In Vitro Susceptibility of *Campylobacter jejuni* to 27 Antimicrobial Agents and Various Combinations of β -Lactams with Clavulanic Acid or Sulbactam

P. VAN DER AUWERA^{†*} AND B. SCORNEAUX

Clinical Microbiology Laboratory, Hôpital Civil de Charleroi, Charleroi, Belgium

Received 26 December 1984/Accepted 9 April 1985

The in vitro susceptibility of human isolates of *Campylobacter jejuni* was investigated with 27 antibiotics and 8 combinations of β -lactams with clavulanic acid or sulbactam. Ansamycin, the new quinolones, erythromycin, and cefpirome were the most active drugs against *C. jejuni*; amoxicillin, ampicillin, cefotaxime, and ceftazidime were moderately active. Among the β -lactams, cefazolin, cefamandole, and temocillin were inactive (MIC for 90% of the isolates, ≥ 50 mg/liter). The activity of various β -lactams was unchanged by the addition of clavulanic acid or sulbactam.

The purpose of the present study was to compare the in vitro activities of drugs of known activity against *Campylobacter jejuni* with those of recently developed drugs. It seems worthwhile to evaluate the in vitro activity of sulfasalazine against *C. jejuni* because of the use of this drug in chronic inflammatory bowel diseases and the possible role of *C. jejuni* in these conditions (3).

MATERIALS AND METHODS

C. jejuni strains recently isolated from the feces of patients with acute diarrhea were selected. We included two strains isolated from bacteremic patients with severe cirrhosis of the liver. The strains were kept as stock cultures in thioglycolate broth. After the strains were checked for noncontamination on Mueller-Hinton agar, they were cultivated at 42°C in thioglycolate broth until a concentration of 10^8 CFU/ml was achieved. MICs were determined by the agar dilution method (2) with Mueller-Hinton agar and a multipoint inoculator that delivered 10 μ l, i.e., 10^6 CFU per spot. The plates were incubated for 48 h at 42°C in an atmosphere containing 10% CO₂ and 5% O₂. The MIC was defined as the lowest concentration of drug allowing no visible growth. Stock solutions of drugs were prepared as needed in accordance with the recommendations of the manufacturers. The following drugs were tested: sulfasalazine, 5-aminosalicylic acid, and sulfapyridine (Pharmacia Belga S.A.); rifampin (Lepetit); ansamycin (LM 427) (Farmitalia Carlo Erba); erythromycin (Abbott Laboratories); trimethoprim, co-trimoxazole, and sulfamethoxazole (Roche Diagnostics); oxolinic acid and enoxacin (Warner-Lambert Co.); pefloxacin (Rhône-Poulenc); norfloxacin (Merck Sharp & Dohme); ciprofloxacin (Bayer); cefazolin, cefamandole, and cinoxacin (Eli Lilly & Co.); cefotaxime and cefpirome (Hoechst-Roussel Pharmaceuticals, Inc.); ceftazidime (Glaxo Pharmaceuticals, Ltd.); amoxicillin, ampicillin, temocillin, ticarcillin, and clavulanic acid (Beecham Laboratories); and sulbactam (Pfizer Inc.).

Combinations of β -lactams with the β -lactamase inhibitors clavulanic acid and sulbactam were tested at a fixed ratio of

5:1; the results are expressed for the β -lactam concentration within the combination.

The inoculum effect was studied with 10 strains and the following antibiotics or combinations: erythromycin, ampicillin, amoxicillin, cefotaxime, ampicillin plus clavulanic acid, amoxicillin plus clavulanic acid, cefotaxime plus clavulanic acid, sulfamethoxazole, co-trimoxazole, enoxacin, ciprofloxacin, and ansamycin; the inocula tested were 10^4 , 10^5 , and 10^6 CFU per spot.

