Characterization of High-Level Quinolone Resistance in *Campylobacter jejuni*

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High-level resistance to quinolones has previously been shown to occur in *Campylobacter* spp. both in vitro and in patients treated with quinolones. We have selected isolates that are resistant to quinolones by plating cells from a susceptible *C. jejuni* strain, UA535, on medium containing nalidixic acid at 32 µg/ml. Fluctuation analysis indicated that resistance occurred by mutation at a frequency of 5×10^{-8} per cell plated. Unlike what is observed with other gram-negative organisms, the nalidixic acid-resistant mutants demonstrated high-level cross-resistance (MIC, ≥ 4 µg/ml) to newer quinolones, including ciprofloxacin, norfloxacin, and temafloxacin, yet remained susceptible to coumermycin A1 and several other unrelated antibiotics. Mutants with an identical resistance phenotype could also be selected from UA535 with ciprofloxacin and norfloxacin at a similar frequency. To study the mechanism of quinolone resistance, DNA gyrases were purified from *C. jejuni* UA535 and two resistant mutants by heparin-agarose and novobiocin-Sepharose chromatography. After the respective enzyme concentrations were adjusted to equivalent units of activity in the DNA supercoiling reaction, the DNA gyrases from the resistant mutants were found to be 100-fold less susceptible than the wild-type enzyme to inhibition by quinolones. Subunit switching experiments with purified A and B subunits from the wild type and one of the quinolone-resistant mutants indicated that an alteration in the A subunit was responsible for resistance. These results show that a single-step mutation can occur in vitro in the gene encoding DNA gyrase in *C. jejuni*, producing clinically relevant levels of resistance to the newer quinolones.

Campylobacter jejuni is a gram-negative, microaerophilic organism that has been cited as being the most common etiologic agent of infectious diarrhea in the United States (5). *C. jejuni*, like other *Campylobacter* spp., can colonize the intestinal tract of many animals, including poultry, cattle, and swine, without causing illness (3). In humans, ingestion of as few as 500 organisms can produce illness, normally characterized as diarrhea associated with abdominal pain, fever, and vomiting. Grossly bloody stools containing mucus and leukocytes are often present, but bacteremia is infrequent in individuals from developed countries (6). Infectious diarrhea caused by *C. jejuni* is usually a self-limiting disease lasting 3 to 5 days but can persist for up to 2 weeks or longer. Although the organism is susceptible to several antimicrobial agents (25), the effects of these agents on the course of the disease remain unclear.

Several studies have shown that *C. jejuni* isolates are susceptible in vitro to the new fluoroquinolones (4, 13, 23), and ciprofloxacin has been compared with other antimicrobial agents in a randomized, double-blinded fashion for treating patients with *Campylobacter*-associated diarrhea (12). Given this interest in quinolones for treatment of diarrhea caused by *C. jejuni*, the question of resistance to these agents becomes an issue, particularly given the reported increases in isolation rates of quinolone-resistant strains observed in Europe (8). In this regard, an earlier study (26) demonstrated that nalidixic acid-resistant mutants of *C. jejuni* UA535 could be selected in vitro in a single step from this quinolone-susceptible strain; these mutants demonstrated cross-resistance to enoxacin and ciprofloxacin, with MICs of 8 to 32 µg/ml. The purpose of the current study was to further characterize resistance to the newer quinolones in *C. jejuni* UA535. This investigation culminated in

the first isolation of DNA gyrase from this organism and identified the A subunit of the enzyme as the determinant of high-level resistance to nalidixic acid, ciprofloxacin, temafloxacin, and norfloxacin.

(This work was presented in part at the 3rd International Symposium on New Quinolones [13a].)

