ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2003, p. 2027–2029 Vol. 47, No. 6
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.6.2027–2029.2003

Antimicrobial Resistance of *Campylobacter jejuni* subsp. *jejuni* Strains Isolated from Humans in 1998 to 2001 in Montréal, Canada

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Received 30 August 2002/Returned for modification 29 December 2002/Accepted 25 February 2003

The rates of resistance of 51 to 72 human strains of *Campylobacter jejuni* subsp. *jejuni* isolated annually from 1998 to 2001 in Montréal, Québec, Canada, varied from 1 to 12% for erythromycin, 43 to 68% for tetracycline, and 10 to 47% for ciprofloxacin. In the last years of the study, there was a significant increase in the rate of resistance to ciprofloxacin ($P = 0.00003$) but not in the rate of resistance to erythromycin ($P = 0.056$) or tetracycline ($P = 0.095$) compared to the rate obtained in the first years. All 51 *C. jejuni* strains isolated in 2001 were susceptible to gentamicin, amoxicillin–clavulanic acid, imipenem, and meropenem. From 1999 to 2001, 74 strains of *C. jejuni* acquired abroad were significantly more resistant to ciprofloxacin than 109 strains of *C. jejuni* acquired locally (66 versus 9%, $P < 0.00001$) but were not significantly more resistant to erythromycin (1 versus 6%, $P = 0.15$) or to tetracycline (55 versus 58%, $P = 0.87$).

Campylobacter jejuni subsp. *jejuni* is a major worldwide cause of enterocolitis (1, 3, 10). Fewer than 50% of cases of *C. jejuni* enterocolitis need to be treated with an antimicrobial agent (1). A macrolide is the first-choice agent, and a fluoroquinolone is the second-choice agent (1, 3, 10). The resistance of *C. jejuni* and *Campylobacter coli* alone (or that of both strains together) to fluoroquinolone increased significantly over the last 15 years, with a rate of 75 to 88% in Spain and Thailand (3, 9). In patients treated with a fluoroquinolone for *C. jejuni* enterocolitis, those infected with a resistant strain had a significantly longer disease than those infected with a susceptible strain (13). Up to 28% of the patients infected with a susceptible *C. jejuni* strain and treated with a fluoroquinolone harbor a resistant strain after treatment, with or without clinical failure (9). Many countries have reported *C. jejuni* strains with a low and stable rate of resistance to erythromycin; Taiwan and Spain, however, have reported that, respectively, 10 and 11% of their strains are resistant to macrolides (3).

The objectives of this study were to determine and to compare the annual rates of resistance to erythromycin, tetracycline, and ciprofloxacin of human strains of *C. jejuni* isolated from 1998 to 2001 in Montréal, Québec, Canada. The annual rates of resistance of these *C. jejuni* strains to two or three of these antimicrobial agents (multidrug resistance) were determined. The role of the nalidixic acid disk, as it is used for the identification of *Campylobacter* spp., as a marker of ciprofloxacin susceptibility was studied. Another purpose of this study was to determine whether there was a link between the resistance to ciprofloxacin and the resistance to tetracycline of *C. jejuni* strains. The resistance rate of the *C. jejuni* strains acquired abroad from 1999 to 2001 was compared to the resistance rate of those acquired locally in the same years. The *C. jejuni* strains were isolated at Hôpital Saint-Luc du Centre Hospitalier de l'Université de Montréal. All the *Campylobacter* sp. strains included in this study were microaerobic spiral gram-negative rods that were hippurate positive and resistant to cephalothin and were isolated at 42°C and grew well at this temperature, which confirmed their identification as *C. jejuni* subsp. *jejuni*. The identification tests were done by using the methods recommended by Nachamkin in the *Manual of Clinical Microbiology* (10). Nalidixic acid susceptibility was determined by using 30-μg disks as described previously for the identification of *Campylobacter* spp. (10): strains with an inhibition zone were considered susceptible, and those without such a zone were considered resistant (10). The strains were preserved at −70°C in a Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) to which 15% (vol/vol) glycerol was added.

