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Antimicrobial Resistance of Clinical Strains of *Campylobacter jejuni* subsp. *jejuni* Isolated from 1985 to 1997 in Quebec, Canada

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The antimicrobial resistance of 158 *Campylobacter jejuni* strains isolated from humans in Quebec, Canada, from 1995 to 1997 was compared to the resistance of 47 and 86 strains of *C. jejuni* isolated in 1985 and 1986 and in 1992 and 1993, respectively. Of the 291 *C. jejuni* strains tested, no strain was resistant to erythromycin. Compared to the *C. jejuni* strains isolated in 1985 and 1986, the *C. jejuni* strains isolated in 1992 and 1993 were more resistant to tetracycline (40.7 versus 19.1%, respectively; $P = 0.01$) but not to nalidixic acid or ciprofloxacin ($P > 0.05$). Compared to the *C. jejuni* strains isolated in 1992 and 1993 and in 1985 and 1986, the *C. jejuni* strains isolated from 1995 to 1997 were more resistant to tetracycline (55.7% versus 40.7 and 19.1%, respectively; $P = 0.03$ and $P < 0.001$, respectively) to nalidixic acid (13.9% versus 4.7 and 0%, respectively; $P = 0.02$ and $P = 0.007$, respectively), and to ciprofloxacin (12.7% versus 3.5 and 0%, respectively; $P = 0.02$ and $P = 0.009$, respectively).

Campylobacter jejuni subsp. *jejuni* is a major cause of human bacterial diarrhea and, occasionally, of systemic illness (1, 10). Studies in many European countries had shown that *C. jejuni* has increased levels of resistance to the newer fluoroquinolones but an almost stable level of resistance to erythromycin (2, 14, 15). Antimicrobial susceptibility testing of *Campylobacter* spp. is not standardized (10, 11), and the agar dilution method is the reference method (11). The levels of resistance to ciprofloxacin, erythromycin, and tetracycline were 0, 2, and 55%, respectively, for 130 *C. jejuni* and 12 *Campylobacter coli* strains isolated from 1982 to 1992 in Pennsylvania (9). In Ontario, Canada, 13.6% of 309 *C. jejuni* strains isolated from May 1992 to December 1994 were reported to be resistant to nalidixic acid, and 84% of these isolates were resistant to ciprofloxacin (5).

The aim of the study described here was to determine the frequency of resistance of 158 consecutive *C. jejuni* strains isolated from humans in Quebec, Canada, from 1995 to 1997 and to compare these results with those for 47 and 86 consecutive *C. jejuni* strains isolated in 1985 and 1986 and in 1992 and 1993, respectively. The *C. jejuni* strains were isolated at Campus Saint-Luc, Centre Hospitalier de l'Université de Montréal. The strains were identified by standard methods (10) and were preserved at −70°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 15% (vol/vol) glycerol.

The resistance of the *C. jejuni* strains was determined by an agar dilution method as described previously (7); the concentrations of the antibiotics tested were 0.06 to 256 μg/ml, and a control plate without antibiotic was inoculated at the end of the procedure. Inocula were prepared in a Mueller-Hinton broth (BBL Microbiology Systems) at a density adjusted to a 0.5 McFarland turbidity standard for the disk diffusion method and diluted 1:10 for the agar dilution method. With a 3-mm Cathra replicator, a final inoculum of about 10^4 CFU was delivered onto Mueller-Hinton agar plates (Difco Laboratories, Detroit, Mich.). The same medium was used for the disk diffusion testing. The inoculated plates were incubated at 35°C under a microaerophilic atmosphere obtained with a gas generator envelope (Difco Laboratories) for 48 h. For the agar dilution method, the endpoint was taken as the complete inhibition of macroscopic growth. The following control strains were used: *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. The following antibiotics were tested by the agar dilution method: ciprofloxacin, erythromycin, and tetracycline (Sigma Chemical Co., St. Louis, Mo.); the susceptibility criteria were those of the National Committee for Clinical Laboratory Standards (11). The *C. jejuni* strains were tested for nalidixic acid susceptibility by the disk diffusion method; strains with an inhibition zone were considered susceptible to nalidixic acid; those without such a zone were considered resistant (3, 10). Nalidixic acid (30 μg; Oxoid, Unipath Ltd., Basingstoke, Hampshire, England) disks were used.