MATERIALS AND METHODS

Bacterial strains. The nalidixic acid-susceptible strain *C. jejuni* UA535 was kindly supplied by Diane Taylor, University of Alberta, Alberta, Canada, and has been described elsewhere (26). Quinolone-resistant mutants were derived from UA535 by the direct plating technique described below. All isolates were cultured on plates containing brain heart infusion agar with 3% sheep blood for 48 h at 37°C in an incubator supplemented with 8% CO_2 with 85% humidity or in gas jars in a CampyPak microaerophilic system (BBL, Cockeysville, Md.). Strains were confirmed as *C. jejuni* by typical reactions for oxidase, catalase, growth differential at 25 and 42°C, resistance to cephalothin, hippurate hydrolysis, and indoxyl acetate utilization.

Selection of resistant mutants. Isolation of quinolone-resistant mutants from *C. jejuni* UA535 was performed as previously described (26). A single colony was inoculated into 4 ml of brain heart infusion broth (BHIB; BBL), and the resulting overnight cell growth was pelleted by centrifugation (7,000 × *g* for 10 min). The cell pellet was suspended in 1/10 of the original volume of fresh medium. Aliquots of 0.1 ml were plated (10^9 CFU) on the surface of brain heart infusion agar containing 3% sheep blood and 32 µg of nalidixic acid per ml. Resistant colonies were counted after 48 h of incubation. Several representative colonies were selected and transferred three times on drug-containing medium to ensure culture purity. Resistant colonies were obtained in an identical manner from UA535 by using

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ciprofloxacin or norfloxacin as the selecting agent at concentrations 4- to 32-fold above the MIC.

Antibiotics. Antibiotics were obtained as follows: ciprofloxacin (Miles Inc., Pharmaceutical Div., West Haven, Conn.); temafloxacin (Abbott Laboratories, Abbott Park, Ill.); norfloxacin (Merck Sharp and Dohme, Rahway, N.J.); and nalidixic acid, oxolinic acid, coumermycin A1, novobiocin, kanamycin, ampicillin, and tetracycline (Sigma Chemical Co., St. Louis, Mo.).

Susceptibility studies. MICs were determined from an overnight culture in Mueller-Hinton broth (Difco, Detroit, Mich.) after dilution in the same medium to obtain an inoculum of 10^4 to 10^5 CFU per spot with a Steers replicator on the surface of Mueller-Hinton agar plates containing twofold dilutions of drug. All drugs were tested by the agar dilution method with incubation for 48 h as described above. Killing curves were generated with *C. jejuni* UA535 and one quinolone-resistant clone in BHIB incubated under static conditions in 8% CO_2 with and without ciprofloxacin as described previously (14).

Isolation of DNA gyrase. Cells from an overnight culture in BHIB were diluted 1:50 in fresh, prewarmed medium and incubated for 24 h in 8% CO_2 without agitation. A total of 20 liters of culture was used for each enzyme isolation. Cells were pelleted by centrifugation (7,000 × *g* for 10 min), and approximately 15 to 20 g (wet weight) of cells was suspended in 20 ml of TGED buffer (20 mM Tris-HCl [pH 7.5], 0.1 M KCl, 0.2 mM EDTA, 10% [wt/vol] glycerol, 5 mM dithiothreitol). The protease inhibitors sodium metabisulfite (10 mM) and phenylmethylsulfonyl fluoride (1 mM) were freshly prepared and added to the TGED buffer. The cell suspension was chilled on ice, and the cells were broken by sonication with 15 30-s pulses with an Ultrasonic Cell Disruptor (Braun Instruments, Burlingame, Calif.). The sonicated material was centrifuged at 12,000 × *g* for 30 min, and the protein content of the supernatant was adjusted to 10 mg/ml with TGED buffer (cleared sonicated material). The isolation of gyrase holoenzyme was accomplished by the method of Stadenbauer and Orr (24). Briefly, the cleared sonicated material was mixed overnight at 4°C with 35 ml of heparin-agarose (washed with TGED). The material was then poured into a column and washed with 2 column volumes of TGED; DNA gyrase was eluted with TGED containing 0.3 M KCl. Active fractions containing DNA supercoiling activity were pooled and dialyzed against HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer. The dialysate was applied to a novobiocin-Sepharose column, the column washed with 2 column volumes of HEPES buffer, and the holoenzyme was eluted with HEPES buffer containing 5 M urea. Individual fractions were dialyzed overnight at 4°C against TGED, and the supercoiling activity of the undiluted fractions was determined. To obtain GyrA and GyrB subunits separately, cleared cell sonicated material was added to a novobiocin-Sepharose column and then washed extensively with HEPES buffer containing 0.1 M KCl until no further UV-absorbing material eluted as determined by a Pharmacia UV absorbance monitor (Pharmacia, Piscataway, N.J.). The A subunit was eluted with a 0.1 to 1.5 M KCl gradient in HEPES buffer. This was followed by an extensive column wash with 2 M KCl in HEPES buffer. The B subunit was then eluted with 5 M urea in HEPES. The individual column fractions were dialyzed overnight against TGED. Active fractions for each subunit were identified in the supercoiling reaction by using a pooled dialysate of the opposite subunit. Active fractions were pooled, aliquoted, and stored at −70°C until needed.

