APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2003, p. 3005–3007 Vol. 69, No. 5
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.5.3005–3007.2003

Antimicrobial-Resistant *Campylobacter* Species from Retail Raw Meats

Beilei Ge,[1] David G. White,[2] Patrick F. McDermott,[2] Webb Girard,[1] Shaohua Zhao,[2] Susannan Hubert,[2] and Jianghong Meng[1]*

Department of Nutrition and Food Science, University of Maryland, College Park, Maryland 20742,[1] and Division of Animal and Food Microbiology, Office of Research, Center for Veterinary Medicine, Food and Drug Administration, Laurel, Maryland 20708[2]

Received 16 October 2002/Accepted 13 February 2003

The antimicrobial susceptibilities of 378 *Campylobacter* isolates were determined. Resistance to tetracycline was the most common (82%), followed by resistance to doxycycline (77%), erythromycin (54%), nalidixic acid (41%), and ciprofloxacin (35%). *Campylobacter coli* displayed significantly higher rates of resistance to ciprofloxacin and erythromycin than *Campylobacter jejuni*, and *Campylobacter* isolates from turkey meat showed a greater resistance than those from chicken meat.

Campylobacter is a leading cause of human gastroenteritis worldwide, with an estimated 2.4 million cases each year in the United States (8). *Campylobacter jejuni* and *Campylobacter coli* are the species most commonly associated with human infections and cause clinically similar illnesses. When an antibiotic is recommended for treatment of patients with severe campylobacteriosis, the most frequently recommended drug is erythromycin or a fluoroquinolone such as ciprofloxacin (16). Tetracycline, doxycycline, and chloramphenicol are sometimes listed as alternative drugs for treatment. However, an increasing number of *Campylobacter* species that exhibit decreased susceptibility to several of these drugs have been isolated from food and water sources as well as from clinical samples in Europe (9, 14, 15, 18), Canada (5), and the United States (2, 17).

Campylobacter is considered mainly a food-borne pathogen, with raw or undercooked poultry serving as an important source of sporadic *Campylobacter* infection (1). The identification of antimicrobial-resistant *Campylobacter* organisms in food has raised concerns that the treatment of food-borne campylobacteriosis may be compromised, because antimicrobial-resistant *Campylobacter* strains cause more prolonged or more severe illness than do antimicrobial-susceptible strains (19). In this study, we examined the antimicrobial susceptibility of *Campylobacter* isolates from retail meats and determined the genetic basis of resistance to ciprofloxacin and erythromycin by studying the mutations in the *gyrA* and 23S rRNA genes, respectively.

Campylobacter was isolated from 159 (22%) of 719 retail meat samples from a previous study (21). To determine whether multiple *Campylobacter* strains were present, more than one colony per sample was selected, which resulted in a total of 378 isolates, including 196 *C. jejuni* isolates, 153 *C. coli* isolates, and 29 isolates from other *Campylobacter* species. The 378 *Campylobacter* isolates were examined for susceptibility to chloramphenicol, ciprofloxacin, doxycycline, erythromycin, gentamicin, nalidixic acid, and tetracycline by using the agar dilution method according to NCCLS recommendations (6, 10).

Approximately 94% of the 159 meat samples (149 samples) were contaminated with *Campylobacter* isolates that were resistant to at least one of the seven antimicrobials tested. Resistance to tetracycline was the most commonly found among 155 poultry isolates (82%), followed by resistance to doxycycline (77%), erythromycin (54%), nalidixic acid (41%), and ciprofloxacin (35%) (Table 1). Two *C. coli* isolates from two turkey samples showed resistance to chloramphenicol. None of the isolates was resistant to gentamicin.

The antimicrobial resistance of *Campylobacter* isolates differed according to the species of the organism and the source of isolation. *C. coli* isolates displayed significantly higher rates of resistance ($P < 0.05$) to ciprofloxacin and erythromycin than did *C. jejuni*. Therefore, it is important to differentiate *Campylobacter* isolates at the species level in order to better monitor trends in antimicrobial resistance. Turkey isolates of both species showed significantly higher rates of resistance ($P < 0.05$) to ciprofloxacin and erythromycin than did chicken isolates (Fig. 1). This may be due to the longer raising period for turkeys (up to 18 weeks) than for chickens (7 weeks). In addition, because turkeys are economically more valuable than chickens, farmers are prone to giving turkeys antibiotics for disease treatment and prevention and for growth promotion (11). Multidrug-resistant *Campylobacter* isolates were common. Coresistance to ciprofloxacin and erythromycin was found in *Campylobacter* isolates from 41 (26%) meat samples. The ciprofloxacin MIC for all ciprofloxacin-resistant *Campylobacter* isolates was ≥16 μg/ml, and that for two of the isolates was 128 μg/ml.

