JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1987, p. 1807–1808
0095-1137/87/091807-02$02.00/0

Vol. 25, No. 9

Problems in Identification of *Campylobacter jejuni* Associated with Acquisition of Resistance to Nalidixic Acid

M. ALTWEGG,[1]* A. BURNENS,[1] J. ZOLLINGER-ITEN,[1] AND J. L. PENNER[2]

Department of Medical Microbiology, University of Zurich, Zurich, Switzerland,[1] and Department of Microbiology, University of Toronto, Toronto, Ontario, Canada[2]

Received 28 April 1987/Accepted 10 June 1987

In a patient with campylobacteriosis, we observed development of resistance to nalidixic acid and norfloxacin under adequate treatment with the latter antibiotic. Isolates before and after treatment differed in their total protein profile but were otherwise identical with respect to enzymatic activity, serotype, crude membrane protein profiles, and other phenotypic characteristics.

Campylobacter jejuni and *Campylobacter coli* are the predominant species associated with human intestinal campylobacteriosis. They can be distinguished from each other as well as from other catalase-positive members of the genus on the basis of a limited number of phenotypic characteristics (2). Susceptibility to nalidixic acid is important for diagnostic purposes as well as for the management of the disease, because this drug and its derivatives are increasingly being used for the treatment of enteric infections with multiresistant *Shigella* strains (9) as well as in situations in which cultures are not readily available, e.g., traveler's diarrhea (1, 3).

(Results of this study were presented at the 87th Annual Meeting of the American Society for Microbiology, Atlanta, Ga., 1 to 6 March 1987 [M. Altwegg and A. Burnens, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C-166, p. 351].)

We observed development of resistance to nalidixic acid and norfloxacin in a *C. jejuni* strain isolated from a 29-year-old female patient who returned from Italy on 15 April 1986. The next day, she suffered from profuse, watery diarrhea and high fever. On 18 April, microscopical stool analysis yielded many fecal leukocytes but no parasites. At the same time, a specimen for bacterial cultures was taken and treatment with norfloxacin (400 mg three times a day for 7 days) was initiated. Cultures were negative for species of the genera *Salmonella*, *Shigella*, and *Yersinia*, but nalidixic acid-susceptible *C. jejuni* was isolated (isolate P1). On 19 April, the patient became afebrile and diarrhea stopped. Control stool cultures taken from an asymptomatic patient on 28, 29, and 30 April each yielded *C. jejuni* resistant to nalidixic acid in disk diffusion tests (isolates P2 to P4, respectively).

The question arose whether the patient was infected simultaneously with two different *Campylobacter* strains, as has been described previously (7), or whether the strains isolated after clinically successful therapy had developed resistance to nalidixic acid and norfloxacin under therapy with the latter antibiotic. In addition to the characteristics documented in Table 1, the strains were shown to be identical by their enzymatic activity (API ZYM; API SA, La Balme-Les Grottes, France) and their crude membrane protein profiles (data not shown). The only difference found between isolate P1 and isolates P2 to P4 was a single band of approximately 50,000 daltons in the total protein profiles analyzed on a sodium dodecyl sulfate-12.5% polyacrylamide gel (Fig. 1).

From these results, we conclude that all four strains are identical and that development of resistance occurred after adequate treatment with norfloxacin. Whether the difference in whole-cell protein patterns is functionally related to this change in susceptibility is not known.

After storage of the strains for 6 months in fetal bovine serum-glycerol (1:1) at −70°C, only isolate P3 had survived and was available for determination of norfloxacin and nalidixic acid MICs (Table 2). Unexpectedly, P3 proved to be resistant to nalidixic acid but not to norfloxacin. These results had a correlation in the altered zone of inhibition in the disk test, the result of which was now 30 mm for norfloxacin (compared with only 6 mm corresponding to resistance upon initial testing). This zone size is very similar to that produced by isolate P1 in the initial test. Obviously, strain P3 had lost resistance to norfloxacin but not to nalidixic acid upon prolonged storage. This phenomenon points toward a two-step event involved in the development of resistance against newer quinolones. The exact mechanism(s) remains to be elucidated.

Until now there were only a few strains of *C. jejuni* known to be resistant to nalidixic acid (6, 10). Upon increased use of this drug and related compounds, as is recommended by different authors for prophylaxis and treatment of traveler's

TABLE 1. Characteristics of four *C. jejuni* isolates from one patient[a]

Isolate	Susceptibility to antimicrobial agents (disk test, diam in mm)[b]			
	CF-30	E-15	NA-30	NOR-10
P1	6	31	21	34
P2	6	32	6	6
P3	6	33	6	6
P4	6	33	6	6

[a] Hydrolysis of hippurate was determined by the method of Hwang and Ederer (5); all isolates produced positive results. Serotyping was done on the basis of thermostable (O) antigens by passive hemagglutination (8); all isolates were serotype O:37. CF, Cephalothin; E, erythromycin; NA, nalidixic acid; NOR, norfloxacin. The numbers after the drug abbreviations indicate the amounts of antibiotic per disk in micrograms.

