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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.

(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 plated 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 (Campyloplate, BioMerieux, 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), cephalexin (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 diameters of the 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, mefA, and mefE/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
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 (35 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. jejuni 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)
results were obtained from PCR amplification of possible mechanism of resistance involved. In all cases, negative strains were analyzed by PCR in order to determine the pos-

strains). Ten highly erythromycin-resistant Campylobacter with most of them belonging to the resistance to clindamycin but were susceptible to erythromycin, Twenty-three strains showed an intermediate or high level of studied (except one C. coli) were resistant to virginiamycin, with the increase in ciprofloxacin resistance 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>
<thead>
<tr>
<th>MLS resistance phenotypes*</th>
<th>Species (no. of strains)</th>
</tr>
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<tbody>
<tr>
<td>Ery Azm Spy Cli Vrg</td>
<td>C. jejuni</td>
</tr>
<tr>
<td>R R R R R R</td>
<td>7</td>
</tr>
<tr>
<td>R R R R R S</td>
<td>19</td>
</tr>
<tr>
<td>S S S S I R/I R</td>
<td>10</td>
</tr>
<tr>
<td>I S I S I R/I R</td>
<td>10</td>
</tr>
<tr>
<td>S S S S S R</td>
<td>55</td>
</tr>
</tbody>
</table>

* MLS, macrolide-lincosamin-streptogramin; Ery, erythromycin; Azm, azithro-

mycin; Spy, spiramycin; Cli, clindamycin; Vrg, virginiamycin; R, resistant; I, intermediate; S, susceptible.

**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. Con-
tamination 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 non-existent (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, F. Pérez-Trallero, F. Otero, C. López-Lopategui, M. Montes, J. M. García-Arenzana, and M. Gomariz, Prog. Abstr. 37th In-

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.

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

<table>
<thead>
<tr>
<th>Ampicillin susceptibility</th>
<th>β-Lactamase production</th>
<th>C. jejuni</th>
<th>C. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>+</td>
<td>203</td>
<td>59</td>
</tr>
<tr>
<td>Resistant</td>
<td>−</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Susceptible</td>
<td>+</td>
<td>77</td>
<td>10</td>
</tr>
<tr>
<td>Susceptible</td>
<td>−</td>
<td>77</td>
<td>10</td>
</tr>
</tbody>
</table>
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 (C14, C15, and C16) 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 235 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 strains isolated from broilers and pigs (40.8%). 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 to gentamicin) 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 humans or 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.

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