The drugs' MICs for the *C. jejuni* strains were determined by using an agar dilution method as described previously (5). The concentrations of the antimicrobial agents tested were 0.06 to 128 μg/ml; a control plate without an antimicrobial agent was inoculated at the end of the procedure. The inocula were prepared in saline at a density adjusted to a 0.5 McFarland turbidity standard and diluted 1:10 for the agar dilution method. With a 3-mm-diameter Cathra replicator, a final inoculum of about 10^4 CFU, as determined previously by colony counting, was delivered onto Mueller-Hinton agar (Difco, Becton Dickinson Microbiology Systems, Sparks, Md.) plates without blood. The inoculated plates were incubated at 35°C under a microaerobic atmosphere obtained with a *Campylobacter* gas generator envelope (Becton Dickinson Microbiology Systems) for 48 h. The end point was taken as the complete inhibition of macroscopic growth. *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as control strains for the antimicrobial concentrations in the agar, as such an American Type Culture Collection *C. jejuni* quality control strain has become available only recently (2, 12). All of the strains were tested for their resistance and susceptibility to erythromycin, tetracycline (Sigma Chemical Co., St. Louis, Mo.), and cipro-

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TABLE 1. Annual single-drug and multidrug rates of resistance to three antimicrobial agents of *C. jejuni* strains isolated from 1998 to 2001

Agent(s)	Resistance rate[a] (%) of tested strains in:				*P* value[b]
	1998 (n = 62)	1999 (n = 60)	2000 (n = 72)	2001 (n = 51)	
Erythromycin[c]	3	2	1	12	0.056
Tetracycline[c]	43	48	68	51	0.095
Ciprofloxacin[c]	10	27	26	47	0.00003
Erythromycin-ciprofloxacin	0	2	1	4	0.14
Ciprofloxacin-tetracycline	6	12	18	25	0.0029
Erythromycin-tetracycline	0	0	0	6	0.013
Erythromycin-tetracycline-ciprofloxacin	0	0	0	2	0.37

[a] *C. jejuni* strains for which the erythromycin, tetracycline, and ciprofloxacin MICs were ≥8, ≥16, and ≥4 μg/ml, respectively, were reported to be resistant. *n,* number of strains tested (one strain from each patient was tested).
[b] Chi-square test for trends from 1998 to 2001.
[c] MICs ranged from ≤0.06 to ≥128 μg/ml.

floxacin (Bayer Leverkusen). The strains isolated in 2001 were tested for their resistance and susceptibility to gentamicin (Sigma Chemical Co.), imipenem (Merck & Co., West Point, Pa.), meropenem (AstraZeneca), and amoxicillin-clavulanic acid (Sigma Chemical Co. and SmithKline Beecham Pharmaceuticals). The MIC interpretive criteria were those of the National Committee for Clinical Laboratory Standards for erythromycin MICs for *Staphylococcus* spp. and for MICs of all other drugs for *Enterobacteriaceae* (11). These are the criteria used in most studies (9), since no such criteria are available for *C. jejuni* (11, 12).

The significance of differences in resistance was analyzed by using the chi-square test or by using the chi-square test for trends or Fisher's exact two-tailed test with Epi Info software (version 6.0; Centers for Disease Control and Prevention, Atlanta, Ga.). A *P* value of 0.05 or less was considered statistically significant.

