ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2000, p. 267–271 Vol. 44, No. 2
0066-4804/00/$04.00+0

Antibiotic Resistance in *Campylobacter* Strains Isolated from Animals, Foods, and Humans in Spain in 1997–1998

YOLANDA SÁENZ,[1] MYRIAM ZARAZAGA,[1] MARTA LANTERO,[2] M. JOSÉ GASTAÑARES,[2] FERNANDO BAQUERO,[3] AND CARMEN TORRES[1]*

Area de Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño,[1] Sección de Microbiología, Hospital San Millán, Logroño,[2] and Servicio de Microbiología, Hospital Ramón y Cajal,[3] Madrid, Spain

Received 16 March 1999/Returned for modification 23 June 1999/Accepted 29 October 1999

Colonization by *Campylobacter* strains was investigated in human, broiler, and pig fecal samples from 1997-1998, as well as in foods of animal origin, and antibiotic susceptibility testing was carried out for these strains. *Campylobacter* strains were isolated in the foods of animal origin (55 of 101 samples; 54.4%), intestinal samples from broilers (85 of 105; 81%), and pigs (40 of 45; 88.9%). A total of 641 *Campylobacter* strains were isolated from 8,636 human fecal samples of clinical origin (7.4%). *Campylobacter jejuni* was the most frequently isolated species from broilers (81%) and humans (84%), and *Campylobacter coli* was most frequently isolated from pigs (100%). An extremely high frequency of ciprofloxacin resistance was detected among *Campylobacter* strains, particularly those isolated from broilers and pigs (99%), with a slightly lower result for humans (72%); cross-resistance with nalidixic acid was almost always observed. A higher frequency of resistance to erythromycin (81.1%), ampicillin (65.7%), gentamicin (22.2%), and amikacin (21.6%) was detected in *C. coli* strains isolated from pigs compared to those isolated from humans (34.5, 29.3, 8.6, and 0%, respectively). A low frequency of erythromycin resistance was found in *C. jejuni* or *C. coli* isolated from broilers. A greater resistance to ampicillin and gentamicin (47.4 and 11.9%, respectively) was detected in *C. jejuni* isolated from broilers than in human strains (38 and 0.4%, respectively). β-Lactamase production was found in 81% of the *Campylobacter* strains tested, although 44% of them were characterized as ampicillin susceptible. The increasing rates of *Campylobacter* resistance make advisable a more conservative policy for the use of antibiotics in farm animals.

Thermophilic *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are recognized as one of the most frequent causes of acute diarrheal disease in humans throughout the world (28, 54). *Campylobacter* infections usually occur following ingestion of improperly handled or cooked food. Campylobacteriosis is considered a zoonotic disease, and animals such as poultry and pigs may act as reservoirs for *Campylobacter*. In patients in which treatment is indicated, erythromycin or fluoroquinolones are often recommended. Several studies have linked the use of antimicrobial agents in veterinary medicine or as feed additives with the emergence and spread of resistance among *Campylobacter* strains, with potentially serious effects on food safety and in both veterinary and human health (13, 20, 51).

This study was conducted to compare the isolation frequency and the antimicrobial resistance rate of thermophilic *Campylobacter* strains isolated in clinical infections in humans, from foods of animal origin, and from feces of healthy broilers or pigs.

(This work was presented in part at the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, Calif., September 1998.)

MATERIALS AND METHODS

Bacterial sampling. During the 2-year period from 1997 to 1998, 101 samples from foods (mainly products of chicken origin) were obtained from 18 local supermarkets and poultry shops in Logroño, Spain (Table 1). In addition, 105 samples from the intestinal content of broilers and 45 from pig feces were collected during evisceration in slaughterhouses in the same region. Clinical human fecal samples (n = 8,636) collected in the San Millán General Hospital (Logroño) during the same period were also analyzed for *Campylobacter* detection.