Pulsed-field gel electrophoresis (PFGE) was used for subtyping *Campylobacter* isolates (12). Among the *C. jejuni* and *C. coli* isolates, 81 and 67 PFGE patterns were identified, respectively. Multiple *Campylobacter* species were present in 16 (10%) meat samples, whereas multiple strains with distinct PFGE patterns were recovered from 41 (26%) of the 159 meat samples. The PFGE and antimicrobial susceptibility profiles correlated well. *Campylobacter* isolates with identical PFGE patterns displayed the same or similar antimicrobial susceptibility profiles. Several of the *Campylobacter* isolates were found in chicken and turkey products from different chains or stores

* Corresponding author. Mailing address: Department of Nutrition and Food Science, University of Maryland, College Park, MD 20742. Phone: (301) 405-1399. Fax: (301) 314-3313. E-mail: jm332@umail.umd.edu.

TABLE 1. Number of retail poultry meat samples containing *C. jejuni, C. coli*, and other *Campylobacter* species resistant to antimicrobial agents

Antimicrobial agent	MIC breakpoint (μg/ml)	No. (%) of meat samples that contain resistant isolates of:			
		C. jejuni (n = 88)	*C. coli* (n = 75)	Other species (n = 12)	Total[a] (n = 155)
Chloramphenicol	32	0	2 (3)	0	2 (1)
Ciprofloxacin	4	22 (25)	30 (40)	4 (33)	55 (35)
Doxycycline	16	66 (75)	57 (76)	7 (58)	119 (77)
Erythromycin	8	37 (42)	46 (61)	3 (25)	84 (54)
Gentamicin	16	0	0	0	0
Nalidixic acid	32	28 (32)	32 (43)	5 (42)	64 (41)
Tetracycline	16	71 (81)	58 (77)	9 (75)	127 (82)

[a] Multiple species of *Campylobacter* were isolated from 15 poultry meat samples.

TABLE 2. Point mutations observed for sequences from ciprofloxacin-resistant *C. jejuni* (n = 42) and *C. coli* (n = 56) isolates

Species	Mutation(s)	Ciprofloxacin MIC (μg/ml)	No. of isolates
C. jejuni	Ser-22-Gly, Thr-86-Ile, Val-149-Ile, Asn-203-Ser	16–64	22
	Ser-22-Gly, Thr-86-Ile, Asn-203-Ser, Ala-206-Val	32–64	3
	Ser-22-Gly, Thr-86-Ile, Asn-203-Ser	16–64	7
	Thr-86-Ile, Asn-203-Ser, Ala-206-Thr	16	1
	Thr-86-Ile, Asn-203-Ser	64–128	8
	Asn-203-Ser, Ala-206-Thr	64	1
C. coli	Thr-86-Ile	≥16	56

throughout the 12-month sampling period. Nineteen (68%) of the 28 meat samples that contained isolates of the same species with different PFGE patterns also showed different antibiograms.

For *Campylobacter*, the resistance to fluoroquinolones and macrolides appears to be due mostly to mutations in genes encoding DNA gyrase and 23S rRNA, respectively (4). DNA sequence analyses of the *gyrA* gene from 98 ciprofloxacin-resistant *Campylobacter* isolates and of the 23S rRNA gene from 44 erythromycin-resistant and 15 erythromycin-susceptible *Campylobacter* isolates were performed to detect mutations associated with resistance. The genes were amplified by using PCR as described in published reports (22, 23). Sequence analyses of the *gyrA* genes of the ciprofloxacin-resistant *C. jejuni* isolates identified 14 point mutations, 8 of which were silent mutations, compared to the sequence for ciprofloxacin-susceptible *C. jejuni* strain UA580 (GenBank accession no. L04566). In addition to the previously reported Thr-86-Ile mutation in 41 of the 42 isolates (13, 20), mutations at several other positions were identified (Table 2). All isolates showed a mutation from Asn at position 203, and 32 had an additional mutation from Ser at position 22. Interestingly, the one isolate lacking the Thr-86-Ile mutation contained novel double mutations, from Asn at position 203 and from Ala at position 206. Up to four mutations have been identified in some *C. jejuni* isolates. In *C. coli*, although six point mutations were observed in the *gyrA* gene of the 58 ciprofloxacin-resistant isolates relative to the sequence for the wild-type *C. coli* strain ATCC 33559 (accession no. AF092101), the mutation at position 86 was the only one that resulted in an amino acid sequence change (from Thr). However, one ciprofloxacin-resistant *C.*

