J Clin Microbiol. 1999 Oct; 37(10): 3276–3280.
PMCID: PMC85547

Ciprofloxacin Resistance in *Campylobacter jejuni* Isolates: Detection of *gyrA* Resistance Mutations by Mismatch Amplification Mutation Assay PCR and DNA Sequence Analysis

Gerald Zirnstein,* Yu Li, Bala Swaminathan, and Frederick Angulo
Author information ► Article notes ► Copyright and License information ►

ABSTRACT

The *gyrA* quinolone resistance determining region was sequenced from 13 ciprofloxacin-resistant and 20 ciprofloxacin-susceptible *Campylobacter jejuni* isolates. All isolates resistant to ciprofloxacin had Thr-86-to-Ile mutations, a mutation frequently associated with the acquisition of resistance to fluoroquinolones. A mismatch amplification mutation assay (MAMA) PCR protocol was developed that detects this *gyrA* mutation in quinolone-resistant isolates. The MAMA PCR provides a means for routine detection of the *gyrA* mutation without the need for sequencing the *gyrA* gene.

The National Antimicrobial Resistance Monitoring System (NARMS) was initiated on 1 January 1996 as a collaborative study conducted by the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration Center for Veterinary Medicine, and 14 state and local health departments to prospectively monitor the antimicrobial resistance of human nontyphoidal *Salmonella* and *Escherichia coli* O157 isolates. In 1997, the study was expanded to include *Campylobacter* isolates from five state health departments.

Two to four million persons are infected with *Campylobacter* each year in the United States. Most of these infections are caused by *Campylobacter jejuni*. Most *Campylobacter* infections cause gastroenteritis, but invasive disease (e.g., bacteremia and meningitis) may also occur. While gastroenteritis caused by *C. jejuni* usually is self-limiting, treatment with antibiotics is often necessary for young children, pregnant women, or immunosuppressed patients because of the possibility of greater severity or duration of infection (11). Furthermore, antibiotics are essential for patients with invasive disease, which occurs in approximately 1% of culture-confirmed infections. Fluoroquinolones, particularly ciprofloxacin, have been widely used for the treatment of *Campylobacter* infections and for empiric treatment of patients with gastroenteritis, including traveler's diarrhea. In recent years, however, an increased proportion of *Campylobacter* isolates, both in the United States and other countries, have been reported to be resistant to ciprofloxacin (2, 4, 8, 13, 15). In 1997, 13% of *Campylobacter* isolates received at the CDC via the NARMS were ciprofloxacin resistant (18).

In *Campylobacter*, *E. coli*, and other gram-negative bacteria, fluoroquinolones work by interfering with DNA gyrase, a type II topoisomerase that catalyzes the negative supercoiling of relaxed or positively supercoiled, double-strand, covalently closed circular DNA (5, 10, 14). Mutations in the *gyrA* gene of gram-negative bacteria cause resistance to fluoroquinolones by altering the amino acid sequence near the putative active site of the GyrA protein (3). In *E. coli*, the quinolone resistance determining region (QRDR) of the *gyrA* gene, including codons 67 to 106, near the Tyr-122 catalytic site of DNA gyrase (16, 17), is the principal location for mutations leading to quinolone resistance. A similar QRDR exists in the *C. jejuni gyrA* gene, but the *gyrA* Tyr-125 residue appears to be the catalytic site involved in the transient, covalent DNA-protein bridge that forms during the DNA strand passage process of DNA topoisomerization (3).

The purpose of this study was to investigate the type and frequency of *gyrA* mutations in a sample of ciprofloxacin-resistant *C. jejuni* isolates, particularly those obtained in NARMS in 1997. Furthermore, we describe a mismatch amplification mutation assay (MAMA), which utilizes PCR technology to allow the rapid and specific characterization of *gyrA* mutations without performing DNA sequencing.

Go to:

MATERIALS AND METHODS

Strains, culture conditions, and antimicrobial susceptibility testing.