In Table 1 are reported, for each year of the study, the rates of resistance of the *C. jejuni* strains to each or to a combination (multidrug resistance) of the three antimicrobial agents tested. Fifty-one to 72 *C. jejuni* strains, one strain from each patient being tested, were isolated annually from 1998 to 2001. The annual resistance rates of *C. jejuni* strains varied from 1 to 12% for erythromycin, 43 to 68% for tetracycline, and 10 to 47% for ciprofloxacin. There was a significant increase in the rate of resistance to ciprofloxacin ($P = 0.00003$) but not to erythromycin ($P = 0.056$) or to tetracycline ($P = 0.095$) among *C. jejuni* strains isolated in the last years of the study compared to the resistance rates of *C. jejuni* strains isolated in the first years. The annual rate of multidrug resistance varied from 0 to 4% for erythromycin and ciprofloxacin, 6 to 25% for ciprofloxacin and tetracycline, 0 to 6% for erythromycin and tetracycline, and 0 to 2 for erythromycin, ciprofloxacin, and tetracycline. When multidrug rates of resistance of the *C. jejuni* strains isolated in the first years of the study were compared to the rates of the strains isolated in the last years of the study, there were significant increases in the rates of multidrug resistance to ciprofloxacin and tetracycline ($P = 0.0029$) and to erythromycin and tetracycline ($P = 0.013$) but not to erythromycin and ciprofloxacin ($P = 0.14$) or to erythromycin, ciprofloxacin, and tetracycline ($P = 0.37$). From 1999 to 2001, 10 patients infected with a locally acquired erythromycin- and ciprofloxacin-resistant, tetracycline-susceptible *C. jejuni* strain were documented; nine of these patients' infections were from an outbreak documented by molecular studies (data not shown). One of these cluster strains was included in each of the annual resistance datum reports produced in 1999, 2000, and 2001. In 2001, one patient was infected with a locally acquired erythromycin-, tetracycline-, and ciprofloxacin-resistant strain. All 51 *C. jejuni* strains isolated in 2001 were susceptible to gentamicin, amoxicillin-clavulanic acid, imipenem, and meropenem; MICs at which 90% of the strains were inhibited were 1 μg/ml for gentamicin and ≤0.06 μg/ml for the three other antimicrobial agents. All 65 *C. jejuni* strains resistant to nalidixic acid were resistant to ciprofloxacin, and all 180 *C. jejuni* strains susceptible to nalidixic acid were susceptible to ciprofloxacin (MIC ≤ 1 μg/ml). Thirty-seven of the 65 *C. jejuni* strains resistant to ciprofloxacin and 93 of the 180 *C. jejuni* strains susceptible to ciprofloxacin were resistant to tetracycline ($P = 0.56$). The results of the rates of resistance to three antimicrobial agents of *C. jejuni* strains acquired abroad compared to the rates of resistance of those acquired locally from 1999 to 2001 are reported in Table 2. The 74 *C. jejuni* strains acquired abroad were significantly more resistant to ciprofloxacin than the 109 *C. jejuni* strains acquired locally (66 versus 9%; $P < 0.00001$) but were not significantly more resistant to erythromycin (1 versus 6%; $P = 0.15$) or to tetracycline (55 versus 58%; $P = 0.87$).

Antimicrobial susceptibility testing with an agar dilution of *Campylobacter* spp. has been standardized recently with regard to the medium, inoculum, incubation characteristics, and American Type Culture Collection *C. jejuni* quality control strain (2, 12). Antimicrobial susceptibility testing using the disk diffusion test and the Etest or β-lactamase test with *Campylobacter* spp. is not standardized (9, 10, 12). The disk diffusion test and the Etest have been demonstrated to be reliable and convenient methods (4, 7, 9). Since 1995, for each *C. jejuni* strain isolated in our laboratory, the nalidixic acid disk test has been performed for identification as well as used as a marker of quinolone susceptibility. After the 24-h subculture of each strain, the disk diffusion test is performed for erythromycin, tetracycline, and ciprofloxacin. If a strain is resistant to nalidixic acid, a ciprofloxacin Etest is done. The erythromycin resistance as determined by disk diffusion is confirmed by the

TABLE 2. Comparison between the rates of resistance to three antimicrobial agents of *C. jejuni* strains acquired abroad and the rates of resistance of those acquired locally from 1999 to 2001

Agent	Resistance rate[a] (%) of strains acquired:		*P* value[b]
	Abroad (n = 74)	Locally (n = 109)	
Erythromycin	1	6	0.15
Tetracycline	55	58	0.87
Ciprofloxacin	66	9	<0.00001

[a] *C. jejuni* strains for which the erythromycin, tetracycline, and ciprofloxacin MICs were ≥8, ≥16, and ≥4 μg/ml, respectively, were reported to be resistant. One strain from each patient was tested.
[b] Chi-square or Fisher's exact two-tailed test.