RESULTS

Table 1 shows the MICs for 50 and 90% of the isolates and the ranges of MICs for the *C. jejuni* strains. Among the quinolones, the two most active drugs were ciprofloxacin and pefloxacin, followed by norfloxacin and ofloxacin; enoxacin was slightly less active, and oxolinic acid and cinoxacin were the least active drugs. Among the β -lactams, cefpirome and cefotaxime were the most active, followed by amoxicillin, ampicillin, and ceftazidime; ticarcillin was only moderately active, and temocillin, cefazolin, and cefamandole were inactive. Interestingly, clavulanic acid was two to four times more active than sulbactam. Rifampin was essentially inactive, although ansamycin, a closely related drug, was the most active drug in vitro against *C. jejuni*, with all strains being susceptible at 0.62 mg/liter. Sulfamethoxazole and co-trimoxazole had the same activity as a consequence of the lack of activity of trimethoprim. Sulfapyridine was at least 10-fold less active than sulfamethoxazole. Sulfasalazine was inactive, and 5-aminosalicylic acid, as expected, was also inactive. Erythromycin had an MIC for 90% of the isolates of 2.5 mg/liter, although 8% of the strains were resistant.

The addition of a β -lactamase inhibitor, sulbactam or clavulanic acid, to a β -lactam had no effect on the in vitro susceptibility of *C. jejuni*.

Table 2 shows a comparison among the MICs for 10 *C. jejuni* strains at three different inocula. Only a minimal effect was observed between 10^4 and 10^6 CFU per spot.

DISCUSSION

The value of antimicrobial therapy has not been established for diarrhea caused by *C. jejuni*. Erythromycin has been recommended for the treatment of campylobacteriosis; however, ca. 8% of *C. jejuni* strains are resistant. This

* Corresponding author.

[†] Present address: Service de Médecine et Laboratoire d'Investigation Clinique H. J. Tagnon (Laboratoire de Biologie), Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Brussels, Belgium.

TABLE 1. In vitro activity of various antibiotics against *C. jejuni*

Antibiotic	No. of strains tested	MIC (mg/liter)			Geometric mean
		50%	90%	Range	
Rifampin	200	20	20	20	20
Ansamycin	200	0.31	0.62	0.31-0.62	0.30
Erythromycin	200	1.25	2.5	0.62-20	0.71
Trimethoprim	200	20	20	20	20
Co-trimoxazole	200	50	50	12.5-100	35
Sulfamethoxazole	200	25	50	25-100	21.5
Sulfapyridine	100	500	500	500-1,000	500
Sulfasalazine	100	2,000	2,000	2,000-4,000	2,000
5-Aminosalicylic acid	100	2,000	4,000	2,000-4,000	1,800
Cinoxacin	100	>50	>50	>50	>50
Oxolinic acid	100	1.25	2.5	0.31-2.5	0.74
Norfloxacin	200	0.62	1.25	0.31-2.5	0.39
Pefloxacin	100	0.31	0.62	0.31-0.62	0.23
Enoxacin	200	0.62	1.25	0.62-2.5	0.52
Ciprofloxacin	100	0.31	0.62	0.31-0.62	0.20
Ofloxacin	100	0.62	1.25	0.15-1.25	0.44
Cefazolin	40	50	50	50	50
Cefamandole	40	>50	>50	>50	>50
Cefotaxime	40	3.12	6.25	3.12-6.25	2.4
Ceftazidime	40	3.12	6.25	6.25	3.8
Cefpirome	100	1.50	3.12	0.75-6.25	1.2
Amoxicillin	40	6.25	6.25	1.56-6.25	4.1
Ampicillin	40	6.25	6.25	3.12-6.25	4.5
Ticarcillin	40	12.5	12.5	6.25-25	10.1
Temocillin	40	>50	>50	>50	>50
Clavulanic acid	40	12.5	25	6.25-25	9.8
Sulbactam	40	25	50	25-50	20.5
Ampicillin + clavulanic acid	40	6.25	12.5	6.25-12.5	5.0
Amoxicillin + clavulanic acid	40	6.25	12.5	6.25-12.5	6.2
Ticarcillin + clavulanic acid	40	25	25	12.5-50	21
Cefazolin + clavulanic acid	40	50	50	50	37
Ampicillin + sulbactam	40	12.5	12.5	6.25-12.5	7.8
Amoxicillin + sulbactam	40	25	25	12.5-25	15
Ticarcillin + sulbactam	40	50	50	25-50	30
Cefazolin + sulbactam	40	50	50	50	37

resistance is probably not clinically relevant as far as the treatment of diarrhea caused by this organism is concerned, since >500 µg of erythromycin per g was obtained in the feces of patients receiving 3 g orally (4). Controlled studies of this drug failed to show any significant effect on the clinical course of the diarrhea (16, 18, 20), although *C. jejuni* was usually eradicated from all the patients treated.