Twenty-five NARMS 1997 *C. jejuni* isolates, 12 ciprofloxacin resistant and 13 ciprofloxacin susceptible by E test, were chosen for analysis; their CS (*Campylobacter* Study) numbers were 5, 7, 8, 10, 17, 18, 34, 40, 42, 50, 54, 57, 58, 59, 61, 64, 71, 82, 89, 120, 133, 143, 161, 165, and 166. In addition, isolate D4344, a CDC ciprofloxacin-resistant *C. jejuni* isolate from 1992, was included. Seven additional quinolone-susceptible control *Campylobacter* spp. employed in this study were *Campylobacter jejuni* subsp. *jejuni* ATCC 33560 (CDC number D133), D125, D135, and D2589 and *Campylobacter jejuni* subsp. *doylei* ATCC 49349 (CDC number D2295), D3820, and D3836. The NARMS isolates and isolate D4344 were identified to species level by the methods below. Quality control strains used for antimicrobial susceptibility testing were *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

Campylobacter isolates for NARMS antibiotic resistance screening were streaked for isolation on plates of Trypticase soy agar containing 5% sheep blood at 37°C in an atmosphere of ~5% O_2 for 48 h. Microaerophilic growth conditions were created in a model A143 vacuum incubator (VWR Scientific Products, Bridgeport, N.J.) by drawing a vacuum to 21 in. of mercury then flushing the system with an anaerobic gas mixture (5% H_2, 10% CO_2, and 85% N_2) until the vacuum was lowered to, and maintained at, 5 in. of mercury. After incubation, the plates were examined for characteristic morphology and motility by dark-field microscopy, followed by biochemical tests for catalase, oxidase, and hippurate hydrolysis. Heavy growth from each isolate was suspended in Trypticase soy broth containing 20% glycerol and was stored at −70°C.

NARMS screening for antimicrobial resistance was performed by the use of E-test strips (AB Biodisk, Solna, Sweden). Overnight growth of campylobacters isolated on Trypticase soy agar containing 5% sheep blood was suspended in sterile saline and was adjusted to a turbidity matching a 0.5 McFarland standard. Sterile swabs were used to inoculate plates of Mueller-Hinton agar containing 5% lysed horse blood. E-test strips were placed on the plates, the plates were incubated at 37°C for 48 h under the microaerobic conditions described above, and the results were read according to the manufacturer's instructions. With every test run, the American Type Culture Collection standard strains were inoculated with E-test strips into duplicate sets of Mueller-Hinton plates containing 5% lysed horse blood. One set was incubated aerobically at 37°C and read at 18 h, while the other set was incubated microaerobically at 37°C and read at 48 h.

The nalidixic acid and ciprofloxacin antibiotypes of all *Campylobacter* isolates chosen for molecular characterization were confirmed by agar dilution testing as described by the National Committee for Clinical Laboratory Standards (12). Overnight growth on Trypticase soy agar containing 5% sheep blood was suspended in sterile saline and adjusted to a turbidity matching a 0.5 McFarland standard. Aliquots (450 μl) of each saline suspension were pipetted into the seeding wells of a Cathra replicator (Oxoid, Inc., Nepean, Ontario, Canada). Freshly prepared plates of Mueller-Hinton agar containing 5% sheep blood and doubling dilutions of nalidixic acid (Sigma Chemical Co., St. Louis, Mo.) or ciprofloxacin (Bayer Corp., Pharmaceutical Division, West Haven, Conn.) were then inoculated by using 3-

mm pins in the inoculating head of the replicator. The inoculated plates were incubated at 37°C for 48 h in the microaerobic environment generated by the use of three BBL CampyPaks in one BBL Gas Pack 150 anaerobic jar (Becton Dickinson, Cockeysville, Md.).

DNA isolation, PCR, DNA sequencing, and nucleotide sequence analysis.