Isolation and identification. Bacterial isolation from the food, broiler and pig samples, was carried out by suspending a representative fragment of each sample in a sterile saline solution (dilution 1/10 [wt/vol]). A 0.5-ml portion of the suspension was then placed in a 4.5-ml Preston broth tube and enriched in a microaerophilic atmosphere (GasPack; Oxoid, Basingstoke, England) at 42°C for 18 to 24 h. A selective Skirrow agar plate (Campylosel; BioMérieux, La Balme, France) was inoculated with 100 μl of the enriched suspension and incubated at 42°C in a microaerophilic atmosphere for 48 h. Stool samples collected from human sources were directly plated on Skirrow selective agar plates and incubated for 48 h at 42°C in a microaerophilic atmosphere. Identification of suspected colonies was undertaken by conventional biochemical tests (32). Only one strain per sample was kept for further studies.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was done by using the agar disk diffusion standard method (29) with Mueller-Hinton agar supplemented with 5% sheep blood; the plates were incubated at 37°C for 24 h in a microaerophilic atmosphere. The antibiotics tested included nalidixic acid (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), azithromycin (15 μg), spiramycin (100 μg), clindamycin (2 μg), virginiamycin (15 μg), tetracycline (30 μg), ampicillin (10 μg), chloramphenicol (30 μg), cephalothin (30 μg), amikacin (30 μg), gentamicin (10 μg), kanamycin (30 μg), and fosfomycin (50 μg). Susceptibility categorization was carried out according to National Committee of Clinical Laboratory Standards (NCCLS) recommendations (29), except for with spiramycin and virginiamycin (not included in the NCCLS), for which the diameter halos considered for resistance were those recommended by the Société Française de Microbiologie (47).

Analysis of the molecular mechanisms of antibiotic resistance. The mechanism of macrolide resistance was analyzed by PCR amplification with degenerate *erm* primers and specific primers for *ermA*, *ermB*, *ermC*, *msrA*, and *mefA*/*E* genes by using the conditions described by other authors (4, 10, 49, 50, 61). β-Lactamase detection was carried out with nitrocefin disks (Becton Dickinson Microbiology Systems, Cockeysville, Md.).

RESULTS

Prevalence of *Campylobacter*. During the study period, 641 *Campylobacter* strains were isolated from 8,636 clinical human fecal samples (7.4%) submitted to the San Millán Hospital in

* Corresponding author. Mailing address: Area Bioquímica y Biología Molecular, Universidad de La Rioja, Avenida de la Paz 105, 26004 Logroño, Spain. Phone: 34-941-299284. Fax: 34-941-299274. E-mail: carmen.torres@daa.unirioja.es.

TABLE 1. Origin of samples analysed and prevalence of *Campylobacter* strains

Samples studied and their origin (no. of isolates)	*Campylobacter* strains isolated			
	Total no. (%)	*C. jejuni*	*C. coli*	*Campylobacter* spp.
Foods (101)	55 (54.4)	42	12	1
Hamburger, sausage, and minced chicken (32)	14 (43.7)	11	2	1
Breast of chicken (5)	0			
Cecum sample (16)	7 (43.8)	6	1	
Skin of chicken (37)	32 (86.5)	23	9	
Precooked chicken food (3)	1	1		
Boiled ham (5)	0			
Turkey products (3)	1	1		
Pigs[a] (45)	40 (88.9)		40	
Broilers[a] (105)	85 (81)	69	10	6
Human feces (8,636)	641 (7.4)	537	58	46

[a] Intestinal content samples.

Logroño (La Rioja, Spain). The species isolated were *C. jejuni* (84%), *C. coli* (9%), and *Campylobacter* spp. (7%) (Table 1).

The frequency of isolation of *Campylobacter* strains was 54.4% in food samples of animal origin (55 of 101 samples), 81% in intestinal samples from broilers (85 of 105 samples), and 88.9% in fecal samples from pigs (40 of 45 samples). The respective frequencies of *C. jejuni* and *C. coli* among the positive samples were as follows: food of animal origin (76 and 22%) and intestinal samples from broilers (81 and 12%) and from pigs (0 and 100%). A higher frequency of *C. coli* was observed among isolates from pigs compared to those isolated from broilers, foods, or human feces. *C. jejuni* was the most frequently isolated species from broilers (Table 1).

Among all the food samples of animal origin tested, chicken skin had the highest rate of isolation of *Campylobacter* (86.5%). Nevertheless, high rates were also found in other samples of chicken origin such as sausage, hamburger, or minced chicken (14 of 32 samples) but not in chicken breasts (0 of 5 samples) (Table 1).

Antimicrobial susceptibility. The results of the susceptibility testing for *C. jejuni* and *C. coli* strains from different origins are shown in Table 2. Resistance to ciprofloxacin in *C. jejuni* and *C. coli* was higher for strains isolated from broilers (98.7 and 100%) and pigs (100% for *C. coli*) than in those isolated from foods (74.4 and 72.7%) or from human fecal samples (75 and 70.7%, respectively). Cross-resistance between nalidixic acid and ciprofloxacin was found in all quinolone-resistant strains except one *C. jejuni* strain, which showed a nalidixic acid-resistant ciprofloxacin-susceptible phenotype.