Antimicrobial agents will continue to be used in the treatment of acute bacterial diarrhea on an empiric basis. Since the use of effective agents may contribute to the development of resistance in enteropathogens, it is worthwhile to evaluate new antibiotics suitable for this indication. New quinolones are very active on an in vitro basis against *C. jejuni* and have also been shown to be very active against many enteropathogens (6), such as *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Vibrio* spp., and *Aeromonas* spp. The concentration of the quinolones in the feces is usually very high. Oxolinic acid is recovered in high concentrations in the feces (11), and this is true also for norfloxacin (8), pefloxacin (14), and ciprofloxacin (5). Overall, concentrations in the feces are higher than 300 µg/g. New β-lactams have also been shown to have very high in vitro activities against most enteropathogens, including *C. jejuni*. The concentrations of β-lactam antibiotics in the gastrointestinal tract depend mostly on three parameters, intestinal absorption after oral

administration, biliary excretion of active drug or metabolites, and stability against hydrolysis by the β-lactamases produced by the resident flora. Ampicillin is absorbed less after oral administration than amoxicillin, and high concentrations of active drug are obtained in the feces (1; E. A. P. Croydon and A. J. Swaisland, Proc. 13th Int. Congr. Chemother., p. 22/26-22/32, 1984; W. Stille, A. Rahmel-Pitzer, and P. M. Shah, Proc. 13th Int. Congr. Chemother., p. 3/1-3/13, 3/56-3/59, 1984). After the administration of 500 mg of ampicillin intravenously, a fecal concentration of 6.4 µg/g was obtained (13). Most parenteral cephalosporins achieve high concentrations in the bile and significantly influence the fecal flora (9). Clavulanic acid and sulbactam were found to be moderately active in vitro against *C. jejuni*. The mean concentration of sulbactam in the feces of volunteers was 5.3 µg/g after the administration of 500 mg of ampicillin plus sulbactam; it is worth noting that the concentration in the mucosa was 13 µg/g (13). Trimethoprim and co-trimoxazole remain the drugs of choice for the treatment of shigellosis and have been shown to be effective in the treatment of traveler's diarrhea (12, 15), although no significant in vitro activity was demonstrated against *C. jejuni* (21, 22). After the oral administration of co-trimoxazole, both drugs were found in the feces, although precise concentrations were not obtained (Stille et al., 13th Int. Congr. Chemother.). More-

TABLE 2. Effect of inocula on the in vitro susceptibility of 10 *C. jejuni* strains

Antibiotic	Inoculum	No. of strains for which there was increase in the MIC of the indicated dilution in comparison with the MIC obtained at 10 ⁴ CFU/spot		
		0	1	2
Erythromycin	10 ⁵	9	1	
	10 ⁶	0	9	1
Ampicillin	10 ⁵	7	3	
	10 ⁶	4	6	
Amoxicillin	10 ⁵	5	5	
	10 ⁶	2	8	
Cefotaxime	10 ⁵	6	4	
	10 ⁶	1	9	
Sulfamethoxazole	10 ⁵	7	3	
	10 ⁶	1	9	
Co-trimoxazole	10 ⁵	0	9	
	10 ⁶	0	7	
Ampicillin + clavulanic acid	10 ⁵	7	3	
	10 ⁶	6	4	
Amoxicillin + clavulanic acid	10 ⁵	7	3	
	10 ⁶	2	8	
Cefotaxime + clavulanic acid	10 ⁵	7	3	
	10 ⁶	3	7	
Enoxacin	10 ⁵	9	1	
	10 ⁶	0	9	1
Ciprofloxacin	10 ⁵	8	2	
	10 ⁶	3	7	
Ansamycin	10 ⁵	8	2	
	10 ⁶	0	10	

over, the rapid colonization of the gut with co-trimoxazole-resistant organisms in decontaminated neutropenic patients suggests that significant amounts of the active drugs are present (23).