DNA supercoiling assay. Relaxed plasmid pBR322 substrate DNA was prepared by treatment with calf thymus topoisomerase I as recommended by the manufacturer (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The DNA supercoiling assay was performed as described previously (16, 17). One unit of gyrase is defined as the amount of enzyme that completely supercoils 0.5 µg of relaxed pBR322 in 60 min at 30°C in the standard gyrase reaction mix. Approximately 1 U each of gyrase A and B subunit was used per reaction. Gyrase A and B subunits purified from *Escherichia coli* K-12 by the method of Mitzuuchi et al. (17) were a generous gift from M. Norcia. To test for interspecies complementation, 1 U of *E. coli* A subunit was mixed with 1 U of *C. jejuni* B subunit.

IC_{100} determinations. To determine the inhibitory effect of quinolones on gyrase supercoiling activity, each antibiotic was added to a series of supercoiling reaction mixes before the addition of enzyme (final volume, 25 µl). After incubation for 60 min at 30°C, the reaction was stopped by the addition of 3 µl of a solution containing 4.7% (wt/vol) sodium dodecyl sulfate, 55.7 mM disodium EDTA, 50% (wt/vol) glycerol, and 0.125 mM bromphenol blue. The entire sample was loaded onto a 0.7% TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA [pH 8.3])–agarose gel and electrophoresed at 60 to 70 V for 3 h. After staining with ethidium bromide, the amount of supercoiled DNA product formed was compared with that obtained in a drug-free control. The minimum effective concentration (MEC) was defined as the lowest concentration of drug to show any inhibition of supercoiling, whereas the IC_{100} was defined as the minimum concentration required to completely inhibit supercoiling.

RESULTS

Selection of resistant mutants. Nalidixic acid-resistant mutants of *C. jejuni* UA535 arose at a frequency of 5.0 × 10^{-8} per cell when plated on selective media containing 32 µg of nalidixic acid per ml. Fluctuation analysis performed on UA535 at this concentration of nalidixic acid indicated that resistance occurred by spontaneous mutation, rather than by adaptation (data not shown). Quinolone-resistant mutants were selected from UA535 in an identical manner with ciprofloxacin or norfloxacin as the selecting agent at concentrations up to 8-fold (ciprofloxacin) and 16-fold (norfloxacin) above the MIC. Mutants could not be isolated with either drug at concentrations above these levels with the direct plating method. The frequency of mutant isolation when UA535 was plated on medium containing norfloxacin at 2, 4, or 8 µg/ml was 1.3 × 10^{-8} to 2.5 × 10^{-8} per cell plated, whereas the frequency of mutation with ciprofloxacin at 1 or 2 µg/ml was 1.4 × 10^{-8} to 1.8 × 10^{-8}. For each selecting drug concentration that produced resistant colonies, 56 typical colonies were transferred three times on fresh medium containing the same concentration of selecting agent. Twelve isolates from each group were chosen for susceptibility testing with ciprofloxacin, norfloxacin, coumermycin A1, and tetracycline. All of the clones were cross-resistant to quinolones and remained susceptible to coumermycin A1 and tetracycline. Quinolone resistance in representative mutants was stably expressed after 10 sequential transfers on drug-free medium. UA535 and all quinolone-resistant mutants gave identical biochemical reactions and were oxidase and catalase positive, were positive for hippurate hydrolysis, hydrolyzed indoxyl acetate, were resistant to cephalothin, and grew at 42°C but not at 25°C.