Chromosomal DNA was isolated by using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minn.) according to the manufacturer's instructions. The QRDRs of the *gyrA* genes of the *Campylobacter* isolates were amplified by PCR. Primers GZ*gyrA*5 and GZ*gyrA*6 (Table (Table1)1) were chosen for PCR amplification of a 673-bp product containing the QRDR of the *gyrA* gene of quinolone-resistant and -susceptible isolates of *C. jejuni* after analysis of the L04566 *C. jejuni gyrA* gene sequence in GenBank (15). PCRs (100 μl each) contained 5 μl of Puregene purified chromosomal DNA (~75 ng in sterile, deionized H_2O), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, a 200 μM concentration of each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 2.5 U of *AmpliTaq* DNA polymerase (The Perkin-Elmer Corp., Foster City, Calif.), and 20 pmol of each primer. PCR cycling conditions were as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, with a final step at 72°C for 5 min. Primers were synthesized at the CDC Biotechnology Core Facility. Five-microliter aliquots of each PCR mixture were loaded onto horizontal agarose gels and stained with ethidium bromide for analysis after electrophoresis.

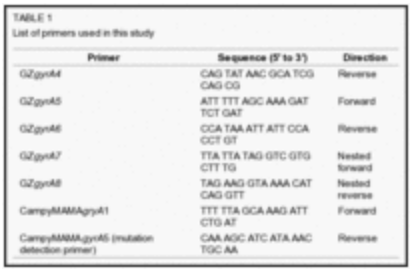

TABLE 1
List of primers used in this study

Primer	Sequence (5′ to 3′)	Direction
GZgyrA4	CAG TAT AAC GCA TCG CAG CG	Reverse
GZgyrA5	ATT TTT AGC AAA GAT TCT GAT	Forward
GZgyrA6	CCA TAA ATT ATT CCA CCT GT	Reverse
GZgyrA7	TTA TTA TAG GTC GTG CTT TG	Nested forward
GZgyrA8	TAG AAG GTA AAA CAT CAG GTT	Nested reverse
CampyMAMAgyrA1	TTT TTA GCA AAG ATT CTG AT	Forward
CampyMAMA-gyrA5 (mutation detection primer)	CAA AGC ATC ATA AAC TGC AA	Reverse

The remaining 673-bp *gyrA* PCR products were purified by the use of QIAquick purification columns (QIAGEN, Inc., Hilden, Germany) for use in sequencing reactions. *Taq* dye terminator cycle sequencing was performed with the ABI Prism dRhodamine Ready Reaction Kit (Applied Biosystems). Nested primers GZ*gyrA*7 and GZ*gyrA*8 (Table (Table1),1), which are internal to the 673-bp *gyrA* PCR product, were used for sequencing. Labeled sequencing reactions were purified by using CENTRI-SEP columns (Princeton Separations, Adelphia, N.J.) before analysis on an Applied Biosystems 377 automated sequencer.

Conserved primers were chosen with OLIGO Primer Analysis Software version 5.0 (National Biosciences, Inc., Plymouth, Minn.). Mutation primers were chosen after a manual analysis of the DNA sequence near codon 86 of the *C. jejuni gyrA* gene of fluoroquinolone-susceptible and -resistant isolates and the *C. jejuni* L04566 *gyrA* DNA sequence in GenBank (1, 15). DNA sequences were analyzed with DNASIS version 2.5 (Hitachi Software Engineering Co., Ltd., San Francisco, Calif.).

MAMA PCR protocol.

A conserved, forward primer, CampyMAMA*gyrA*1, and a reverse, mutation detection primer, CampyMAMA*gyrA*5 (Table (Table1),1), were used together in a PCR to generate a 265-bp PCR product that was a positive indication of the presence of the Thr-86-to-Ile (ACA→ATA) mutation in the *C. jejuni gyrA* gene. Primer GZ*gyrA*4, a conserved reverse primer, was used in conjunction with primer CampyMAMA*gyrA*1 to produce a positive PCR control product of 368 bp with any *C. jejuni gyrA* gene. PCRs (100 μl each) were the same as above except the PCR cycling conditions were as follows: initial denaturation was at 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 20 s. Twenty-microliter aliquots of each PCR product were loaded onto horizontal, 2.0%, 0.5× Tris-borate-EDTA agarose gels and were stained with ethidium bromide for analysis after electrophoresis.