The possible implication of *C. jejuni* in the pathogenesis of chronic inflammatory bowel diseases prompted us to evaluate the in vitro activity of sulfasalazine (3). This drug is split in the gut into 5-aminosalicylic acid and sulfapyridine, a sulfonamide. This hydrolysis is nearly complete, since the fecal concentration of sulfasalazine is very low, and the fecal concentrations of sulfapyridine and 5-aminosalicylic acid are >1,000 mg/liter (P. Hoffmann, Pharmacia Belga S.A., personal communication). None of these molecules had any in vitro activity against *C. jejuni*, even at the concentrations achieved in stools.

C. jejuni has been implicated in more severe diseases, such as bacteremia, in compromised patients (7, 17, 19). In this setting, parenteral antibiotic treatment is mandatory. In the absence of comparative clinical studies on the treatment of *C. jejuni* bacteremia, erythromycin is usually considered to be the drug of choice, despite the 8% rate of resistance. Based on results of the present study, ampicillin, amoxicillin, new cephalosporins, or new quinolones might represent

useful alternatives. The addition of a β -lactamase inhibitor like clavulanic acid or sulbactam could not enhance the susceptibility of *C. jejuni* to β -lactam antibiotics, suggesting that the resistance of *C. jejuni* to cephalosporins and the moderate activity of penicillins could mainly be due to a permeability barrier. Although rifampin was totally inactive against *C. jejuni*, ansamycin, a new rifamycin compound (10), was shown to be the most active drug in vitro against *C. jejuni*.

The inoculum effect was found to be negligible in the range of 10⁴ to 10⁶ CFU per spot.

Most of the new drugs we tested demonstrated excellent in vitro activity against *C. jejuni*; some of them also have broad-spectrum activity against most bacterial enteropathogens. Therefore, clinical trials with these agents should be initiated to evaluate their efficacy in the treatment of human campylobacteriosis.