* Corresponding author.

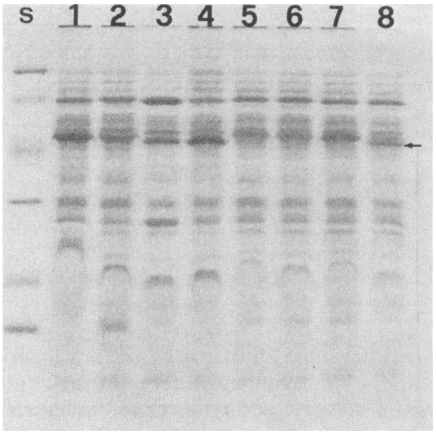

FIG. 1. Coomassie brilliant blue-stained sodium dodecyl sulfate-polyacrylamide gel of total protein preparations from *C. jejuni* strains. Lanes: 1 to 4, four different *C. jejuni* strains for controls; 5, *C. jejuni* P4; 6, *C. jejuni* P3; 7, *C. jejuni* P2; 8, *C. jejuni* P1; S, standard proteins of molecular weights of 92,500, 66,200, 45,000, 31,000, 21,500, and 14,400 (top to bottom). Note that the 50,000-molecular-weight band (arrow) in *C. jejuni* P1 is absent in *C. jejuni* P2 to P4.

TABLE 2. Determination of nalidixic acid and norfloxacin MICs after storage of strains (only strain P3 viable)

Strain	MIC (mg/liter)[a]		Disk test result (mm)	
	Nalidixic acid	Norfloxacin	Nalidixic acid	Norfloxacin
C. jejuni P3	32	0.25	6	30
C. jejuni control	8	0.25		
Escherichia coli ATCC 25922	2	0.06		

[a] MIC determined by agar dilution method.

diarrhea or with resistant *Shigella* strains (4), the number of *C. jejuni* strains resistant to quinolones may increase and therefore cause problems not only with respect to treatment but also with respect to species identification in the diagnostic laboratory. To circumvent the latter problems, testing for hippurate hydrolysis is necessary to identify nalidixic acid-resistant *C. jejuni*, whereas additional tests such as tolerance to 2,3,5-triphenyltetrazolium chloride (1 g/liter) (2) will have to be used to differentiate *C. coli* from *Campylobacter laridis*.

LITERATURE CITED

1. **Altwegg, M.** 1986. Resistance patterns in *Shigella*. Schweiz. Med. Wochenschr. **116**:1848–1851.
2. **Benjamin, J., S. Leaper, R. J. Owen, and M. B. Skirrow.** 1983. Description of *Campylobacter laridis*, a new species comprising the nalidixic acid resistant thermophilic *Campylobacter* (NARTC) group. Curr. Microbiol. **8**:231–238.
3. **DuPont, H. L., C. D. Ericsson, R. R. Reves, and E. Galindo.** 1986. Antimicrobial therapy for travelers' diarrhea. Rev. Infect. Dis. **8**(Suppl. 2):217–222.
4. **Holmes, B., R. N. Brogden, and D. M. Richards.** 1985. Norfloxacin: a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs **30**:482–513.
5. **Hwang, M.-N., and G. M. Ederer.** 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. J. Clin. Microbiol. **1**:114–115.
6. **Lariviere, L. A., C. L. Gandreau, and F. F. Turgeon.** 1986. Susceptibility of clinical isolates of *Campylobacter jejuni* to twenty-five antimicrobial agents. J. Antimicrob. Chemother. **18**:681–685.
7. **Lastovica, A. J., E. LeRoux, and J. L. Penner.** 1986. Mixed infections with different species and serotypes of *Campylobacter*. J. Infect. Dis. **154**:375.
8. **Penner, J. L., J. N. Hennessy, and R. V. Congi.** 1983. Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. Eur. J. Clin. Microbiol. **2**:378–383.
9. **Rogerie, F., D. Ott, J. Vandepitte, L. Verbist, P. Lemmens, and I. Habiyaremye.** 1986. Comparison of norfloxacin and nalidixic acid for treatment of dysentery caused by *Shigella dysenteriae* type 1 in adults. Antimicrob. Agents Chemother. **29**:883–886.
10. **Walder, M., K. Sandstedt, and J. Ursing.** 1983. Phenotypic characteristics of thermotolerant *Campylobacter* from human and animal sources. Curr. Microbiol. **9**:291–296.