Go to:

RESULTS

Detection of *gyrA* QRDR mutations associated with quinolone resistance.

An examination of the *gyrA* DNA sequence data, some of which are shown in Fig. Fig.1,1, enabled us to determine the presence or absence of amino acid mutations in the QRDR and to compare this information with the nalidixic acid and ciprofloxacin MICs for the 1997 NARMS isolates (Table (Table2).2). All 13 ciprofloxacin-resistant isolates had a substitution at amino acid position 86 of the GyrA protein due to a mutation of the DNA codon from ACA (threonine) to ATA (isoleucine). Furthermore, one isolate had an additional GyrA proline-to-serine mutation at amino acid position 104, but this mutation did not appear to dramatically alter the ciprofloxacin or nalidixic acid MICs for this isolate when compared to other ciprofloxacin-resistant isolates.

FIG. 1
Comparison of *C. jejuni gyrA* QRDR DNA sequences. Sequence L04566 is a portion of the *gyrA* QRDR of *C. jejuni* UA580 (GenBank no. **...**

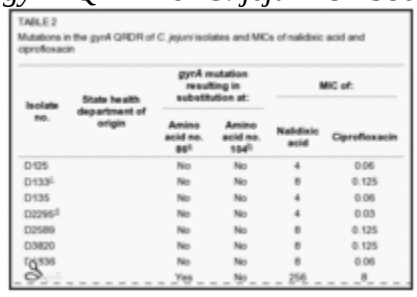

TABLE 2
Mutations in the *gyrA* QRDR of *C. jejuni* isolates and MICs of nalidixic acid and ciprofloxacin

Detection of the Thr-86-to-Ile mutations (ACA→ATA) by use of MAMA PCR.

A rapid PCR method was developed for the detection of the Thr-to-Ile (ACA-to-ATA) mutation at amino acid position 86 in the DNA sequence of *C. jejuni* isolates. Isolates with the wild-type amino acid 86 codon (ACA, ciprofloxacin susceptible) were not amplified with the reverse mutation primer CampyMAMA*gyrA*5, whereas the isolates with the mutated amino acid 86 codon (ATA, ciprofloxacin resistant) generated a 265-bp PCR product with the CampyMAMA*gyrA*5 reverse mutation primer and the CampyMAMA*gyrA*1 forward conserved primer (Fig. (Fig.2).2). Conserved primers GZ*gyrA*4 and CampyMAMA*gyrA*1 generated a 368-bp *gyrA* PCR product with DNA isolated from all of the isolates listed in this study. Primers used for MAMA PCR are listed in Table Table1.1.

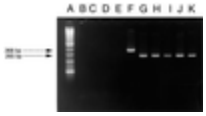

FIG. 2
Agarose gel of *C. jejuni* MAMA PCR products. Lanes contain the following: A, 0.8 μg of a 100-bp DNA ladder (Bethesda Research Laboratories, Inc., Gaithersburg, Md.); B (control lane), no DNA plus CampyMAMA*gyrA*1 and GZ*gyrA*4 conserved primers; C **...**
Go to:

DISCUSSION

Fluoroquinolone resistance among *Campylobacter* isolates tested in this study appears to have been caused by a threonine-to-isoleucine mutation of amino acid 86 of the GyrA protein. Although other mechanisms, such as changes in membrane permeability or mutations in the *parC* and *parE* genes, may be involved, this point mutation appears to be sufficient to cause fluoroquinolone resistance. The relationship of the Thr-86-to-Ile mutation to the acquisition of fluoroquinolone resistance in *Campylobacter* isolates has been previously characterized (2, 4, 13, 15). This mutation appears to be equivalent to the serine-to-alanine mutation of amino acid 83 in the *E. coli* GyrA protein (2, 4, 7, 8, 13, 15). Other mutations in the *gyrA* gene have also been reported to cause fluoroquinolone resistance in *C. jejuni* isolates. Wang et al. (15) reported *C. jejuni* Asp-90-to-Asn (GAT-to-AAT) and Ala-70-to-Thr (GCC-to-TCC) *gyrA* mutations in two laboratory mutants with MICs of 4 and 1 μg of ciprofloxacin per ml, respectively. Ruiz et al. (13) reported a unique *C. jejuni* Thr-86-to-Lys *gyrA* mutation from a clinical isolate with a ciprofloxacin MIC of ≥16 μg/ml and a nalidixic acid MIC of ≥128 μg/ml. Eleven other *C. jejuni* isolates from the same study (13) had Thr-86-to-Ile *gyrA* mutations resulting in similar ciprofloxacin and nalidixic acid MICs, but the actual DNA sequence was not included for either type of Thr-86 mutation. In addition, Ruiz et al. mention “transitions” at codon 119 of the *gyrA* gene, but since they were present in eight fluoroquinolone-resistant strains and nine fluoroquinolone-susceptible strains, they appear to be irrelevant to the matter of fluoroquinolone resistance phenotypes (13). Factors other than *gyrA* QRDR mutations, such as mutations in *gyrB*, *parC*, and *parE* and efflux pumps, permeability factors, or detoxification (3), may be responsible for some of the variability in nalidixic acid and ciprofloxacin MICs among groups of resistant and susceptible isolates in our study.

In addition to the Thr-86-to-Ile mutation, several silent mutations were evident in the *gyrA* QRDR of fluoroquinolone-resistant and -susceptible *C. jejuni* isolates. While most of the silent mutations appear to be somewhat random, the silent mutations at *gyrA* codons 82, 110, and 117 may be conserved, because all of the isolates with these mutations were *C. jejuni* subsp. *doylei* isolates. Also, 11 of the 12 ciprofloxacin-resistant *C. jejuni* isolates from the

NARMS study shared the same DNA sequence in the QRDRs of their *gyrA* genes as NARMS isolates CS5 and CS34, a sequence which is unlike the DNA sequence of any of the other *C. jejuni* isolates of either subspecies examined. Isolates with this particular *gyrA* QRDR DNA sequence were received from five state health laboratories, indicating the distribution of isolates with this DNA sequence is not localized. Although the number of isolates examined in this study is too small to draw any definite conclusions, comparison of *gyrA* QRDR sequences suggests the possibility that many of the ciprofloxacin-resistant *C. jejuni* isolates are derived from the same clone. However, it is also possible that *C. jejuni* isolates with this particular *gyrA* DNA sequence developed the quinolone resistance mutation at amino acid position 86 due to separate environmental events. If the second possibility is true, perhaps certain *C. jejuni* strains are more prone than other strains to develop *gyrA* quinolone resistance mutations. NARMS isolate CS50 has a *gyrA* QRDR DNA sequence unlike that of the other ciprofloxacin-resistant NARMS isolates but which is identical to the DNA sequences of *C. jejuni* isolates UA580, ATCC 33560, and D135, except for mutations at amino acid positions 86 and 104. It is likely that these additional mutations arose in isolate CS50 following exposure to quinolone or fluoroquinolone antibiotics. Unique DNA sequences found in the *gyrA* QRDRs of ciprofloxacin-resistant isolates may be useful during an outbreak investigation for determining if isolates are related.