The susceptibilities of UA535 and six representative mu-

TABLE 1. Susceptibilities of *C. jejuni* UA535 and quinolone-resistant mutants to several antibiotics[a]

Organism	MIC (μg/ml)								
	NAL	OXO	NFX	CPX	TEM	COU	KAN	AMP	TET
UA535	2	0.5	0.5	0.25	<0.125	0.5	8	8	0.25
UA535 Nal 32[b]	64	64	>32	16	4	0.5	8	8	0.25
UA535 Cpx 1	32	64	>32	16	4	0.5	8	8	0.25
UA535 Cpx 2	64	64	>32	16	4	0.5	8	8	0.25
UA535 Nfx 2	32	64	>32	16	4	0.5	8	8	0.25
UA535 Nfx 4	32	64	>32	16	4	0.5	8	8	0.25
UA535 Nfx 8	64	64	>32	16	4	0.5	8	8	0.25

[a] NAL, Nalidixic acid; OXO, oxolinic acid; NFX, norfloxacin; CPX, ciprofloxacin; TEM, temafloxacin; COU, coumermycin A1; KAN, kanamycin; AMP, ampicillin; TET, tetracycline.
[b] Mutants selected from quinolone-susceptible UA535 with nalidixic acid, ciprofloxacin, or norfloxacin at concentrations between 1 and 32 μg/ml.

tants to quinolones and several other antimicrobial agents are shown in Table 1. *C. jejuni* UA535 was susceptible to nalidixic acid and the newer quinolones, which had MICs of ≤2 μg/ml. Coumermycin A1, ampicillin, and tetracycline were also highly active against this strain. In contrast, the mutants isolated with nalidixic acid, ciprofloxacin, or norfloxacin were clearly resistant to all of the quinolones, with MICs ranging from 4 (temafloxacin) to >32 (norfloxacin) μg/ml. The MICs of nalidixic and oxolinic acid ranged from 32 to 64 μg/ml. The resistance levels observed for each drug appeared to be independent of the selecting agent. It was noteworthy that the susceptibility of the mutants, compared with that of UA535, did not change for the DNA gyrase B subunit inhibitor coumermycin A1 or for unrelated agents such as kanamycin, ampicillin, and tetracycline.

The selection of resistance in *C. jejuni* UA535 to DNA gyrase A subunit inhibitors raised the question of whether these agents were still bactericidal in vitro against such mutants when tested at the MIC. Figure 1 shows kill curve results for strain UA535 and the resistant strain UA535 Nal 32 grown in BHIB with and without ciprofloxacin. Results indicate that in the absence of drug, both isolates have very similar growth kinetics and that the respective MICs of ciprofloxacin produce a bactericidal effect on each.

Isolation of DNA gyrase holoenzymes. The antibiotic resistance phenotype of the mutants suggested that resistance was due to an alteration in DNA gyrase rather than to a general permeability defect, which would be expected to alter susceptibility to several unrelated drugs. To test this hypothesis, DNA gyrase was isolated from susceptible UA535 and two representative mutants, UA535 Nal 32 and UA535 Cpx 1. The holoenzyme was isolated from cell-free sonicated material by heparin-agarose and novobiocin-Sepharose chromatography. This treatment was necessary to separate active gyrase from nucleases present in the extracts that degrade substrate DNA in the supercoiling reaction. As shown in Fig. 2 and Table 2, gyrase isolated from resistant UA535 Nal 32 or UA535 Cpx 1 was significantly more resistant than that from UA535 to inhibition by ciprofloxacin or norfloxacin in the supercoiling assay. In all instances, the MECs were significantly higher than the corresponding MICs of each drug. In contrast, the supercoiling activity of the UA535 and mutant gyrases was equally susceptible to inhibition by the B-subunit inhibitor novobiocin (Table 2). These results agree with MIC data, which indicated that resistance was confined to nalidixic acid and quinolones.