LITERATURE CITED

- Adam, D., I. de Visser, and P. Koeppe. 1982. Pharmacokinetics of amoxicillin and clavulanic acid administered alone and in combination. *Antimicrob. Agents Chemother.* 22:353-357.
- Barry, A. L. 1980. Procedures for testing antibiotics in agar media: theoretical considerations, p. 1-23. In V. L. Lorian (ed.), *Antibiotics in laboratory medicine*. The Williams & Wilkins Co., Baltimore.
- Blaser, M. J., D. Hoverson, I. G. Ely, D. J. Duncan, W.-L. L. Wang, and W. R. Brown. 1984. Studies of *Campylobacter jejuni* in patients with inflammatory bowel disease. *Gastroenterology* 86:33-38.
- Bornside, G. H., and I. Cohn, Jr. 1969. Intestinal antiseptics. *Gastroenterology* 57:569-573.
- Brumfitt, W., I. Franklin, D. Grady, J. M. T. Hamilton-Miller, and A. Iliffe. 1984. Changes in the pharmacokinetics of ciprofloxacin and fecal flora during administration of a 7-day course to human volunteers. *Antimicrob. Agents Chemother.* 26:757-761.
- Carlson, J. R., S. A. Thornton, H. L. DuPont, A. H. West, and J. J. Mathewson. 1983. Comparative in vitro activities of ten antimicrobial agents against bacterial enteropathogens. *Antimicrob. Agents Chemother.* 24:509-513.
- Cavanagh, P., and A. Ryden. 1978. *Campylobacter* isolated from hospital patients. *Med. J. Aust.* 2:435.
- Cofsky, R. D., L. duBouchet, and S. H. Landesman. 1984. Recovery of norfloxacin in feces after administration of a single oral dose to human volunteers. *Antimicrob. Agents Chemother.* 26:110-111.
- Conly, J. M., K. Ramotar, H. Chubb, E. J. Bow, and T. J. Louie. 1984. Hypoprothrombinemia in febrile, neutropenic patients with cancer; association with antimicrobial suppression of intestinal flora. *J. Infect. Dis.* 150:202-212.
- Della Bruna, C., G. Schioppacassi, D. Ungheri, D. Jabes, E. Morvillo, and A. Sanfilippo. 1983. LM 427, a new spiro-piperidyl-rifamycin: *in vitro* and *in vivo* studies. *J. Antibiot.* 36:1502-1506.
- Dicarlo, F. J., M. C. Crew, M. D. Melgar, S. Roemer, S. M. Ringer, L. J. Haynes, and M. Wilson. 1968. Oxolinic acid metabolism by man. *Arch. Int. Pharmacodyn. Ther.* 174: 413-417.
- Dupont, H. L., R. R. Reeves, E. Galindo, P. S. Sullivan, L. V. Wood, and J. G. Mendiola. 1982. Treatment of traveller's diarrhea with trimethoprim, sulfamethoxazole and with trimethoprim alone. *N. Engl. J. Med.* 307:841-844.
- Kager, L., L. Liljeqvist, A. S. Malmberg, C. E. Nord, and R. Pieper. 1982. Effects of ampicillin plus sulbactam on bowel flora in patients undergoing colorectal surgery. *Antimicrob. Agents Chemother.* 22:208-212.
- Muytjens, H. L., G. L. van Veldhuizen, G. W. Welling, J. van der Ros-van de Repe, H. B. J. Boerema, and D. van der Waaij. 1983. Selective decontamination of the digestive tract by pipemidic acid. *Antimicrob. Agents Chemother.* 24:902-904.
- Nelson, J. D., H. Kusmiesz, L. H. Jackson, and E. Woodman.

1976. Trimethoprim-sulfamethoxazole therapy for shigellosis. *J. Am. Med. Assoc.* **235**:1239-1243.
16. **Pai, C. H., F. Gillis, E. Tuomanen, and M. I. Marks.** 1983. Erythromycin in treatment of *Campylobacter* enteritis in children. *Am. J. Dis. Child.* **137**:286-288.
17. **Pepersack, F., T. Prigogyne, J. P. Butzler, and E. Yourassowsky.** 1979. *Campylobacter jejuni* post-transfusional septicaemia. *Lancet* **ii**:911.
18. **Pitkanen, T., T. Petterson, A. Ponka, and T. U. Kosunen.** 1982. Effects of erythromycin on the fecal excretion of *Campylobacter fetus* subspecies *jejuni*. *J. Infect. Dis.* **145**:128.
19. **Rettig, P. J.** 1979. *Campylobacter* infections in human beings. *J. Pediatr.* **94**:855-864.
20. **Robins-Browne, R. M., M. K. R. Mackenzie, M. N. Bodasing, and H. M. Cooradia.** 1983. Treatment of *Campylobacter*-associated enteritis with erythromycin. *Am. J. Dis. Child.* **137**:282-285.
21. **Vanhoof, R., M. P. Vanderlinden, R. Dierickx, S. Lauwers, E. Yourassowsky, and J. P. Butzler.** 1978. Susceptibility of *Campylobacter fetus* subsp. *jejuni* to twenty-nine antimicrobial agents. *Antimicrob. Agents Chemother.* **14**:553-556.
22. **Walder, M.** 1979. Susceptibility of *Campylobacter fetus* subsp. *jejuni* to twenty antimicrobial agents. *Antimicrob. Agents Chemother.* **16**:37-39.
23. **Wells, C. L., R. P. Podzorski, P. K. Peterson, N. K. Ramsay, R. L. Simmons, and F. S. Rhame.** 1984. Incidence of trimethoprim-sulfamethoxazole-resistant *Enterobacteriaceae* among transplant patients. *J. Infect. Dis.* **150**:699-706.