Regarding macrolide resistance, a higher prevalence erythromycin resistance was found in *C. jejuni* and *C. coli* strains isolated from foods of chicken origin (17.1 and 50%, respectively) or from pig samples (81.1% for *C. coli*) than in those isolated from human fecal samples (3.2 and 34.5%). The frequency of resistance to azithromycin and spiramycin was similar to those of erythromycin. One-third of *Campylobacter* strains from broilers showed an intermediate level of erythromycin resistance, with intermediate or full resistance to clindamycin and susceptibility to azithromycin and spiramycin. Clindamycin resistance was higher in *C. jejuni* and *C. coli* strains isolated from pigs (86.5% for *C. coli*), broilers (13.6 and 50%), and foods (18 and 45.4%) than in those isolated from humans (2.8 and 41.4%, respectively).

The different MLS (macrolide-lincosamin-streptogramin)

TABLE 2. Resistance to 15 antibiotics in 637 *C. jejuni* and 117 *C. coli* strains isolated from different sources

Antibiotic	Resistance (%) in *C. jejuni* strains			Resistance (%) in *C. coli* strains			
	Broilers (n = 59)	Foods (n = 41)	Humans (n = 537)	Broilers (n = 10)	Pigs (n = 37)	Foods (n = 12)	Humans (n = 58)
Nalidixic acid	98.7	76.9	ND[a]	100	100	72.7	ND
Ciprofloxacin	98.7	74.4	75	100	100	72.7	70.7
Erythromycin[b]	0/20.3	17.1/12.2	3.2	0/90	81.1/5.4	50/0	34.5
Azithromycin	1.7	17.5/5.0	ND	0	81.1	45.5	ND
Spiramycin[b]	0	17.5	ND	0	81.1/2.7	45.5/9.1	ND
Clindamycin[b]	13.6/27.1	18/20.5	2.8	50/30	86.5/5.4	45.4/18.2	41.4
Virginiamycin	100	100	ND	100	94.6	100	ND
Tetracycline[b]	31.8/9.1	65	ND	0	94.4/2.8	90.9	ND
Ampicillin[b]	47.4/19.3	40/15	38/0.4	90/0	65.7/14.3	45.4/27.3	29.3
Chloramphenicol[b]	0	2.4/0	ND	0	0/2.7	0/16.7	ND
Cephalothin	100	100	98.7	100	100	100	98.3
Amikacin	0	2.4	0.2	0	21.6	0	0
Gentamicin	11.9	4.9	0.4	80	22.2	33.3	8.6
Kanamycin[b]	11.8/6.8	19.5/14.6	ND	80/0	55.9/17.6	33.3/16.7	ND
Fosfomycin[b]	0/1.9	10.8/2.7	4.8/0.6	0/10	8.1	40/0	13.8

[a] ND, not determined.
[b] % Resistant/% intermediate values are indicated.

TABLE 3. MLS resistance phenotypes in 153 *Campylobacter* strains from broilers, pigs, and foods

MLS resistance phenotypes[a]					Species (no. of strains)	
Ery	Azm	Spy	Cli	Vrg	*C. jejuni*	*C. coli*
R	R	R	R	R	7	34
R	R	R	R	S		1
S	S	S	R/I	R	19	4
S/I	S	S/I	S	R	5	2
I	S	I/S	R/I	R	10	10
S	S	S	S	R	55	6

[a] MLS, macrolide-lincosamin-streptogramin; Ery, erythromycin; Azm, azithromycin; Spy, spiramycin; Cli, clindamycin; Vrg, virginiamycin; R, resistant; I, intermediate; S, susceptible.

resistance phenotypes found in 153 *Campylobacter* strains (96 *C. jejuni* and 57 *C. coli*) are shown in Table 3. All the strains studied (except one *C. coli*) were resistant to virginiamycin. Twenty-three strains showed an intermediate or high level of resistance to clindamycin but were susceptible to erythromycin, with most of them belonging to the *C. jejuni* species (19 strains). Ten highly erythromycin-resistant *Campylobacter* strains were analyzed by PCR in order to determine the possible mechanism of resistance involved. In all cases, negative results were obtained from PCR amplification of *ermA*, *ermB*, *ermC*, *msrA*, *mefA*, and *mefE* genes.

Combined resistance to both quinolones and macrolides was frequently detected in *Campylobacter* strains, mainly in *C. coli* isolated from pigs (80.6%). High rates of coresistance were also obtained in *C. coli* from foods and humans (33 and 38%, respectively). With respect to the *C. jejuni* species, 10.5% of the strains isolated from foods showed resistance to both antibiotics versus 1.6% of the strains of human origin.