DNA gyrase subunit switching. To identify the gyrase subunit responsible for conferring quinolone resistance, DNA gyrase subunits were isolated from UA535 and UA535

Nal 32 by novobiocin-Sepharose column chromatography. Studies confirmed that the individual A or B subunit alone could not supercoil relaxed pBR322, whereas reconstituted homologous and heterologous A and B subunits possessed significant supercoiling activity (data not shown). The effect of ciprofloxacin on the supercoiling activity of holoenzyme reconstituted from the separate subunits is shown in Fig. 3 and Table 3. Reconstituted holoenzyme containing mutant GyrA, in each instance, demonstrated resistance to inhibition by ciprofloxacin. Interestingly, the heterologous system of mutant GyrA combined with the UA535 GyrBs subunit was more resistant to supercoiling inhibition by ciprofloxacin than was gyrase reconstituted from homologous mutant subunits (Table 3).

Characterization of DNA gyrase holoenzyme. Isolates of *C. jejuni* have, in general, been shown to be less susceptible to
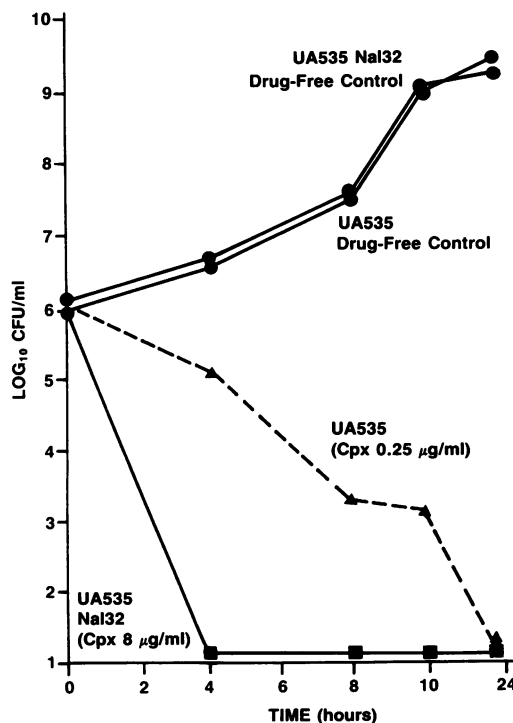

FIG. 1. Killing curve studies of *C. jejuni* UA535 and UA535 Nal 32 with and without ciprofloxacin (Cpx) at concentrations (in parenthesis) equal to the MICs in broth.
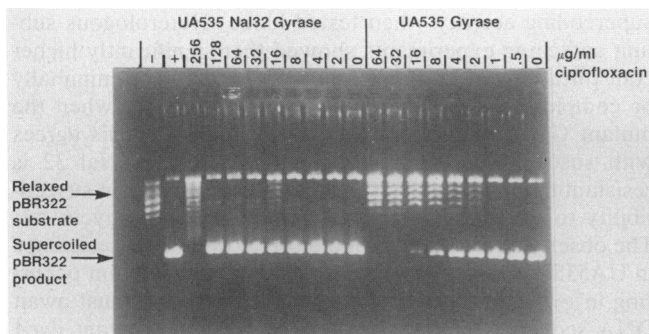
FIG. 2. Inhibition by ciprofloxacin of DNA gyrase supercoiling activity of holoenzyme purified from *C. jejuni* UA535 and UA535 Nal 32. First and second lanes represent relaxed substrate and supercoiled product pBR322, respectively.
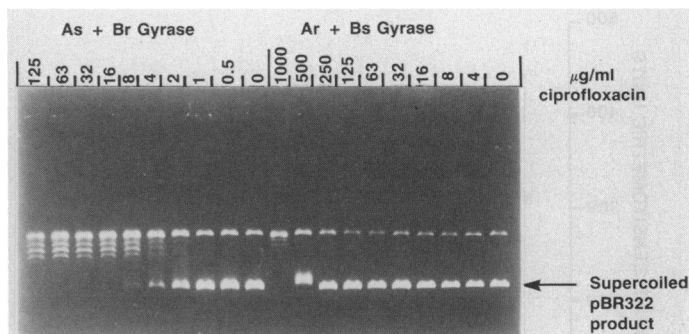