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

The results of the resistance of the *C. jejuni* strains isolated from 1995 to 1997 to four antibiotics are reported in Table 1 and are compared to the results of the resistance of the *C. jejuni* strains isolated in 1985 and 1986 and in 1992 and 1993. Of the 291 *C. jejuni* strains from the three time periods tested, no strain was found to be resistant to erythromycin. Compared to *C. jejuni* strains isolated in 1985 and 1986, the *C. jejuni* strains isolated in 1992 and 1993 were significantly more resistant to tetracycline (40.7 and 19.1%, respectively; $P = 0.01$) but not to nalidixic acid or ciprofloxacin ($P > 0.05$). *C. jejuni* strains isolated from 1995 to 1997 were significantly more resistant than the *C. jejuni* strains isolated in 1992 and 1993 and in 1985 and 1986 to tetracycline (55.7% versus 40.7 and 19.1%, respectively; $P = 0.03$ and $P < 0.001$, respectively), nalidixic acid (13.9% versus 4.7 and 0%, respectively; $P = 0.02$ and $P = 0.007$, respectively), and ciprofloxacin (12.7% versus 3.5 and 0%, respectively; $P = 0.02$ and $P = 0.009$, respectively). The *C. jejuni* strains isolated in 1996 were significantly more resistant than those isolated in 1995 to nalidixic acid (11 of 51 versus 4

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TABLE 1. Resistance of *C. jejuni* subsp. *jejuni* to four antibiotics[a]

Antimicrobial agent	Rate of resistance (%) of *C. jejuni* strains from the following time periods[b]:		
	1985–1986 (n = 47)	1992–1993 (n = 86)	1995–1997 (n = 158)
Erythromycin	0	0	0
Tetracycline	19.1	40.7[c]	55.7[d]
Nalidixic acid	0	4.7	13.9[e]
Ciprofloxacin	0	3.5	12.7[f]

[a] One strain from each patient was tested.

[b] The resistance breakpoints are those of the National Committee for Clinical Laboratory Standards (11) for the agar dilution method: ≥8 μg/ml for erythromycin, ≥16 μg/ml for tetracycline, and ≥4 μg/ml for ciprofloxacin and nalidixic acid (see text).

[c] For *C. jejuni* strains from 1992 and 1993 versus *C. jejuni* strains from 1985 and 1986, $P = 0.01$.

[d] For *C. jejuni* strains from 1995 to 1997 versus *C. jejuni* strains from 1992 and 1993 ($P = 0.03$) and *C. jejuni* strains from 1985 and 1986, $P = 0.03$ and $P < 0.001$, respectively.

[e] For *C. jejuni* strains from 1995 to 1997 versus *C. jejuni* strains from 1992 and 1993 and *C. jejuni* strains from 1985 and 1986, $P = 0.02$ and $P = 0.007$, respectively.

[f] For *C. jejuni* strains from 1995 to 1997 versus *C. jejuni* strains from 1992 and 1993 and *C. jejuni* strains from 1985 and 1986, $P = 0.02$ and $P = 0.009$, respectively.

of 52, respectively; $P = 0.046$) and ciprofloxacin (10 of 51 versus 3 of 52, respectively; $P = 0.03$). The *C. jejuni* strains isolated in 1997 were not more resistant than the *C. jejuni* strains isolated in 1996 and in 1995 to nalidixic acid (7 of 55 versus 11 of 51 and 4 of 52, respectively; $P = 0.2$ and $P = 0.4$, respectively) and ciprofloxacin (7 of 55 versus 10 of 51 and 3 of 52, respectively; $P = 0.3$ and $P = 0.3$, respectively). Ten of the 23 strains resistant to ciprofloxacin and 122 of the 268 strains susceptible to ciprofloxacin were resistant to tetracycline ($P = 0.9$). In 1996, two strains of *C. jejuni* were isolated from the same patient 1 month apart. For the first strain there was a zone around the nalidixic acid disk and the ciprofloxacin MIC was 0.06 μg/ml or less; for the second strain there was no zone around the nalidixic acid disk and the ciprofloxacin MIC was 2 μg/ml by the agar dilution method. This patient was treated with ciprofloxacin after the isolation of the first *C. jejuni* strain. The pattern of susceptibility to nalidixic acid for these two strains was confirmed by the Laboratoire de Santé Publique du Québec and by the Laboratory Centre for Disease Control (LCDC), Ottawa, Ontario, Canada. These two strains were not typeable by the Lior serotyping system but were shown by LCDC to be the same strain by pulsed-field gel electrophoresis.