A higher prevalence of ampicillin resistance was observed in *C. jejuni* and *C. coli* strains from broilers (47.4 and 90%) and pigs (65.7% for *C. coli*) than in strains isolated from foods (40 and 45.4%) and human samples (38 and 29.3%, respectively). β-Lactamase production was detected in 468 of 578 *Campylobacter* strains studied (Table 4). Interestingly, 23 ampicillin-resistant *Campylobacter* strains (21 *C. jejuni* and 2 *C. coli*) were β-lactamase negative (all of them isolated from humans), and 206 ampicillin-susceptible strains were β-lactamase positive (176 *C. jejuni* and 30 *C. coli*). Chloramphenicol resistance was low in strains from all origins.

All *C. coli* and all but one of *C. jejuni* strains tested from human feces were susceptible to amikacin, but 21.6% of *C. coli* strains isolated from pigs were resistant to this antibiotic. In relation to gentamicin resistance in *C. jejuni* and *C. coli*, large differences were found depending on the origin of the strains: it was high in broilers (11.9 and 80%), pigs (22.2 for *C. coli*), and foods (4.9 and 33.3% respectively) but low in strains isolated from humans (0.4 and 8.6%, respectively). High frequencies of kanamycin resistance were detected in *C. jejuni* and *C. coli* strains from broilers (11.8 and 80%) and also in *C. coli* obtained from pigs (55.9%). Tetracycline resistance was also high in *C. jejuni* and *C. coli* strains from foods (65 and 90.9%) and in *C. coli* from pigs (94.4%). In most cases, kanamycin-resistant *Campylobacter* strains also showed tetracycline resistance.

DISCUSSION

A high isolation frequency of *Campylobacter* strains (>80%) was found in the intestinal content from broilers and pigs. *C. jejuni* was the most frequently isolated species from broilers (81%), and *C. coli* was the only one obtained from pigs. Contamination frequencies of 60 to 80% of retailed chickens by *Campylobacter* strains have been previously reported (3, 6, 46), and a higher level of isolation of *C. jejuni* than *C. coli* from broilers has also been reported (9, 18, 44). *C. coli* is particularly associated with pigs, both in our study and in others (1, 18, 48). A high isolation frequency of *Campylobacter* strains was also found in this study from foods of chicken origin obtained in the supermarket, particularly from samples such as skin (86.5%). *Campylobacter*-contaminated food may influence the acquisition of these strains by humans. This may occur either by direct ingestion of contaminated undercooked food or by cross-contamination of raw poultry to other ready-to-eat foods via the unwashed hands of the cook or kitchen utensils (31).

The extremely high prevalence of ciprofloxacin resistance found in *Campylobacter* strains isolated from broilers (99%), pigs (100%), and human feces (72%) is of interest. Studies carried out up to 1987 had shown that strains resistant to fluoroquinolones were practically nonexistent (12, 13, 36, 37). In Spain, the absence of ciprofloxacin resistance in human strains was reported in 1987; the first three *C. jejuni*-resistant strains were detected in 1988 (39). Since then a remarkable increase in quinolone resistant strains isolated from humans has been observed for the period 1989 to 1991 (3 to 8% to 19 to 50%, respectively) (25, 26, 38, 43). During that period, this increase in quinolone resistance was also observed in other countries, such as Finland (36), The Netherlands (13), England (5, 16, 24), and Canada (15). However, no ciprofloxacin resistance among *Campylobacter* strains was found in that period in studies carried out in Sweden (30, 45), the United States (27), or Austria (19). High rates of ciprofloxacin resistance (ca. 80%) have been reported recently in different studies (17; E. Pérez-Trallero, F. Otero, C. López-Lopategui, M. Montes, J. M. García-Arenzana, and M. Gomariz, Prog. Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-21, 1997).

In our study, ciprofloxacin resistance was higher in *Campylobacter* strains isolated from animals and foods than in those isolated from human fecal samples. The use of quinolones, mainly enrofloxacin, in veterinary practice, has been correlated with the increase in ciprofloxacin resistance in *Campylobacter* strains in different European countries (13, 33) as well as in Spain (57). Gaunt and Piddock (16) found that poultry imported into the United Kingdom (from countries that use quinolones in animals) was more likely to harbor ciprofloxacin-resistant *Campylobacter* strains than those bred in the United Kingdom, where quinolones were not allowed for veterinary use before November 1993.