FIG. 3. Inhibition by ciprofloxacin of DNA gyrase supercoiling activity of reconstituted A and B subunits from *C. jejuni* UA535 and UA535 Nal 32. As and Bs, gyrase A and B subunits from UA535; Ar and Br, gyrase A and B subunits from UA535 Nal 32.

the newer quinolones than other enteric pathogens such as *E. coli* and *Salmonella* and *Shigella* species (4, 27). In an attempt to understand this phenomenon, isolated DNA gyrase from UA535 was compared with gyrase from *E. coli* K-12. This comparison indicated that the *Campylobacter* gyrase is intrinsically more resistant to supercoiling inhibition by ciprofloxacin than the *E. coli* enzyme (IC$_{100}$s of 32 and 1.0 μg/ml, respectively). Basic similarities exist between the two enzymes, however, since A and B subunits isolated from each were complementary in supercoiling experiments (data not shown). Supercoiling activity of the UA535 gyrase was ATP dependent and demonstrated an optimum MgCl$_2$ concentration between 4 and 6 mM (Fig. 4), which is similar to that reported for DNA gyrase from *E. coli*. Polyacrylamide gel electrophoretic analysis of the individual gyrase subunits isolated from UA535 indicated monomer molecular masses of approximately 95 KDa for GyrA and 90 KDa GyrB, which are similar to reported values for the *E. coli* subunits (17). Scanning of the subunit lanes indicated that each was at least 90% pure.

DISCUSSION

Quinolones have been proposed for use in therapy of a wide variety of bacterial infections, due to their broad spectrum and oral administration. This has recently included proposed therapy of diarrhea resulting from infection with *C. jejuni* (1, 9, 12, 21). Although this is generally a self-limiting disease, the severity or duration of symptoms can necessitate antibacterial therapy. Isolates of *C. jejuni* have been shown to be susceptible to a number of antibacterial agents in vitro and erythromycin has been suggested for use in severe cases, based on susceptibility data. In addition, a randomized, double-blinded study showed that ciprofloxacin therapy shortened the duration of diarrhea (compared with that in patients given a placebo) in patients infected with

enteric pathogens including *C. jejuni* (12). These results encourage further investigation into the activity of newer quinolone agents such as ciprofloxacin against *Campylobacter* spp.

In an earlier study Taylor et al. (26) described the susceptibility patterns of spontaneous nalidixic acid-resistant mutants of *C. jejuni*. These investigators found that spontaneous mutants could be selected from *C. jejuni* UA535 by plating on media containing 32 μg of nalidixic acid per ml. Mutants arose at a frequency of 2.5 × 10^{-8} and were also cross-resistant to enoxacin and ciprofloxacin (26). Mutants with an identical resistance phenotype were also selected from quinolone-susceptible strains of *Campylobacter coli*. However, nalidixic acid-resistant strains of *Campylobacter fetus* subsp. *fetus* and *C. hyointestinalis* were not cross-resistant to the newer quinolones. We have confirmed these observations with *C. jejuni* UA535, and we have established that resistance in this case is attributable to a change in the DNA gyrase A subunit. In addition, mutants with an identical phenotype could be selected in vitro from UA535 with ciprofloxacin or norfloxacin at approximately the same frequency. All of the drug-selected mutants had MICs of ciprofloxacin, norfloxacin, and temafloxacin at least 64-fold higher than those observed for UA535. A similar phenomenon has been demonstrated in other gram-negative organisms, although the absolute levels of resistance were not as high as the MICs observed with resistant mutants derived from UA535. Mutations in the *gyrA* and *gyrB* genes were shown to occur with equal frequency in spontaneous nalidixic acid-resistant mutants of *E. coli* KL16; in other reports, mutations in *gyrA* were more common than those in *gyrB* in resistant clinical isolates of *E. coli* (7, 18, 28–30). DNA sequence analysis has shown that all of the mutations