Since the Thr-86-to-Ile (ACA→ATA) mutation in codon 86 of the *C. jejuni gyrA* gene was the most commonly encountered mutation leading to fluoroquinolone resistance (for which the DNA sequence has been reported [references 4, 8, and 15 and this study]) and since many laboratories do not have the equipment, time, or expertise to sequence genes for the investigation of mutations relevant to antibiotic resistance, we felt it was worthwhile to develop a MAMA protocol for the specific detection of this mutation. When the MAMA protocol is used, however, isolates should be confirmed as being *C. jejuni* by hippurate hydrolysis or PCR assay (6, 9). This is important because the 3′ end of the CampyMAMA*gyrA*5 mutation detection primer, designed for the Thr-86-to-Ile mutation (ACA→ATA) in codon 86 of the *C. jejuni gyrA* gene, is homologous to codon 86 of the wild-type *C. coli gyrA* gene and will generate a false-positive PCR product. The 3′-terminal nucleotide of primer CampyMAMA*gyrA*5 pairs correctly with the thymine in codon 86 of the ciprofloxacin-resistant *C. jejuni gyrA* mutants from this study, but a mismatch in the primer base immediately 5′ to the base at the 3′ end reduces amplification efficiency to about 70% of what could be expected with perfectly conserved primers (1). Neither the nucleotide at the 3′ end nor the nucleotide immediately 5′ to it pairs with the wild-type, fluoroquinolone-susceptible *C. jejuni gyrA* DNA sequence, and thus no PCR product is amplified (1).

While the MAMA PCR assay described here is undoubtedly simpler than DNA sequencing to determine the presence of mutations relevant to fluoroquinolone resistance, it does have some disadvantages. Although the majority of fluoroquinolone-resistant *C. jejuni* isolates have the Thr-86-to-Ile mutation, which the mutation primer in this assay was developed to detect, we have not yet developed additional primers to detect other reported mutations in the *C. jejuni gyrA* gene (15). However, it will be a simple matter to develop additional MAMA PCR mutation detection primers as isolates with other mutations become more widely available to test. We also concede that, for the present time, many research laboratories will still wish to sequence the *gyrA* genes of any fluoroquinolone-resistant *C. jejuni* isolates they obtain in order to detect new or additional mutations. In addition, clinical laboratories will undoubtedly still prefer to perform phenotypic susceptibility tests as a means for detecting resistant strains. The most relevant use for this assay, at this point in time, will be as a quick screening method for public health laboratories interested in quickly characterizing the resistance profiles of

resistant outbreak isolates or in public health laboratories with little access to DNA sequencing equipment or the funds to perform sequencing. Fluoroquinolone-resistant *C. jejuni* isolates that test negative by the MAMA PCR assay can be further examined by DNA sequencing of the *gyrA*, *gyrB*, *parC*, and *parE* genes (3). Other resistance mechanisms, such as efflux pumps, permeability factors, or detoxification, could be studied if no other obvious mechanism appears to be causing fluoroquinolone resistance in the isolates being examined (3). We believe the MAMA PCR method is a simple, specific, rapid, inexpensive, and portable alternative to the nonradioisotopic single-strand conformation polymorphism method previously published (2) and DNA sequencing for the detection of this important fluoroquinolone resistance mutation.

Go to:

REFERENCES

1. Cha R S, Zarbl H, Keohavong P, Thilly W G. Mismatch amplification mutation assay (MAMA): application to the c-H-*ras* gene. PCR Methods Appl. 1992;2:14–20. [PubMed]
2. Charvalos E, Peteinaki E, Spyridaki I, Manetas S, Tselentis Y. Detection of ciprofloxacin resistance mutations in *Campylobacter jejuni gyrA* by nonradioisotopic single-strand conformation polymorphism and direct DNA sequencing. J Clin Lab Anal. 1996;10:129–133. [PubMed]
3. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev. 1997;61:377–392. [PMC free article] [PubMed]
4. Gaunt P N, Piddock L J V. Ciprofloxacin resistant *Campylobacter* spp. in humans: an epidemiological and laboratory study. J Antimicrob Chemother. 1996;37:747–757. [PubMed]
5. Gellert M, Mizuuchi K, O'Dea M H, Nash H A. Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. Proc Natl Acad Sci USA. 1977;74:4772–4776. [PMC free article] [PubMed]
6. Gonzalez I, Grant K A, Richardson P T, Park S F, Collins M D. Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the *ceuE* gene encoding a putative virulence determinant. J Clin Microbiol. 1997;35:759–763. [PMC free article] [PubMed]
7. Hallett P, Maxwell A. Novel quinolone resistance mutations of the *E. coli* DNA gyrase A protein: enzymatic analysis of the mutant proteins. Antimicrob Agents Chemother. 1991;35:335–340. [PMC free article] [PubMed]
8. Husmann M, Feddersen A, Steitz A, Freytag C, Bhakdi S. Simultaneous identification of campylobacters and prediction of quinolone resistance by comparative sequence analysis. J Clin Microbiol. 1997;35:2398–2400. [PMC free article] [PubMed]
9. Linton D, Owen R J, Stanley J. Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. Res Microbiol. 1996;147:707–718. [PubMed]
10. Maxwell A. The molecular basis of quinolone action. J Antimicrob Chemother. 1992;30:409–414. [PubMed]
11. Nachamkin I. Campylobacter infections. Curr Opin Infect Dis. 1993;6:72–76.
12. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. 4th ed. Wayne, Pa: National Committee for Clinical Laboratory Standards; 1997.
13. Ruiz J, Goni P, Marco F, Gallardo F, Mirelis B, Jimenez De Anta T, Vila J. Increased resistance to quinolones in *Campylobacter jejuni*: a genetic analysis of *gyrA* gene mutations in quinolone-resistant clinical isolates. Microbiol Immunol. 1998;42:223–226. [PubMed]