Cross-resistance between nalidixic acid and ciprofloxacin was found in all but one of the strains studied, which was nalidixic acid resistant and ciprofloxacin susceptible. This unusual phenotype in our series of strains corresponds to the type 2 previously described by Reina et al. (39, 40), which represented 10.9% of their quinolone-resistant strains. Why has *Campylobacter* readily acquired fluoroquinolone resistance? This genus may have a certain level of intrinsic resistance that may be due to the presence of an efflux pump (8). The appear-

TABLE 4. Ampicillin resistance phenotypes and β-lactamase production in 578 *Campylobacter* strains of different sources

Ampicillin susceptibility	β-Lactamase production	*C. jejuni*	*C. coli*
Resistant	+	203	59
Resistant	−	21	2
Susceptible	+	176	30
Susceptible	−	77	10

ance of *gyrA* mutation (8) may lead to a fully resistant phenotype (60).

A high level of erythromycin resistance was detected in *C. jejuni* strains isolated from foods of animal origin compared with the level obtained in strains from humans or broilers. Previous studies also observed low-level erythromycin resistance in *C. jejuni* isolated from humans or broilers (14, 15, 22, 38, 43). In our study, no erythromycin-resistant *C. jejuni* strains were found among those isolated from broilers. In the case of *C. coli*, a higher rate of erythromycin resistance was obtained in strains from pigs (81.1%) or foods (50%) compared to those from humans (34.5%). Data similar to ours were obtained with *C. coli* strains from pigs by other researchers (2, 53, 59). Macrolides (such as tylosin) have been permitted as growth promoters in swine (Council Directive 70/524/EEC), and this use could help to explain the selection of erythromycin resistance in the strains isolated from pigs. On the other hand, tylosin has not been used as a growth promoter in broilers, and this could be the reason for the low level of resistance obtained in strains of this origin (2). A recent resolution of the European Union banned the use of tylosin as a growth promoter in animal feeds (from January 1999). Our erythromycin resistance levels confirm that the *C. coli* species is much more resistant to this antibiotic than *C. jejuni*, a fact which has also been observed by others (2, 39). Cross-resistance among all macrolides (C_{14}, C_{15}, and C_{16}) was observed in all previously reported cases (39, 43). The mechanism of macrolide resistance was studied in 10 erythromycin-resistant *C. jejuni* and *C. coli* strains. Negative results were regularly obtained by PCR when degenerate *erm* primers and specific primers for *ermA*, *ermB*, *ermC*, and *mefA*/*mefE* genes were used. Nevertheless, we detected in the genome of *C. jejuni* a gene of high homology with a 23S rRNA methyltransferase (http://www.sanger.ac.uk/projects/*C. jejuni*). Information about the mechanism involved in *Campylobacter* macrolide resistance remains scarce (39, 55, 58). An efflux pump may also be involved in macrolide resistance (8). Almost all *Campylobacter* strains tested were resistant to virginiamycin, including even those strains that were susceptible to erythromycin.

The frequency of ampicillin resistance among human strains was 39%, a level similar to that found by other researchers (52). A higher level of ampicillin resistance was obtained in strains from broilers (55%) and pigs (66%), in contrast to observations made by Prasad et al. (35), who found a higher resistance to ampicillin in human strains (22.2%) than in chicken strains (6.7%). Resistance is usually associated with β-lactamase production; such enzymatic activity was detected in 81% of our strains. Nevertheless, 206 *Campylobacter* strains were susceptible to ampicillin as determined by disk diffusion even though they were β-lactamase producers. Similar data were obtained by others (21, 22, 52). The β-lactamase-positive strains are significantly less susceptible to ampicillin even if the MICs detected in these strains are not high enough to be regarded as resistant (21, 22). In our study, 23 *Campylobacter* strains were resistant to ampicillin but did not produce β-lactamase. Other researchers have found that sonic disruption of cells could improve β-lactamase detection in some *Campylobacter* strains (11), although other mechanisms of resistance could be involved, such as modified penicillin-binding proteins or impermeability (52).

Low percentages of resistance to chloramphenicol in our series of *Campylobacter* strains were found, in accordance with the results presented in previous publications (39, 45).

In this study, the prevalence of gentamicin-resistant strains was 1% in human isolates in contrast to 25, 22, and 11% in broilers, pigs, and food strains, respectively. A higher resistance frequency was found in *C. coli* than in *C. jejuni*, mainly in *C. coli* isolated from broilers (8 of 10 strains). Different studies have reported that gentamicin is an effective agent for the treatment of *Campylobacter* enteritis in humans (1, 23, 34, 57). The emergence of gentamicin resistance in strains isolated from animals could be related to the use of apramycin (aminoglycoside, structurally related with gentamycin) for veterinary treatment. The use of this antibiotic may have selected strains producing an aminoglycoside modifying enzyme, AAC(3′)-IV, that modifies both aminoglycosides (7, 42). In strains isolated from animals by other research groups, no gentamicin-resistant strains were detected (1, 23).

