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Antimicrobial Susceptibility of *Campylobacter jejuni* and *Campylobacter fetus* subsp. *fetus* to Eight Cephalosporins with Special Reference to Species Differentiation

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Agar dilution antimicrobial susceptibility testing showed that *Campylobacter jejuni* was significantly more resistant than *Campylobacter fetus* subsp. *fetus* (*intestinalis*) to cephalosporin C, cephaloridine, cephalothin, cefazolin, and cefamandole. No species differences in susceptibility were noted with cephalexin, cefotaxime, and cefoxitin. Rapid species differentiation on the basis of an antibiogram could be achieved with the disk diffusion method. *C. jejuni* failed to produce a zone of inhibition around a 30-μg cephalothin disk but produced a significant zone around a 30-μg nalidixic acid disk. *C. fetus* subsp. *fetus* (*intestinalis*) produced exactly the reverse pattern.

The genus *Campylobacter* contains a number of species and subspecies, only two of which are known to be associated with human disease (3). *Campylobacter jejuni* (7), corresponding to the *C. jejuni-Campylobacter coli* group described by Véron and Chatelain (14) and to *Campylobacter fetus* subsp. *jejuni* described by Smibert (11), has become established as a major bacterial cause of diarrhea (3, 8, 10). *C. fetus* subsp. *fetus* (Véron and Chatelain) is an opportunistic pathogen that sometimes causes septicemic illness in compromised hosts (3) and recently has occasionally been isolated from stools of patients with diarrhea (2, 6). It should be noted that the term *C. fetus* subsp. *fetus* (Véron and Chatelain) as used in this report is not synonymous with the term *C. fetus* subsp. *fetus* as used by Smibert (11), but corresponds instead to the term *C. fetus* subsp. *intestinalis* as used by the latter author. To avoid confusion, *C. fetus* subsp. *fetus* (Véron and Chatelain) will be referred to in this communication as *C. fetus* subsp. *fetus* (*intestinalis*).

The differentiation of *C. jejuni* from *C. fetus* subsp. *fetus* (*intestinalis*) in clinical laboratories remains unsatisfactory. The principle criteria for differentiating the two species (11, 14) are the ability of strains to grow at different temperatures and susceptibility of strains to nalidixic acid. *C. fetus* subsp. *fetus* (*intestinalis*) grows at 25 and 37°C but not at 42°C and is resistant to 40 μg of nalidixic acid per ml, whereas *C. jejuni* fails to grow at 25°C, grows at 37°C, grows better at 42°C, and is inhibited by 40 μg of nalidixic acid per ml. Recently, Smibert (12) reported that some strains of *C. fetus* subsp. *fetus* (*intestinalis*) are also able to grow at 42°C, and Vanhoof et al. (13) found that about 5% of *C. jejuni* strains are resistant to nalidixic acid. There is, therefore, clearly a need for extending the criteria for differentiating the two species so that their role in disease can be more accurately defined.

Our preliminary investigations (M. A. Karmali, A. K. Allen, and P. C. Fleming, Can. J. Public Health 71:204, 1980) showed that catalase-positive campylobacters could be differentiated morphologically on the basis of size. We also noted (Karmali et al., Can. J. Public Health 71:204, 1980) that species differences in antimicrobial susceptibility occurred with certain cephalosporins. Smibert (personal communication) also noted species differences in susceptibility to cephalothin.

The purpose of this study was to compare the susceptibility patterns of *C. jejuni* and *C. fetus* subsp. *fetus* (*intestinalis*) to seven cephalosporins and cefoxitin to determine which agents showed species differences in susceptibility patterns and to evaluate a disk diffusion method for rapidly differentiating the two species in average clinical laboratories on the basis of an antibiogram.

MATERIALS AND METHODS

Bacteria. The bacteria used in this study included 60 strains of *C. jejuni* and 12 strains of *C. fetus* subsp. *fetus* (*intestinalis*). The 12 strains of *C. fetus* subsp. *fetus* (*intestinalis*) were eight human blood culture isolates obtained from N. Chalvardjian, S. McDonald, M. Tischler, J. L. Whitby, W. J. Martin, and J. Righter, one isolate obtained from A. J. Winter, and three reference strains obtained from the American Type Culture Collection (ATCC) and designated ATCC 15296, ATCC 25936, and ATCC 27374. The 60 strains of *C. jejuni* were 59 human fecal isolates and 1 reference strain (ATCC 29428). The identification to species of the strains was based on methods outlined by

Véron and Chatelain (14) and Smibert (11). All strains were small, strictly microaerophilic, gram-negative bacteria which had a curved, S-shaped, or spiral morphology and a rapid, darting, corkscrew-like motility. All strains were catalase and oxidase positive. Strains which grew at 37 and 42°C but not at 25°C and were inhibited by 40 μg of nalidixic acid per ml were considered to be *C. jejuni*. Strains which grew at 25 and 37°C but not at 42°C, grew in the presence of 1% glycine, and were resistant to 40 μg of nalidixic acid per ml were considered to be *C. fetus* subsp. *fetus* (*intestinalis*).