14. Sugino A, Peebles C L, Kreuzer K N, Cozzarelli N R. Mechanism of action of nalidixic acid: purification of *Escherichia coli nalA* gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc Natl Acad Sci USA. 1977;74:4767–4771. [PMC free article] [PubMed]
15. Wang Y, Huang W M, Taylor D E. Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. Antimicrob Agents Chemother. 1993;37:457–463. [PMC free article] [PubMed]
16. Yoshida H, Bogaki M, Nakamura M, Nakamura S. Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. Antimicrob Agents Chemother. 1990;34:1271–1272. [PMC free article] [PubMed]
17. Yoshida H, Kojima T, Yamagashi J, Nakamura S. Quinolone-resistant mutations of the *gyrA* gene of *Escherichia coli*. Mol Gen Genet. 1988;211:1–7. [PubMed]
18. Zirnstein G, Bopp C, Dabney P, Voetsch D, Swaminathan B, Hatmaker J, Miller M, Tollefsen L, Angulo F the NARMS Working Group. abstracts of the 1998 International Conference on Emerging Infectious Diseases. 1998. The national antimicrobial resistance monitoring system (NARMS): 1996–1997, abstr. 9906; p. 138.

Formats:

- Article

  |

- PubReader

  |

- ePub (beta)

  |

- PDF (229K)

  |

- Citation

Share

- Facebook
- Twitter
- Google+

Save items

View more options

Similar articles in PubMed

- Detection of ciprofloxacin resistance mutations in Campylobacter jejuni gyrA by nonradioisotopic single-strand conformation polymorphism and direct DNA sequencing.[J Clin Lab Anal. 1996]
- Characterization of gyrA mutations associated with fluoroquinolone resistance in Campylobacter coli by DNA sequence analysis and MAMA PCR.[FEMS Microbiol Lett. 2000]
- Identification of ciprofloxacin-resistant Campylobacter jejuni by use of a fluorogenic PCR assay.[J Clin Microbiol. 2000]
- Molecular epidemiological analysis of quinolone-resistant Escherichia coli causing bacteremia in neutropenic patients with leukemia in Korea.[Clin Infect Dis. 1997]
- Quinolone resistance and Campylobacter spp.[J Antimicrob Chemother. 1995]

See reviews...See all...

Cited by other articles in PMC

- Antimicrobial resistance of Campylobacter isolates from small scale and backyard chicken in Kenya[Gut Pathogens. 2016]
- Genotyping and antibiotic resistance of thermophilic Campylobacter isolated from chicken and pig meat in Vietnam[Gut Pathogens. 2016]
- High genetic similarity of ciprofloxacin-resistant Campylobacter jejuni in central Europe[Frontiers in Microbiology. 2015]
- Development and application of loop-mediated isothermal amplification for detection of the F167Y mutation of carbendazim-resistant isolates in Fusarium graminearum[Scientific Reports. 2014]
- Molecular Analysis of Ciprofloxacin Resistance Mechanisms in Malaysian ESBL-Producing Klebsiella pneumoniae Isolates and Development of Mismatch Amplification Mutation Assays (MAMA) for Rapid Detection of gyrA and parC Mutations[BioMed Research International....]