TABLE 2. Inhibition of DNA gyrase supercoiling activity[a]

Compound	UA535		UA535 Nal 32		UA535 Cpx 1	
	MEC	IC$_{100}$	MEC	IC$_{100}$	MEC	IC$_{100}$
Ciprofloxacin	1	32	250	>250	125	1,000
Norfloxacin	2	63	>500	>500	125	1,000
Novobiocin	0.7	1.3	0.7	1.3	0.3	1.3

[a] MECs and IC$_{100}$s are given in micrograms per milliliter.

TABLE 3. Concentrations of ciprofloxacin that inhibit supercoiling activity of reconstituted *C. jejuni* DNA gyrases[a]

DNA gyrases[b]	MEC (μg/ml)	IC$_{100}$ (μg/ml)
As + Bs	5	64
Ar + Br	43	417
As + Br	2	21
Ar + Bs	500	1,000

[a] Results are means of at least three determinations.
[b] As and Bs, gyrase A and B subunits from isolate UA535; Ar and Br, gyrase A and B subunits from isolate UA535 Nal 32.
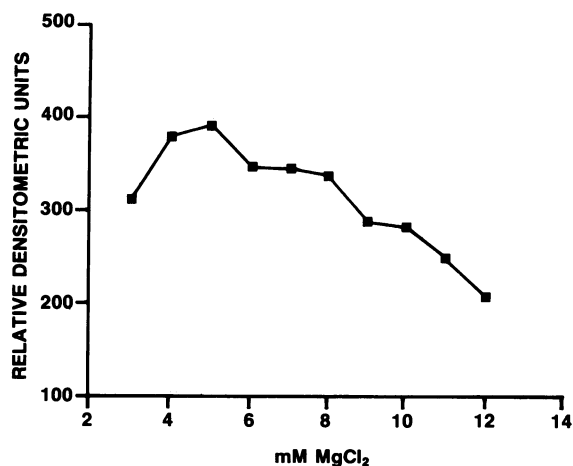
FIG. 4. Relationship of $MgCl_2$ concentration and supercoiling activity of DNA gyrase holoenzyme isolated from *C. jejuni* UA535. The substrate was relaxed pBR322 DNA. The supercoiled pBR322 product band was quantitated by densitometry from a photograph of an ethidium bromide-stained gel; supercoiled DNA is expressed in relative densitometric units.

in *gyrA* produce amino acid changes near Tyr-122 (residues 67 to 106), which is the residue involved in covalent binding to DNA during gyrase-mediated DNA strand passage (15). In *E. coli*, single amino acid changes have been shown to occur in the A subunit, most frequently at Ser-83, producing a 5- to 10-fold increase in the MICs of the newer quinolones (18, 28–30). In most instances the susceptibility of *E. coli gyrA* mutants remains such that attainable blood levels with the newer quinolones would still be above the MIC (27). The MICs for the resistant *Campylobacter* mutants described in this paper are considerably higher (4 to >32 μg/ml) than those observed to date in *E. coli*. Our isolation of *Campylobacter* DNA gyrase helps to explain these differences. In the in vitro DNA supercoiling reaction, the IC_{100} of ciprofloxacin with the UA535 gyrase was initially severalfold higher than the IC_{100} observed with gyrase isolated from *E. coli* K-12, despite the fact that equivalent units of enzyme activity were employed. This finding is consistent with literature reports, which indicate that isolates of *C. jejuni* are intrinsically less susceptible to quinolones than are other enteric pathogens such as *E. coli*, *Salmonella enteritidis*, *Shigella* spp., and *Vibrio* spp. (4, 27). It appears that this decreased susceptibility involves some fundamental difference between the DNA gyrases of these organisms, in terms of their interaction with quinolones. In general, the DNA gyrase isolated from UA535 demonstrates biochemical properties similar to those of gyrase isolated from *E. coli* K-12, in that both holoenzymes show optimum supercoiling activity in vitro at $MgCl_2$ concentrations between 4 and 6 mM (11) and both enzymes have similar A and B subunit monomer molecular weights (17). Also, heterologous switching experiments indicate that the GyrA and GyrB subunits from UA535 and *E. coli* K-12 show interspecies complementation for supercoiling activity.