Agar dilution antimicrobial susceptibility tests. Before testing, each strain was subcultured for two to three passages onto blood agar (BA) plates, which were then incubated for 48 h in a carbon dioxide incubator (CO_2 tension, 7%) set at 36°C. The BA used consisted of Columbia blood agar base (GIBCO Diagnostics) with 7% defibrinated horse blood. Antimicrobial susceptibility tests were carried out by the agar dilution method. Suspensions of 48-h BA cultures of each strain were made in Penassay broth (Difco antibiotic medium no. 3) to a density approximating a McFarland no. 2 turbidity standard and introduced into the wells of a Steers replicator apparatus. A 5-μl volume of each suspension, corresponding to a final inoculum size of approximately 10^6 viable organisms, was then inoculated onto the antibiotic media by means of the replicator head. The medium used for antibiotic susceptibility testing was diagnostic sensitivity test agar (Oxoid) containing 5% lysed horse blood. The inoculated plates were incubated at 36°C in the carbon dioxide incubator and read after 48 h. The endpoint was taken as complete inhibition of growth. It should be noted that the majority of campylobacter strains adapted well to growth in the carbon dioxide incubator and grew satisfactorily on non-antibiotic-containing control plates. A few strains of *C. jejuni* which did not adapt well to the carbon dioxide incubator were rejected from the study. The antibiotics tested were cephalosporin C (reference no. CT 738, Glaxo Laboratories), cephaloridine (Ceporan, Glaxo Laboratories), cephalothin sodium B.P. (Ceporacin, Glaxo Laboratories), cephalexin (Glaxo Laboratories), cefamandole nafate (Mandol, Eli Lilly & Co.), cefazolin sodium (Kefzol, Eli Lilly & Co.), cefotaxime (HR 756, Roussel Laboratories), and cefoxitin sodium (Mefoxin, Merck Sharp & Dohme).

Differential disk diffusion test. All strains were subcultured onto BA plates, which were then incubated for 48 h at 37°C under reduced oxygen tension. The reduced oxygen tension was obtained by evacuating two-thirds of the air from an anaerobic jar (without catalyst) and replacing the evacuated air with a carbon dioxide-hydrogen mixture. Suspensions of each culture were made in Penassay broth (Difco antibiotic medium no. 3) to a density approximating a McFarland no. 2 turbidity standard and inoculated onto a BA plate with a swab by the method of Bauer et al. (1). A 30-μg nalidixic acid disk and a 30-μg cephalothin disk (Sensi-disc Sensitivity Discs, Becton, Dickinson & Co., Ltd., Mississauga, Canada) were placed on the surface of the inoculated BA plates, which were then incubated at 37°C under reduced oxygen tension and examined after 48 h.

RESULTS

The minimum inhibitory concentrations (MICs) of seven cephalosporins and cefoxitin for the organisms tested are shown in Table 1. *C. jejuni* was significantly more resistant to cephaloridine, cephalothin, and cefazolin than was *C. fetus* subsp. *fetus* (*intestinalis*). A significant but less marked species difference in susceptibility occurred with cephalosporin C and cefamandole, but no such difference occurred with cephalexin, cefotaxime, or cefoxitin.

Of the 60 strains of *C. jejuni*, 59 produced no zone of inhibition around the 30-μg cephalothin disk (Fig. 1). The reference strain of *C. jejuni* (ATCC 29428) produced a very narrow zone of inhibition that was barely visible. In contrast, all strains of *C. fetus* subsp. *fetus* (*intestinalis*) produced significant zones of inhibition (Fig. 2) around the cephalothin disks, with zone diameters ranging from 16 to 25 mm (mean, 21 mm). None of the *C. fetus* subsp. *fetus* (*intestinalis*) strains produced any zone around a 30-μg nalidixic acid disk (Fig. 2), whereas all strains of *C.*

TABLE 1. *MICs of eight cephalosporins for C. fetus* subsp. *fetus* and *C. jejuni*[a]

Antibiotic	*C. fetus* subsp. *fetus*		*C. jejuni*	
	MIC range (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{90} (μg/ml)
Cephalosporin C	≦0.5–2	1	4–32	16
Cephaloridine	≦0.5–1	≦0.5	8–128	64
Cephalothin	16–32	32	128–≧512	≧512
Cefazolin	8–32	32	128–≧512	≧512
Cefamandole	16–64	64	128–≧512	≧512
Cephalexin	32–128	128	16–≧512	≧512
Cefotaxime	4–16	16	2–32	8
Cefoxitin	32–64	64	32–≧512	256

[a] There were 12 strains of *C. fetus* subsp. *fetus* (*intestinalis*) and 60 strains of *C. jejuni*. Antibiotic susceptibility tests were carried out by the agar dilution method with diagnostic sensitivity test agar (Oxoid) containing 5% lysed horse blood. Inoculum size, ≈10^6 organisms. MIC_{90}, Minimum concentration (μg/ml) required to inhibit 90% of the strains.