Etest. Agar dilution is done once a year with all the *C. jejuni* strains isolated. If there is a discrepancy between results obtained by disk diffusion and agar dilution, both procedures are repeated with these strains, as is the nalidixic acid disk test if the discrepancy is for ciprofloxacin. If necessary, the Etest will be done for these strains. The susceptibility criteria for disk diffusion are those of the National Committee for Clinical Laboratory Standards for aerobic organisms since such criteria are unavailable for *Campylobacter* spp., and these are the criteria used in most studies (9). The classification of our *C. jejuni* strains as susceptible, intermediate, or resistant is based on the results of two to four methods, and the methodology of these procedures has remained the same in our laboratory since 1985.

The recent development of resistance of strains of *Campylobacter* spp. to erythromycin was reported in Thailand and Sweden (3, 6). In a previous study, we did not find resistance to erythromycin in 47, 86, and 158 human *C. jejuni* strains isolated in 1985 to 1986, 1992 to 1993, and 1995 to 1997, respectively (5). Several worldwide studies showed a significant increase in resistance to the quinolones of the *C. jejuni* strains (3, 9), as was found in our strains isolated during three time periods from 1985 to 1997 (5) and in those isolated from 1998 to 2001. A high resistance level of *C. jejuni* strains to tetracycline has been reported in many studies, and a level up to 95% was reported in Taiwan in a review by Nachamkin et al. (9). In Minnesota, among patients who did not use quinolones before the collection of stool specimens, 59% who had traveled abroad and 11% who had not had a resistant strain and quinolone treatment accounted for no more than 15% of the ciprofloxacin-resistant *C. jejuni* strains (13). In our 1999 to 2001 study, it was impossible to identify the patients who had received a quinolone before the culture of their stools; even if strains isolated from these patients had been excluded, the difference between the rate of resistance to ciprofloxacin of *C. jejuni* strains acquired abroad (66%) and the rate of resistance to ciprofloxacin of strains acquired locally (9%) is so large that it would probably still be significant. In 1994 and 1995 in Thailand, all *Campylobacter* strains resistant to azithromycin (15% of the strains in 1994 and 7% of the strains in 1995) were also resistant to ciprofloxacin (6). The resistance of *C. jejuni* to both macrolides and quinolones is of major concern, these drugs being the first- and second-choice antimicrobial agents for the treatment of *C. jejuni* enterocolitis (3, 9, 14). Alternative agents for antimicrobial treatment of *C. jejuni* enterocolitis are tetracycline, for which a high resistance rate has been reported in most studies (5, 9), and amoxicillin-clavulanic acid, for which no, or a low, resistance rate has been reported (8, 15). Even if a low percentage of *C. jejuni* strains are resistant to ampicillin (8), this antimicrobial agent is not recommended for the treatment of *C. jejuni* infections (1). The β-lactamase produced by 83 to 92% of these bacteria contributed to the resistance to ampicillin, amoxicillin, and ticarcillin and was inhibited by clavulanic acid but not by tazobactam or by sulbactam (8). In this study, the nalidixic acid disk, as used for the identification of *Campylobacter* spp., was a predictor for *C. jejuni* susceptibility to ciprofloxacin, as was found in other surveys (5, 9). There was no link between ciprofloxacin and tetracycline resistance in these strains, as reported previously (5).

With the increased drug resistance of *C. jejuni* strains, it becomes more important that antimicrobial susceptibility testing of each isolate be done routinely by laboratories (9). The monitoring of the antimicrobial resistance of *Campylobacter* spp. from different geographic regions or countries is a priority (3, 6, 9).

We thank Brigite Chevrier for her secretarial services and the personnel of the bacteriology section of the Medical Microbiology Laboratory for its technical assistance.

AstraZeneca Canada Inc. contributed in part to the realization of this study.

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