See all...

Links

- Compound
- MedGen
- Nucleotide
- PubMed
- Substance
- Taxonomy

Recent Activity

ClearTurn Off

- Ciprofloxacin Resistance in Campylobacter jejuni Isolates: Detection of gyrA Re...

  Ciprofloxacin Resistance in Campylobacter jejuni Isolates: Detection of gyrA Resistance Mutations by Mismatch Amplification Mutation Assay PCR and DNA Sequence Analysis

  Journal of Clinical Microbiology. 1999 Oct; 37(10)3276

See more...

- Cloning and nucleotide sequence of the Campylobacter jejuni gyrA gene and characterization of quinolone resistance mutations.[Antimicrob Agents Chemother. 1993]

- Mismatch amplification mutation assay (MAMA): application to the c-H-ras gene.[PCR Methods Appl. 1992]
- Cloning and nucleotide sequence of the Campylobacter jejuni gyrA gene and characterization of quinolone resistance mutations.[Antimicrob Agents Chemother. 1993]

- Detection of ciprofloxacin resistance mutations in Campylobacter jejuni gyrA by nonradioisotopic single-strand conformation polymorphism and direct DNA sequencing.[J Clin Lab Anal. 1996]
- Ciprofloxacin resistant Campylobacter spp. in humans: an epidemiological and laboratory study.[J Antimicrob Chemother. 1996]
- Increased resistance to quinolones in Campylobacter jejuni: a genetic analysis of gyrA gene mutations in quinolone-resistant clinical isolates.[Microbiol Immunol. 1998]
- Cloning and nucleotide sequence of the Campylobacter jejuni gyrA gene and characterization of quinolone resistance mutations.[Antimicrob Agents Chemother. 1993]
- Novel quinolone resistance mutations of the Escherichia coli DNA gyrase A protein: enzymatic analysis of the mutant proteins.[Antimicrob Agents Chemother. 1991]
- Simultaneous identification of campylobacters and prediction of quinolone resistance by comparative sequence analysis.[J Clin Microbiol. 1997]
- Review DNA gyrase, topoisomerase IV, and the 4-quinolones.[Microbiol Mol Biol Rev. 1997]

- Ciprofloxacin resistant Campylobacter spp. in humans: an epidemiological and laboratory study.[J Antimicrob Chemother. 1996]
- Simultaneous identification of campylobacters and prediction of quinolone resistance by comparative sequence analysis.[J Clin Microbiol. 1997]
- Cloning and nucleotide sequence of the Campylobacter jejuni gyrA gene and characterization of quinolone resistance mutations.[Antimicrob Agents Chemother. 1993]
- Specific identification of the enteropathogens Campylobacter jejuni and Campylobacter coli by using a PCR test based on the ceuE gene encoding a putative virulence determinant.[J Clin Microbiol. 1997]

- Rapid identification by PCR of the genus Campylobacter and of five Campylobacter species enteropathogenic for man and animals.[Res Microbiol. 1996]
- Mismatch amplification mutation assay (MAMA): application to the c-H-ras gene.[PCR Methods Appl. 1992]

- Cloning and nucleotide sequence of the Campylobacter jejuni gyrA gene and characterization of quinolone resistance mutations.[Antimicrob Agents Chemother. 1993]
- Review DNA gyrase, topoisomerase IV, and the 4-quinolones.[Microbiol Mol Biol Rev. 1997]
- Detection of ciprofloxacin resistance mutations in Campylobacter jejuni gyrA by nonradioisotopic single-strand conformation polymorphism and direct DNA sequencing.[J Clin Lab Anal. 1996]