Kanamycin resistance was generally associated with tetracycline resistance (all *Campylobacter* strains except two kanamycin-resistant ones showed tetracycline resistance). Tenover and Elvrum (56) found kanamycin resistance associated with plasmids ranging in size from 41 to 132 kb; these plasmids frequently encoded the *tetO* gene that confers resistance to tetracycline. The aminoglycoside phosphotransferase APH(3′) has been implicated in kanamycin resistance in *C. coli* (41).

In conclusion, differences in antibiotic resistance frequencies were observed in *Campylobacter* strains according to the species, the origin of the strains, and the presumed history of antibiotic use of the hosts. The high prevalence of quinolone resistance found in *Campylobacter* strains from broilers and pigs and the high level of macrolide resistance in *C. coli* isolated from pigs is extremely worrisome. The resulting increasing prevalence of these resistances in strains of human origin may have important therapeutic implications. Metronidazole remains as a possible alternative, but there are technical difficulties for a reliable in vitro evaluation of the susceptibility of *Campylobacter* strains to this compound. More restrictive policies on the use of antibiotics in animals may result in an improvement of the current situation in the medium term.

ACKNOWLEDGMENTS

This work was supported in part by grants of the Fondo de Investigaciones Sanitarias (FIS 98/0282), the Universidad de La Rioja (API 98/B28), and the Gobierno de la Rioja (97/453), Spain.

REFERENCES

1. **Aarestrup, F. M., E. M. Nielsen, M. Madsen, and J. Engberg.** 1997. Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. from humans, pigs, cattle, and broilers in Denmark. Antimicrob. Agents Chemother. **41:**2244–2250.
2. **Aarestrup, F. M., F. Bager, N. E. Jensen, M. Madsen, A. Meyling, and H. C. Wegener.** 1998. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. APMIS **106:**606–622.
3. **Aquino, M. H. C., J. C. P. Carvalho, A. Tibana, and R. M. Franko.** 1996. *Campylobacter jejuni-coli* methodology of isolation and possible interfering factors in primary culture. J. Food Protect. **59:**429–432.
4. **Arthur, M., C. Molinas, and C. Mabilat.** 1993. PCR detection of *erm* erythromycin resistance genes by using degenerate oligonucleotide primers, p. 534–538. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology. American Society for Microbiology, Washington, D.C.
5. **Bowler, I., and D. Day.** 1992. Emerging quinolone resistance in *Campylobacters*. Lancet **340:**245.
6. **Bryan, F. L., and M. P. Doyle.** 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. J. Food Protect. **58:**326–344.
7. **Chalus-Dancla, E., J. L. Martel, C. Carlier, J. P. Lafont, and P. Courvalin.** 1986. Emergence of aminoglycoside 3-*N*-acetyltransferase IV in *Escherichia coli* and *Salmonella typhimurium* isolated from animals in France. Antimicrob. Agents Chemother. **29:**239–243.
8. **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.
9. **Chuma, T., K. Makino, K. Okamoto, and H. Yugi.** 1997. Analysis of distribution of *Campylobacter jejuni* and *Campylobacter coli* in broilers by using restriction fragment length polymorphism of flagellin gene. J. Vet. Med. Sci. **59:**1011–1015.