In this study, no strain of *C. jejuni* was found to be resistant to erythromycin. In previous surveys, resistance to erythromycin is reported in 0 to 12.6% of *C. jejuni* strains but most often in fewer than 5% of such strains (9, 10, 13, 14). In Ontario and Alberta, Canada, the rates of *C. jejuni* resistance to erythromycin were reported to be 1 and 0%, respectively, in the 1980s (5). The microbiological and clinical efficacies of erythromycin for the treatment of *C. jejuni* enterocolitis have been demonstrated previously (1, 10). We found resistance to tetracycline in 55.7% of *Campylobacter* spp. isolated from 1995 to 1997. Others reported that between 0 and 60% of *C. jejuni* strains were resistant to tetracycline (9, 14, 16). The *C. jejuni* strains isolated from 1995 to 1997 were significantly more resistant to the quinolones tested than the *C. jejuni* strains isolated in 1985 and 1986 and in 1992 and 1993. The *C. jejuni* strains isolated in 1996 were significantly more resistant than those isolated in 1995 to nalidixic acid (21.6 versus 7.7%) and ciprofloxacin (19.6 versus 5.8%). In previous surveys (2, 12–14), the rate *C. jejuni* resistance to ciprofloxacin increased from 0% to 2 to 50%. In a survey of 155 to 161 *C. jejuni* strains isolated from 1980 to 1983 in five Montréal area centers, 0.6, 0.6, 0, and 14.5% of the strains were resistant to erythromycin, nalidixic acid, norfloxacin, and tetracycline, respectively (8). In the United States, 0, 2, and 55% of *Campylobacter* spp. isolated from 1982 to 1992 were resistant to ciprofloxacin, erythromycin, and tetracycline, respectively (9). In Ontario, of 309 *C. jejuni* strains isolated from May 1992 to December 1994, 13.6% of the strains were resistant to nalidixic acid: 17% in 1992, 10.4% in 1993, and 14.5% in 1994. Eighty-four, 7, and 16% of the nalidixic acid-resistant strains were resistant to ciprofloxacin, erythromycin, and tetracycline, respectively; erythromycin and tetracycline resistance was found only among the quino lone-resistant strains (5). We found no association between ciprofloxacin and tetracycline resistance in *C. jejuni*. Similar to other reports (2, 3, 13), nalidixic acid was a marker for *C. jejuni* susceptibility to ciprofloxacin; in this study, the 265 nalidixic acid-susceptible strains were susceptible to ciprofloxacin; of the 26 nalidixic acid-resistant strains, 23, 2, and 1 of the strains were resistant, intermediate, and susceptible to ciprofloxacin, respectively. The MICs for ciprofloxacin-resistant *C. jejuni* strains are 8 to 256 times higher than those for the susceptible ones (4, 15). Microbiological failure with or without clinical failure has been reported for ciprofloxacin-treated patients infected with *C. jejuni* strains that developed resistance during treatment (12, 15). Even if a low percentage of *Campylobacter* spp. were found to be resistant to ampicillin, between 83 and 92% of the *C. jejuni* strains produced a β-lactamase (7). In a previous study, we found β-lactamase production in 89.3% of 159 *C. jejuni* strains isolated from 1980 to 1983 (8). The β-lactamase-positive strains of *C. jejuni* were significantly less susceptible to ampicillin, amoxicillin, and ticarcillin than the β-lactamase-negative ones (7). Ampicillin is not recommended for use in the treatment of *C. jejuni* infections (1, 10). Amoxicillin-clavulanic acid had good in vitro activity against this bacterium (7, 14). Antimicrobial susceptibility testing of *Campylobacter* spp. needs to be standardized. The agar dilution method is the reference method (11), but the disk diffusion method and the Etest could be reliable and convenient methods (3, 6).

