Journal of Food Protection, Vol. 73, No. 4, 2010, Pages 720–728

Research Note

Prevalence and Biomolecular Characterization of *Campylobacter* spp. Isolated from Retail Meat

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MS 09-336: Received 6 August 2009/Accepted 17 November 2009

ABSTRACT

We estimated the prevalence of *Campylobacter* spp. in retail meat ($n = 352$ samples; 104 chicken, 106 pork, and 142 beef) collected in Campobasso, Italy, comparing two microbiological methods. All the isolates were characterized by biomolecular techniques for epidemiological purposes. *Campylobacter* isolation was performed by selective culture and membrane filtration methods. Phenotypic and genotypic methods for genus and species identification were evaluated together with antimicrobial resistance and plasmid profiling. Sixty-nine (86.2%) samples were positive by selective culture, 49 (61.2%) by membrane filtration, and 38 (47.5%) by both methods. Only 74 of 80 strains were confirmed as *Campylobacter* spp. by PCR, and two *Campylobacter coli* were identified as *Campylobacter jejuni*. Chicken meat was more frequently contaminated than other meats. Selective culture was more sensitive than membrane filtration (85 versus 66%), and specificity of the methods was 98 and 100%, respectively. Among *Campylobacter* isolates from chicken meat, 86.5% were multidrug resistant. Resistance to ciprofloxacin (51.3%) and enrofloxacin (52.7%) was lower than to nalidixic acid (71.6%). *C. coli* strains showed the highest cross-resistance for quinolones (82.6%) and fluoroquinolones (60.9%) as well as a high resistance to tetracycline. Plasmids were isolated from six *C. coli* and two *C. jejuni* isolates, but no association was detected between antimicrobial resistance and plasmid DNA carriage. Selective culture is considered as the optimal method for *Campylobacter* isolation, although it was unable to detect all contaminated samples. Membrane filtration provided more specific results but with low sensitivity. A combination of both techniques may offer better results.

Campylobacter spp. are among the most common agents of acute bacterial gastroenteritis worldwide *(19, 23)*. Preliminary data on infections caused by common foodborne pathogens in the United States indicate that the estimated incidence of *Campylobacter* infections did not change significantly in 2007 compared with 2004 to 2006, confirming the substantial 30% decline in incidence compared with 1996 to 1998 *(9, 10)*. In 2006, as in the previous year, campylobacteriosis was the most commonly reported zoonotic disease in humans in the European Union, with 175,561 cases, which represents a small decrease in confirmed cases from 2005 *(9)*. Incidence in the European Union dropped from 51.6 per 100,000 population in 2005 to 46.1 per 100,000 population in 2006 *(9)*. In Italy, epidemiological surveillance data about the incidence of *Campylobacter* infection in humans and animals and its prevalence in foods are not yet available, since there are limited data submitted to the national surveillance network. Hence, a pilot surveillance study for human infections has been carried out, with results confirming that the pathogen is particularly common in children and adolescents *(15)*.

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Campylobacter spp. were most commonly detected in fresh poultry meat, where on average 35% of samples were positive; they were also frequently found in live poultry, pigs, and cattle *(9, 52)*. The leading route of transmission for human campylobacteriosis in developed countries is through contaminated food consumption, particularly of animal products, especially poultry, in which this microorganism is normally highly prevalent *(9, 11, 24, 25)*.

The intensive use of antimicrobial drugs in human and veterinary medicine and in animal husbandry for disease prevention, treatment, and in subtherapeutic concentrations, for growth promotion, has contributed to the increased resistance to antimicrobial drugs in *Campylobacter* strains that infect humans and animals *(3, 35, 49)*. Fluoroquinolone and macrolide resistance are of particular concern because these drugs are often used for treatment of campylobacteriosis in humans *(1, 9, 16, 30)*. Some authors have reported that plasmid profiling is helpful for *Campylobacter* epidemiological studies *(7, 26)*, and *Campylobacter jejuni* resistance to tetracycline can be plasmid mediated *(44)*. Tracz et al. *(48)* reported an association between the carriage of the pVir plasmid in *C. jejuni* and the development of bloody diarrhea in patients, although these findings were not confirmed elsewhere *(29)*.

The aim of this study was to estimate the prevalence of *Campylobacter* spp. in retail meat using selective microbi-

ological media and selective membrane filtration procedures. In addition, phenotypic and genotypic methods for genus and species identification were evaluated together with antimicrobial resistance patterns (R-types) and plasmid profiling, and the relationship between antibiotic resistance and plasmid carriage was investigated.

MATERIALS AND METHODS

Samples collection. Three hundred fifty-two raw meat samples, purchased from randomly selected markets and butcher shops in Campobasso, Italy, were transported to the laboratory in refrigerated containers and analyzed within 2 h of collection. The raw meat samples consisted of chicken ($n = 104$; 29.5%; breast, wings, and legs with skin and bones), pork ($n = 106$; 30.1%; steaks), and beef ($n = 142$; 40.3%; steaks and thin steaks).