10. **Clancy, J., J. Petitpas, F. Dib-Hajj, W. Yuan, M. Cronan, A. V. Kamath, J. Bergeron, and J. A. Retsema.** 1996. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, *mefA*, from *Streptococcus pyogenes*. Mol. Microbiol. **22:**867–879.
11. **Diker, K. S., M. Akan, G. Hascelik, and G. Emekdas.** 1991. β-Lactamase production in human and animal isolates of *Campylobacter jejuni* in Turkey. J. Antimicrob. Chemother. **27:**693–694.
12. **Endtz, H. P., R. P. Mouton, T. van der Reyden, G. J. Ruijs, and M. Biever.** 1990. Fluoroquinolone resistance in *Campylobacter* spp. isolated from human stools and poultry products. Lancet **335:**787.
13. **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.
14. **Gaudreau, C., and H. Gilbert.** 1997. Comparison of disc diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli*. J. Antimicrob. Chemother. **39:**707–712.
15. **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.
16. **Gaunt, P. N., and L. J. V. Piddock.** 1996. Ciprofloxacin resistant *Campylobacter* spp. in humans: an epidemiological and laboratory study. J. Antimicrob. Chemother. **37:**747–757.
17. **Harnett, N., S. McLeod, Y. A. 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.
18. **Hernández, J.** 1993. Incidence and control of *Campylobacter* in foods. Microbiología SEM **9:**57–65.
19. **Hirschl, A. M., D. Wolf, J. Berger, and M. L. Rotter.** 1990. In vitro susceptibility of *Campylobacter jejuni* and *Campylobacter coli* isolated in Austria to erythromycin and ciprofloxacin. Zentbl. Bakteriol. **272:**443–447.
20. **Jiménez, A., J. B. Velázquez, J. Rodríguez, A. Tinajas, and T. G. Villa.** 1994. Prevalence of fluoroquinolone resistance in clinical strains of *Campylobacter jejuni* isolated in Spain. J. Antimicrob. Chemother. **33:**188–190.
21. **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.
22. **Lachance, N., C. Gaudreau, F. Lamothe, and F. Turgeon.** 1993. Susceptibilities of β-lactamase-positive and -negative strains of *Campylobacter coli* to β-lactam agents. Antimicrob. Agents Chemother. **37:**1174–1176.
23. **Li, C. C., C. H. Chiu, J. L. Wu, Y. C. Huang, and T. Y. Lin.** 1998. Antimicrobial susceptibilities of *Campylobacter jejuni* and *coli* by using E-test in Taiwan. Scand. J. Infect. Dis. **30:**39–42.
24. **McIntyre, M., and M. Lyons.** 1993. Resistance to ciprofloxacin in *Campylobacter* spp. Lancet **341:**188.
25. **Mirelis, B., E. Miro, F. Navarro, C. A. Ogalla, J. Bonal, and G. Prats.** 1993. Increased resistance to quinolone in Catalonia, Spain. Diagn. Microbiol. Infect. Dis. **16:**137–139.
26. **Moles, B., C. Santacruz, C. Gimeno, and M. A. Remacha.** 1992. Resistencia de *Campylobacter jejuni* en Zaragoza. Rev. Esp. Quimioter. **5:**171–172.
27. **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.
28. **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, 6th ed. American Society for Microbiology, Washington, D.C.
29. **National Committee for Clinical Laboratory Standards.** 1999. Performance standards for antimicrobial disk susceptibility tests. Ninth informational supplement. NCCLS document M100-S9. National Committee for Clinical Laboratory Standards, Wayne, Pa.
30. **Olsson-Liljequist, B., and R. Mollby.** 1990. In vitro activity of norfloxacin and other antibacterial agents against gastrointestinal pathogens isolated in Sweden. APMIS **98:**150–155.
31. **Park, R. W. A., P. L. Griffiths, and G. S. Moreno.** 1991. Sources and survival of *Campylobacters*: relevance to enteritis and food industry. J. Appl. Bacteriol. **81:**425–432.
32. **Penner, J. L.** 1988. The genus *Campylobacter*: a decade of progress. Clin. Microbiol. Rev. **1:**157–172.
33. **Piddock, L. J. V.** 1995. Quinolone resistance and *Campylobacter* spp. J. Antimicrob. Chemother. **36:**891–898.
34. **Pigrau, C., R. Bartolomé, B. Almirante, A. M. Planes, J. Gavaldá, and A. Pahissa.** 1997. Bacteremia due to *Campylobacter* species: clinical findings and antimicrobial susceptibility patterns. Clin. Infect. Dis. **25:**1414–1420.
35. **Prasad, K. N., S. K. Mathur, T. N. Dhole, and A. Ayyagari.** 1994. Antimicrobial susceptibility and plasmid analysis of *Campylobacter jejuni* isolated from diarrhoeal patients and healthy chickens in northern India. J. Diarrhoeal Dis. Res. **12:**270–273.
36. **Rautelin, H., O. 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.
37. **Reina, J.** 1991. Incremento progresivo de la resistencia a ciprofloxacina y eritromicina en el grupo de las campilobacterias termotolerantes aisladas en heces. Rev. Esp. Microbiol. Clin. **6:**305–306.