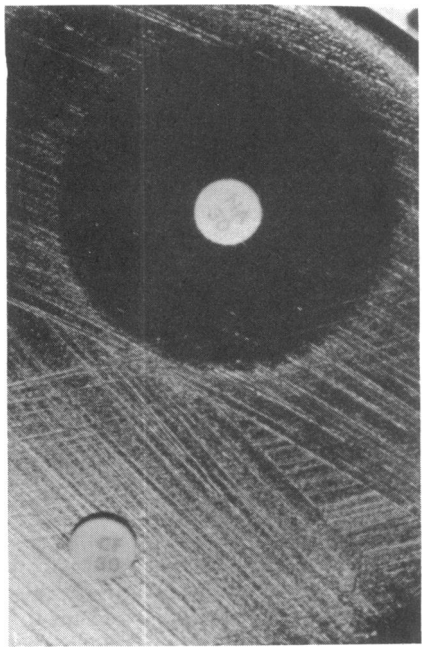

FIG. 1. *Forty-eight-hour BA culture of C. jejuni showing a significant zone of inhibition around a 30-μg nalidixic acid disk but no zone around a 30-μg cephalothin disk.*

jejuni produced significant zones (Fig. 1) ranging from 21 to 38 mm (mean, 31 mm) in diameter.

DISCUSSION

Our findings show that *C. jejuni* and *C. fetus* subsp. *fetus* (*intestinalis*) can be reliably differentiated from each other on the basis of their susceptibilities to cephalosporin C, cephaloridine, cephalothin, cefazolin, and cefamandole. These findings are in keeping with Smibert's (personal communication) observations but contradict the findings of Chow et al. (5) and Butzler (3). The disk diffusion method is satisfactory for testing single isolates in average diagnostic laboratories. Both the 30-μg cephalothin disk and the 30-μg nalidixic acid disk are eminently suitable for this purpose because the results are based on a zone-no-zone phenomenon, so that actual zone sizes need not be measured.

From the therapeutic viewpoint, none of the compounds tested showed very good activity against the two species except for cephaloridine, which on theoretical grounds at least could be used to treat infections caused by *C. fetus* subsp. *fetus* (*intestinalis*). However, good alternative antibiotics such as erythromycin and gentamicin are already known to be highly active and effective in treating campylobacter infections (4, 9).

Butzler's selective medium (4) for isolating

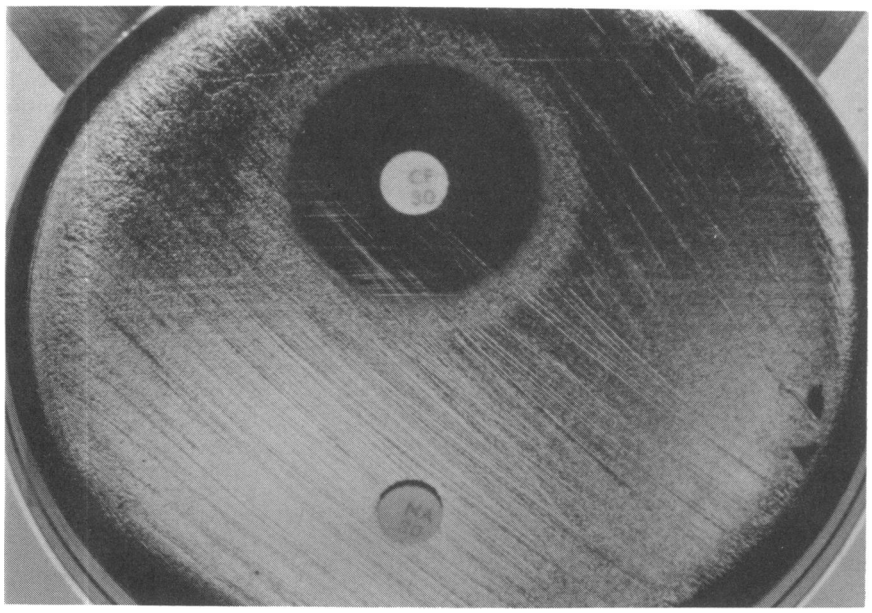

FIG. 2. *Forty-eight-hour BA culture of C. fetus subsp. fetus (intestinalis) showing a significant zone of inhibition around a 30-μg cephalothin disk but no zone around a 30-μg nalidixic acid disk.*

campylobacters from stools contains 15 μg of cefazolin per ml. Although all our strains of *C. jejuni* had MICs greatly in excess of this concentration, it should be noted that the MICs of cefazolin for our strains of *C. fetus* subsp. *fetus* (*intestinalis*) ranged from only 8 to 32 μg/ml, indicating that Butzler's medium may not be reliable for the isolation of the latter species.

In conclusion, susceptibility to certain cephalosporins provides a valuable additional characteristic for differentiating *C. jejuni* from *C. fetus* subsp. *fetus* (*intestinalis*). This observation should be of particular value in classifying strains that react aberrantly in established differential tests. The reasons for the species differences in susceptibility to certain cephalosporins are not known and remain to be investigated.

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