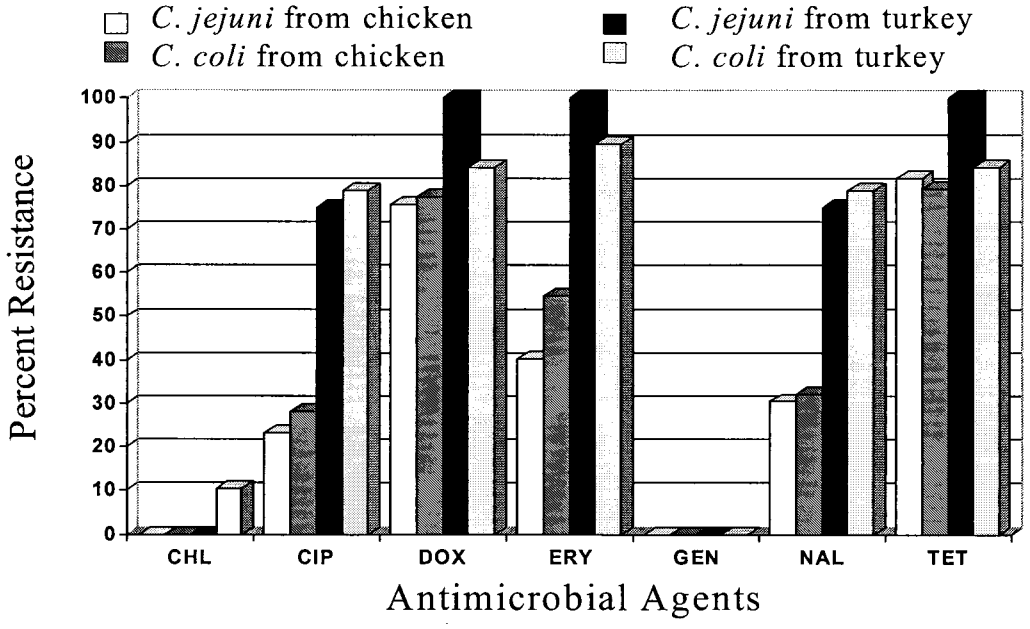

FIG. 1. Comparison of antimicrobial resistance profiles of *C. jejuni* and *C. coli* isolates from chicken (n = 125) and turkey (n = 23) meats. CHL, chloramphenicol; CIP, ciprofloxacin; DOX, doxycycline; ERY, erythromycin; GEN, gentamicin; NAL, nalidixic acid; TET, tetracycline.

TABLE 3. Point mutations observed for 23S rRNA gene sequences from 44 erythromycin-resistant and 15 erythromycin-susceptible *Campylobacter* isolates

Mutation	Erythromycin MIC (μg/ml)	Species	No. of isolates tested	No. of mutated isolates
A-2230-G	>64	*C. coli*	14	12
A-2230-G	64	*C. coli*	1	1
T-2268-C	32	*C. coli*	3	2
	16	*C. jejuni, C. coli*	11	4
	8	*C. jejuni, C. coli*	15	12
	1–4	*C. jejuni*	15	9

coli isolate lacked this mutation but possessed other silent substitutions. Among 44 erythromycin-resistant *Campylobacter* isolates analyzed, 13 of the 15 isolates for which the erythromycin MIC was ≥64 μg/ml had an A-2230-G mutation encoded in the 23S rRNA. The remaining isolates showed a spontaneous mutation at position 2268 (from T to C), which was also found in 15 *Campylobacter* isolates that were susceptible to erythromycin (MIC, ≤4 μg/ml) (Table 3). Other studies have demonstrated an efflux of fluoroquinolones, which also contributes to antimicrobial resistance in *Campylobacter* isolates (3, 7).

Since campylobacteriosis is transmitted primarily through food, particularly food of animal origin, the presence of antimicrobial-resistant *Campylobacter* in raw meat products has important public health implications. Our findings indicated that most *Campylobacter* isolates from retail poultry were resistant to at least one of the seven antimicrobials tested and that multidrug resistance was common. The coresistance to erythromycin and ciprofloxacin must be considered to be highly undesirable, as the two antimicrobials are generally advocated as first-line drugs for the treatment of campylobacteriosis. These findings suggest that cases of campylobacteriosis acquired from undercooked or mishandled retail meats may not respond to empirical therapy. Therefore, the use of in vitro susceptibility testing of *Campylobacter* may take on greater importance in ensuring rapid and appropriate management of patients with food-borne campylobacteriosis.

We are indebted to Robert Walker, from the Division of Animal and, Food Microbiology, Center for Veterinary Medicine, Food and Drug Administration, for his assistance and comments in the preparation of the manuscript.

This study was supported in part by grants from the Maryland Agricultural Experimental Station, the Joint Institute for Food Safety and Applied Nutrition of the University of Maryland and the Food and Drug Administration.