In an attempt to determine the nature of the change in DNA gyrase that was associated with quinolone resistance, the A and B gyrase subunits were isolated from UA535 Nal 32 by novobiocin-Sepharose chromatography. That the subunit preparations were free from significant holoenzyme contamination was confirmed by their lack of detectable

supercoiling activity when tested alone. Heterologous subunit switching experiments showed that significantly higher concentrations of ciprofloxacin were required to minimally or completely inhibit DNA supercoiling activity when the mutant GyrA subunit was involved (Table 3). This agrees with susceptibility data indicating that UA535 Nal 32 is resistant to quinolones but that there is no change in susceptibility to the gyrase B subunit inhibitor coumermycin A1. The observed frequency of mutation to quinolone resistance in UA535 is consistent with a single mutational event occurring in *gyrA*. The exact nature of this mutation must await DNA sequence analysis of the wild-type and mutant *gyrA* genes of *C. jejuni* UA535. It was interesting that the level of resistance of gyrase to supercoiling inhibition by ciprofloxacin was lower when the gyrase Br subunit was a component of the enzyme (Table 3). This may indicate that a secondary mutation has occurred in this subunit, perhaps to stabilize the mutation in the gyrase Ar subunit.

Although other determinants, such as limited access through outer membrane porins (19), cannot be ruled out as potential mechanisms of resistance of *C. jejuni* isolates to quinolones, evidence from this study indicates that in vitro selection of resistance to quinolones involves mutation in DNA gyrase. It is interesting that of the 72 quinolone-resistant clones tested, none demonstrated decreased susceptibility to coumermycin A1 or tetracycline, compared with that of UA535. All of these clones from UA535 could potentially have decreased susceptibility to quinolones as a result of some change in DNA gyrase. The clinical implications of these results cannot be fully assessed at this time. The MICs of ciprofloxacin and temafloxacin range from 4 to 16 μg/ml in the quinolone-resistant mutants, suggesting that, should such mutants be found in humans, therapy of relatively rare extraintestinal *C. jejuni* infections with these agents would not be advisable (1, 2, 10, 20). However, since cases of gastrointestinal illness are more frequently caused by this organism, it could be hypothesized that the very high levels of quinolone present in the gastrointestinal tract of patients after oral administration may prevent selection of resistant mutants of the type described in this study. However, a recent clinical study found that ciprofloxacin (500 mg given orally twice daily) failed to eradicate *Campylobacter* organisms from the gut in 2 (20%) of 10 patients with gastroenteritis (12). Further studies showed that although pretherapy isolates were susceptible to the drug (MIC, 0.06 μg/ml), both *C. jejuni* strains isolated after 3 days of therapy were resistant to ciprofloxacin (MIC, 32 μg/ml). Subsequent isolation and comparison of the DNA gyrases from these pre- and posttherapy strains revealed the same type of enzyme alteration described in this study (22). Another report, from the Netherlands, describes a rise in the frequency of ciprofloxacin-resistant *Campylobacter* spp. isolated from human and poultry sources (8). In 165 strains isolated between 1982 and 1983, no ciprofloxacin-resistant strains were found. Between 1987 and 1988, however, among 243 isolates examined, 8.2% were resistant to ciprofloxacin; this number increased to 12.5% in 1989 (8). This trend toward increased frequency of resistance paralleled the increased use of fluoroquinolones in human and veterinary practice in that country. Although additional studies are clearly needed, the available information suggests that fluoroquinolones may have limited utility for the treatment and prophylaxis of gastrointestinal infections caused by *C. jejuni*.

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