoxacin and enrofloxacin than F5-positive strains.

Two different patterns in the differences between the frequencies of resistance to quinolones could be observed: one for nalidixic acid and the other one for enoxacin and enrofloxacin. One hundred thirty-six strains (69.7%) were sensitive to all the tested antimicrobial agents with recommended breakpoints in the NCCLS guidelines. Twenty-nine strains (14.9%) were resistant to one antibiotic. Thus, a low percentage of the strains studied showed multidrug resistance: 15.4% of the isolates were resistant to at least two antibiotics and 2.6% of the isolates were resistant to at least four antibiotics. A total of nine antibiotic resistance patterns could be distinguished (Table 2).

TABLE 2. Antibiotic resistance patterns of the *E. coli* strains studied

Resistance pattern(s)[a]	Resistant strains	
	No.	%
No resistance	136	69.7
CFL	21	10.8
NA, ENX, ENR	18	9.2
NA	8	4.1
CFL, NA, ENX, ENR	4	2.1
CFL, NA	3	1.5
CFL, CFR	3	1.5
CFL, CFR, NA, ENX, ENR	1	0.5
NA, ENX	1	0.5

[a] CFL, cephalothin; NA, nalidixic acid; ENR, enrofloxacin; ENX, enoxacin; CFR, cefuroxime.

DISCUSSION

The percentages of strains resistant to cephalothin and cefuroxime found in this study are similar to the percentages of *E. coli* strains that were isolated from cattle and that were resistant to the early cephalosporins, as reported previously (4, 7, 13, 31). However, in comparison to data obtained by our research group for *E. coli* strains isolated from diarrheic lambs and goat kids (12), the frequency of resistance to cephalothin was higher among isolates from calves than among isolates from small ruminants.

The newer aminothiazolyl cephalosporins (i.e., cefotaxime) represent major advances in antibacterial therapy because of their broad antibacterial spectra, their resistance to enzymatic hydrolysis by β-lactamases, and the improvements in their pharmacokinetic properties (33). In addition, the later cephalosporins show markedly reduced affinities to β-lactamases and increased levels of outer membrane permeation compared with those for the aminothiazolyl cephalosporins (21). Cefquinome is the first of these cephalosporins developed for use in veterinary medicine. In this study cefotaxime and cefquinome were highly effective against the *E. coli* isolates tested. The results obtained in this study were similar to those reported previously for the activities of cefotaxime, ceftiofur (another expanded-spectrum cephalosporin), and cefquinome against bovine *E. coli* isolates (4, 7). Thus, expanded-spectrum cephalosporins are highly effective against bovine *E. coli* isolates.

The percentage of strains resistant to nalidixic acid found in this study was similar to that found by Pohl et al. (31) for bovine *E. coli* isolates but higher than that reported by Aalbæk et al. (1) for bovine *E. coli* isolates. On the other hand, in a study done by our group with *E. coli* strains isolated from diarrheic lambs and goat kids (12), the in vitro activities of nalidixic and oxolinic acids were higher than those observed in this study.

The fluoroquinolones are an exceptionally important and rapidly developing group of antimicrobial drugs and are being introduced into human and veterinary medicine for a wide variety of antimicrobial purposes (32). In the first reports about fluoroquinolones, resistance of human (25, 36) and bovine (2) *E. coli* strains to these antibiotics was rarely observed. However, in our study, the MICs of the fluoroquinolones were very low but the frequencies of resistance of enoxacin and enrofloxacin were relatively high (about 12%). Recently, other investigators have also described increases in the levels of resistance to these antimicrobial agents among *E. coli* strains isolated from humans (3, 26) and cattle (7, 31). On the other hand, in a study performed recently by our group (11) with diarrheic lambs and goat kids from the same geographic area

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in which the calves used for this study were located, fluoroquinolones proved to be highly effective against *E. coli*. The differences in these results may be due to the introduction of fluoroquinolone therapy in some of the bovine herds but not in the ovine and caprine herds studied.

In this study the level of resistance to enrofloxacin (a fluoroquinolone used for the treatment of infections in domestic animals) was similar to the level of resistance to enoxacin (a fluoroquinolone available for human clinical use). This is due to the fact that resistance to one fluoroquinolone generally confers resistance to the entire class of fluoroquinolone agents (30). The development of cross-resistance among the fluoroquinolones used in veterinary and human medicine is a source of debate on the use of these antibiotics for the treatment of infections in animals and is a source of political fallout (8). Threlfall et al. (35) have suggested that the emergence and spread in the United Kingdom of isolates of *Salmonella typhimurium* DT 104, a salmonella prevalent in humans, with reduced sensitivity to ciprofloxacin has followed the licensing of enrofloxacin for veterinary use in that country in 1993. Because of this, Threlfall et al. (35) have recommended a restriction of the veterinary use of fluoroquinolones.

The increase in the level of resistance of bovine *E. coli* isolates to fluoroquinolones may indicate a risk to public health because some of these strains, principally, VTEC strains, may cause diseases in humans (18, 24) and because resistance to the fluoroquinolones used in veterinary medicine may confer resistance to the fluoroquinolones used in human medicine.

Some reports suggest that pathogenic *E. coli* strains are more likely than nonpathogenic strains to be resistant to antimicrobial agents (20). However, there is no conclusive evidence for this suggestion, since in several studies ETEC strains have been found to be more sensitive to antimicrobial agents than non-ETEC strains (9, 14, 15). On the other hand, among *E. coli* strains isolated from diarrheic calves, González and Blanco (16) found that VTEC strains were significantly more resistant to different antimicrobial agents than NTEC strains and non-VTEC, non-NTEC strains. In this study *eae*-positive, NTEC, and VTEC strains were significantly more sensitive to nalidixic acid (*eae*-positive and NTEC strains only), enoxacin, and enrofloxacin than nonfimbriated, nontoxigenic, *eae*-negative strains, and *eae*-positive strains were significantly more sensitive to enoxacin and enrofloxacin than F5-positive strains. Thus, the results of this study for F5-positive and VTEC strains are in contrast those cited previously. Moreover, these differences in resistance to quinolones were not observed in *E. coli* strains isolated from diarrheic lambs and goat kids (11, 12). Thus, the results of this study suggest that the bovine *E. coli* strains that produce some potential virulence factors are more sensitive to quinolones than those that do not express these factors.

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