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 ceftazidime 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, nontoxicogenic, 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, nontoxicogenic, eae-negative strains. Moreover, eae-positive strains were significantly more sensitive to enoxacin 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 diarrhea in calves (22). Thus, the role of enterotoxigenic *E. coli* (ETEC), which produces enterotoxins and which expresses diarrhea in calves (22). Thus, the role of enterotoxigenic *E. coli* (ETEC), which produces enterotoxins and which expresses diarrhea in calves (22). Thus, the role of enterotoxigenic *E. coli* (ETEC), which produces enterotoxins and which expresses diarrhea in calves (22). Thus, the role of enterotoxigenic *E. coli* (ETEC), which produces enterotoxins and which expresses diarrhea in calves (22). Thus, the role of enterotoxigenic *E. coli* (ETEC), which produces enterotoxins and which expresses diarrhea in calves (22). Thus, the role of enterotoxigenic *E. coli* (ETEC), which produces enterotoxins and which expresses diarrhea in calves (22). 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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), ceftazidime (Hoechst Rousell Vet, San Fernando de Henares, Madrid, Spain), nalidixic acid (Hipra, Girona, Spain), oxolinic acid (Hipra), enoxacin (Almirall, Barcelona, Spain), enrofloxacin (Química Farmaceútica Bayer, Barcelona, Spain), 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 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 cefoxitin, cefotaxime, ceftazidime, 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 (ceftazidime, cephalothin, ceftoxin, nalidixic acid, enoxacin, and enrofloxacin), 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...
antibiotic resistance patterns could be distinguished (Table 2).

Table 1: Antibiotic resistance patterns of the E. coli strains studied

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>No. (%) resistant strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td>Cephalothin (≥32)</td>
<td>1–≥512</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Cefuroxime (≥32)</td>
<td>0.125–64</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Cefotaxime (≥64)</td>
<td>≤0.0625–2</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Ceftiraxone</td>
<td>≤0.0625–2</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Nalidixic acid (≥32)</td>
<td>0.5–≥512</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Oxolinic acid</td>
<td>≤0.0625–512</td>
<td>0.250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>Enoxacin (≥8)</td>
<td>≤0.0625–256</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Enrofloxacin (≥2)</td>
<td>≤0.0625–64</td>
<td>≤0.0625</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Danofloxacin</td>
<td>≤0.0625–64</td>
<td>≤0.0625</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

Number 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.

DISCUSSION

The percentages of strains resistant to cephalothin and cephalosporins are highly effective against bovine E. coli isolates tested. The results obtained in this study were similar to those reported previously for the activities of cefotaxime, ceforfur (another expanded-spectrum cephalosporin), and ceftiofur 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 frequency of resistance to cephalothin was higher among isolates from calves than among isolates from small ruminants.

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 isolates 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
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 typhi-murium 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, nontoxicogen, 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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