REFERENCES

1. **Blaser, M. J.** 1997. Epidemiologic and clinical features of *Campylobacter jejuni* infections. J Infect. Dis. **176**(Suppl. 2)**:**S103–S105.
2. **Centers for Disease Control and Prevention.** 2000. National Antimicrobial Resistance Monitoring System (NARMS)—1999 annual report. [Online.] http://www.cdc.gov/narms.
3. **Charvalos, E., Y. Tselentis, M. M. Hamzehpour, T. Köhler, and J. C. Pechere.** 1995. Evidence for an efflux pump in multidrug-resistant *Campylobacter jejuni*. Antimicrob. Agents Chemother. **39:**2019–2022.
4. **Engberg, J., F. M. Aarestrup, D. E. Taylor, P. Gerner-Smidt, and I. Nachamkin.** 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. Emerg. Infect. Dis. **7:**24–34.
5. **Gaudreau, C., and H. Gilbert.** 1998. Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. Antimicrob. Agents Chemother. **42:**2106–2108.
6. **Ge, B., S. Bodeis, R. D. Walker, D. G. White, S. Zhao, P. F. McDermott, and J. Meng.** 2002. Comparison of the Etest and agar dilution for in vitro antimicrobial susceptibility testing of *Campylobacter*. J. Antimicrob. Chemother. **50:**487–494.
7. **Lin, J., L. O. Michel, and Q. Zhang.** 2002. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. Antimicrob. Agents Chemother. **46:** 2124–2131.
8. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
9. **Moore, J. E., M. Crowe, N. Heaney, and E. Crothers.** 2001. Antibiotic resistance in *Campylobacter* spp. isolated from human faeces (1980–2000) and foods (1997–2000) in Northern Ireland: an update. J. Antimicrob. Chemother. **48:**455–457.
10. **National Committee for Clinical Laboratory Standards.** 2001. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. **National Research Council.** 1999**.** The use of drugs in food animals: benefits and risks. National Academy Press, Washington, D.C.
12. **Ribot, E. M., C. Fitzgerald, K. Kubota, B. Swaminathan, and T. J. Barrett.** 2001. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J. Clin. Microbiol. **39:** 1889–1894.
13. **Ruiz, J., P. Goni, F. Marco, F. Gallardo, B. Mirelis, T. Jimenez De Anta, and J. Vila.** 1998. Increased resistance to quinolones in *Campylobacter jejuni*: a genetic analysis of gyrA gene mutations in quinolone-resistant clinical isolates. Microbiol. Immunol. **42:**223–226.
14. **Sáenz, Y., M. Zarazaga, M. Lantero, M. J. Gastañares, F. Baquero, and C. Torres.** 2000. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997–1998. Antimicrob. Agents Chemother. **44:**267–271.
15. **Sjogren, E., G. B. Lindblom, and B. Kaijser.** 1997. Norfloxacin resistance in *Campylobacter jejuni* and *Campylobacter coli* isolates from Swedish patients. J. Antimicrob. Chemother. **40:**257–261.
16. **Skirrow, M. B., and M. J. Blaser.** 2000. Clinical aspects of *Campylobacter* infection, p. 69–88. *In* I. Nachamkin and M. J. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington, D.C.
17. **Smith, K. E., J. M. Besser, C. W. Hedberg, F. T. Leano, J. B. Bender, J. H. Wicklund, B. P. Johnson, K. A. Moore, M. T. Osterholm, et al.** 1999. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992–1998. N. Engl. J. Med. **340:**1525–1532.
18. **Talsma, E., W. G. Goettsch, H. L. Nieste, P. M. Schrijnemakers, and M. J. Sprenger.** 1999. Resistance in *Campylobacter* species: increased resistance to fluoroquinolones and seasonal variation. Clin. Infect. Dis. **29:**845–848.
19. **Travers, K., and M. Barza.** 2002. Morbidity of infections caused by antimicrobial-resistant bacteria. Clin. Infect. Dis. **34**(Suppl. 3)**:**S131–S134.
20. **Wang, Y., W. M. Huang, and D. E. Taylor.** 1993. Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. Antimicrob. Agents Chemother. **37:**457–463.
21. **Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng.** 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. Appl. Environ. Microbiol. **67:**5431–5436.
22. **Zirnstein, G., L. Helsel, Y. Li, B. Swaminathan, and J. Besser.** 2000. Characterization of gyrA mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. FEMS Microbiol. Lett. **190:**1–7.
23. **Zirnstein, G., Y. Li, B. Swaminathan, and F. Angulo.** 1999. Ciprofloxacin resistance in *Campylobacter jejuni* isolates: detection of *gyrA* resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis. J. Clin. Microbiol. **37:**3276–3280.