38. **Reina, J., N. Borrell, and A. Serra.** 1992. Emergence of resistance to erythromycin and fluoroquinolones in thermotolerant *Campylobacter* strains isolated from feces 1987–1991. Eur. J. Clin. Microbiol. Infect. Dis. **11:**1163–1166.
39. **Reina, J., M. J. Ros, and A. Serra.** 1994. Susceptibilities to 10 antimicrobial agents of 1220 *Campylobacter* strains isolated from 1987 to 1993 from feces of pediatric patients. Antimicrob. Agents Chemother. **38:**2917–2920.
40. **Reina, J., M. J. Ros, and V. Fernandez-Baca.** 1995. Resistance to erythromycin in fluoroquinolone-resistant *Campylobacter jejuni* strains isolated from human faeces. J. Antimicrob. Chemother. **35:**351–352.
41. **Rivera, M. J., J. Castillo, C. Martin, M. Navarro, and R. Gómez-Lus.** 1986. Aminoglycoside-phosphotransferases APH(3′)-IV and APH(3″) synthesized by a strain of *Campylobacter coli*. J. Antimicrob. Chemother. **18:**153–158.
42. **Salauze, D., I. Otal, R. Gómez-Lus, and J. Davies.** 1990. Aminoglycoside acetyltransferase 3-IV (aacC4) and hygromycin B 4-I phosphotransferase (hpbB) in bacteria isolated from human and animal sources. Antimicrob. Agents Chemother. **34:**1915–1920.
43. **Sánchez, R., V. Fernández-Baca, M. D. Díaz, P. Muñoz, M. Rodríguez-Créixems, and E. Bouza.** 1994. Evolution of susceptibilities of *Campylobacter* spp. to quinolones and macrolides. Antimicrob. Agents Chemother. **38:**1879–1882.
44. **Schoeni, J. L., and M. P. Doyle.** 1992. Reduction of *Campylobacter jejuni* colonization of chicks by cecum-colonizing bacteria producing anti-*C. jejuni* metabolites. Appl. Environ. Microbiol. **58:**664–670.
45. **Sjögren, E., B. Kaijser, and M. Werner.** 1992. Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* isolated in Sweden: a 10-year follow-up report. Antimicrob. Agents Chemother. **36:**2847–2849.
46. **Skirrow, M. B., and M. J. Blaser.** 1992. Clinical and epidemiologic considerations, p. 3–8. *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.
47. **Société Française de Microbiologie.** 1996. Report of the Comité de l'Antibiogramme de la Société Francaise de Microbiologie. Clin. Microbiol. Infect. **2**(Suppl. 1)**:**S47–S48.
48. **Stern, N. J.** 1992. Reservoirs for *Campylobacter jejuni* and approaches for intervention in poultry, p. 49–60. *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.
49. **Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
50. **Sutcliffe, J., A. Tait-Kamradt, and L. Wondrack.** 1996. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. Antimicrob. Agents Chemother. **40:**1817–1824.
51. **Svedhem, A., B. Kaijser, and E. Sjögren.** 1981. Antimicrobial susceptibility of *Campylobacter jejuni* isolated from humans with diarrhoea and from healthy chickens. J. Antimicrob. Chemother. **7:**301–305.
52. **Tajada, P., J. L. Gómez-Garcés, J. I. Alós, D. Balas, and R. Cogollos.** 1996. Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* to 12 β-lactam agents and combinations with β-lactamase inhibitors. Antimicrob. Agents Chemother. **40:**1924–1925.
53. **Taylor, D. N., M. J. Blaser, P. Echeverria, C. Pitarangsi, L. Bodhidatta, and W. L. L. Wang.** 1987. Erythromycin-resistant *Campylobacter* infections in Thailand. Antimicrob. Agents Chemother. **31:**438–442.
54. **Taylor, D. N., and M. J. Blaser.** 1991. *Campylobacter* infections, p. 151–172. *In* A. S. Evans and P. S. Brachman (ed.), Bacterial infections in humans. Plenum Publishing Corp., New York, N.Y.
55. **Taylor, D. E.** 1992. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* to tetracycline, chloramphenicol, and erythromycin, p. 74–86. *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.
56. **Tenover, F. C., and P. M. Elvrum.** 1988. Detection of two different kanamycin resistance genes in naturally occurring isolates of *Campylobacter jejuni* and *Campylobacter coli*. Antimicrob. Agents Chemother. **32:**1170–1173.
57. **Velázquez, J. B., A. Jiménez, B. Chomón, and T. G. Villa.** 1995. Incidence and transmission of antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli*. J. Antimicrob. Chemother. **35:**173–178.
58. **Yan, W., and D. E. Taylor.** 1991. Characterization of erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli*. Antimicrob. Agents Chemother. **35:**1989–1996.
59. **Wang, W. L., L. B. Reller, and M. J. Blaser.** 1984. Comparison of antimicrobial susceptibility patterns of *Campylobacter jejuni* and *Campylobacter coli*. Antimicrob. Agents Chemother. **26:**351–353.
60. **Wiedemann, B., and P. Heisig.** 1994. Mechanisms of quinolone resistance. Infection **22:**S73–S79.
61. **Wondrack, L., M. Massa, B. V. Yang, and J. Sutcliffe.** 1996. Clinical strain of *Staphylococcus aureus* inactivates and causes efflux of macrolides. Antimicrob. Agents Chemother. **40:**992–998.