In our 10-year study, no resistance to erythromycin was found for *C. jejuni*, but there was a statistically significant increase in the levels of resistance to tetracycline, nalidixic acid, and ciprofloxacin for the strains studied during the three time periods over those 10 years.

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REFERENCES

1. **Blaser, M. J.** 1995. *Campylobacter* and related species, p. 1948–1956. *In* G. L. Mandell, J. E. Doug, and R. Dolin (ed.), Principles and practice in infectious diseases, 4th ed. Churchill Livingstone, New York, N.Y.
2. **Endtz, H. P., G. J. Ruijs, B. van Klingeren, W. H. Jansen, T. van der Reyden, and R. P. Mouton.** 1991. Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J. Antimicrob. Chemother. **27:**199–208.
3. **Gaudreau, C., and H. Gilbert.** 1997. Comparison of disk diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*. J. Antimicrob. Chemother. **39:**707–712.
4. **Gootz, T. D., and B. A. Martin.** 1991. Characterization of high-level quinolone resistance in *Campylobacter jejuni*. Antimicrob. Agents Chemother. **35:**840–845.
5. **Harnett, N., S. McLeod, Y. Au Yong, C. Hewitt, M. Vearncombe, and C. Krishnan.** 1995. Quinolone resistance in clinical strains of *Campylobacter jejuni* and *Campylobacter coli*. J. Antimicrob. Chemother. **36:**269–270.
6. **Huang, M. B., C. N. Baker, S. Banerjee, and F. C. Tenover.** 1992. Accuracy

of the E test for determining antimicrobial susceptibilities of staphylococci, enterococci, *Campylobacter jejuni*, and gram-negative bacteria resistant to antimicrobial agents. J. Clin. Microbiol. **30:**3243–3248.
7. **Lachance, N., C. Gaudreau, F. Lamothe, and L. A. Larivière.** 1991. Role of the β-lactamase of *Campylobacter jejuni* in resistance to β-lactam agents. Antimicrob. Agents Chemother. **35:**813–818.
8. **Larivière, L. A., C. L. Gaudreau, and F. F. Turgeon.** 1986. Susceptibility of clinical isolates of *Campylobacter jejuni* to twenty-five antimicrobial agents. J. Antimicrob. Chemother. **18:**681–685.
9. **Nachamkin, I.** 1994. Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli* to ciprofloxacin, erythromycin and tetracycline from 1982 to 1992. Med. Microbiol. Lett. **3:**300–305.
10. **Nachamkin, I.** 1995. *Campylobacter* and *Arcobacter*, p. 483–491. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
11. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard. NCCLS publication no. M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. **Piddock, L. J. V.** 1995. Quinolone resistance and *Campylobacter* spp. J. Antimicrob. Chemother. **36:**891–898.
13. **Rautelin, H., O.-V. Renkonen, and T. U. Kosunen.** 1991. Emergence of fluoroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli* in subjects from Finland. Antimicrob. Agents Chemother. **35:**2065–2069.
14. **Reina, J., M. J. Ros, and A. Serra.** 1994. Susceptibilities to 10 antimicrobial agents of 1,220 *Campylobacter* strains isolated from 1987 to 1993 from feces of pediatric patients. Antimicrob. Agents Chemother. **38:**2917–2920.
15. **Segreti, J., T. D. Gootz, L. J. Goodman, G. W. Parkhurst, J. P. Quinn, B. A. Martin, and G. M. Trenholme.** 1992. High-level quinolone resistance in clinical isolates of *Campylobacter jejuni*. J. Infect. Dis. **165:**667–670.
16. **Tenover, F. C., C. N. Baker, C. L. Fennell, and C. A. Ryan.** 1992. Antimicrobial resistance in *Campylobacter* species, p. 66–73. *In* I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni* current status and future trends. American Society for Microbiology, Washington, D.C.