Isolation and biochemical tests. Meat samples (25 g each) were aseptically added to and homogenized with 225 ml of Preston broth (Oxoid, Milan, Italy) containing laked horse blood (5%, vol/vol), growth supplement (Oxoid), and Preston Campylobacter selective supplement (Oxoid). The mixtures were incubated in an AnaeroJar (Oxoid) for 48 h at 42°C in a microaerophilic atmosphere obtained using commercial gas-generating kits (CAMPYgen, Oxoid). After incubation, the samples were analyzed in parallel by selective culture and membrane filtration methods. For culture using selective agar, a loopful of broth was subcultured onto *Campylobacter* blood-free selective agar (mCCDA-Preston, Oxoid) and incubated in a modified atmosphere at 42°C for 48 h. For culture using membrane filtration, sterile 0.45-μm-pore-size cellulose acetate membrane filters (Sartorius, Firenze, Italy) were placed onto the surface of nonselective blood agar plates. Afterward, 100 μl of broth was placed onto the membrane's surface, avoiding spillage around the edges of the filter, and the plates were incubated at room temperature for 30 min until all the liquid had passed through. Membranes were aseptically removed, and the plates were incubated as described for selective culture method. Presumptive *Campylobacter* spp. colonies (gray, watery) from all media were selected for further study and subcultured onto Columbia blood agar containing 5% (vol/vol) laked horse blood. After incubation, presumptive *Campylobacter* strains were initially identified by Gram staining and an oxidase-positive test. Further biochemical species and biotype identification were performed according to Lior's method *(28),* using the following tests: catalase production, hippurate hydrolysis, indoxyl-acetate, rapid production of hydrogen sulphide in FBP broth, and DNA hydrolysis.

Genomic and plasmid DNA extraction. DNA extraction was performed using a modified Boom method *(6).* Briefly, a colony was added to 900 μl of guanidinium thiocyanate L6 buffer, which was then centrifuged and the particulate material discarded. One hundred microliters of activated silica (Severn Biotech, Worcester, UK) was added to the supernatant; it was then incubated for 10 min at room temperature and washed by centrifugation twice with guanidinium thiocyanate L2 buffer, twice with ice-cold 80% ethanol, and once with ice-cold acetone. The silica was heated at 55°C for 10 min, resuspended in 150 μl of sterile distilled water, reincubated at 55°C for 5 min, and centrifuged; the supernatant containing DNA was transferred to a new tube.

Plasmid DNA was extracted by alkaline lyses, as described by Sambrook and Russell *(40),* and visualized on a UV transilluminator after horizontal gel electrophoresis and ethidium bromide staining.

PCR for isolates confirmation. *Campylobacter* spp. identified by biochemical tests were confirmed to genus and species level by three single PCR assays, based on the amplification of a 16S rRNA gene fragment for *Campylobacter* spp. identification (MD16S1 5′ ATC TAA TGG CTT AAC CAT TAA AC 3′; MD16S2 5′ GGA CGG TAA CTA GTT TAG TAT T 3′, 857 bp fragment amplified) and on the amplification of *mapA* and *ceuE* gene fragments for identification of *C. jejuni* and *Campylobacter coli* strains, respectively (MDmapA1 5′ CTA TTT TAT TTT TGA GTG CTT GTG 3′; MDmapA2 5′ GCT TTA TTT GCC ATT TGT TTT ATT A 3′, 589 bp fragment amplified; COL3 5′ AAT TGA AAA TTG CTC CAA CTA TG 3′; MDCOL2 5′ TGA TTT TAT TAT TTG TAG CAG CG 3′, 462 bp fragment amplified) *(13).* PCR amplification was performed in a 30-μl reaction volume containing template (1 μl), deoxynucleoside triphosphates (200 μM), primers (1 μM), and a thermostable DNA polymerase (1 U, Euroclone, Celbio, Milan, Italy). Amplifications were performed using an Eppendorf Mastercycler (Eppendorf, Milan, Italy) with a precycling stage of 10 min at 95°C, followed by 30 cycles consisting of 30 s at 95°C, 1 min at 59°C, 1 min at 72°C, and a final extension step of 10 min at 72°C. The amplified products were separated on a 1.5% agarose gel and detected by UV transillumination after ethidium bromide staining.

For isolates not confirmed as *Campylobacter* spp. by PCR, additional PCR assays were performed as described by Houf et al. *(22)* to identify *Arcobacter* spp. Three positive control strains of *Arcobacter butzleri* were kindly provided from the Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome.

Antimicrobial susceptibility testing. Antimicrobial R-types were assessed by the agar disk diffusion method using Mueller-Hinton agar (Biolife, Milan, Italy) supplemented with 5% horse blood and incubated at 37°C for 24 h under microaerophilic atmosphere. The following antimicrobial disks (Oxoid) were tested: nalidixic acid (30 μg), ampicillin (10 μg), cephalothin (30 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), enrofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), sulphonamides (300 μg), trimethoprim-sulfamethoxazole (25 μg), and tetracycline (30 μg) (Oxoid). Disk diffusion results were interpreted according to Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines *(33). Escherichia coli* ATCC 25922 was used as a control.

Statistical analysis. Statistical analysis was performed using the software SPSS 12.0.1 for Windows (SPSS Italia, Rome). The χ^2 test, at a significance level of less than 0.05, was used to evaluate the results of antimicrobial resistance, while McNemar's test was used to compare the selective culture and membrane filtration methods for their ability to detect genus and the most common pathogenic species *C. jejuni* and *C. coli*. Each method's performance was assessed based on its specificity and sensitivity and its positive and negative predictive values.

RESULTS

***Campylobacter* spp. prevalence.** Overall prevalences of *Campylobacter* spp. in meat samples are shown in Table 1. Using culture methods and biochemical identification, *Campylobacter* spp. were detected in 80 (22.7%) samples (14.1% beef, 5.7% pork, 51.9% chicken meat). The detection rate by membrane filtration was lower than by selective culture. Particularly, 38 (47.5%) samples were positive by selective culture and membrane filtration techniques, 31 (38.7%) by selective culture alone, and 11

TABLE 1. *Prevalence of thermophilic* Campylobacter *spp. isolated from meat samples by selective culture and membrane filtration methods and confirmed by PCR*

Type of sample	No. of samples	*Campylobacter* spp. prevalence by detection and confirmation method, no. (%)		*Campylobacter* spp. prevalence by microbiological isolation methods and after PCR confirmation, no. (%)[a]				
		Culture	PCR	BM	BM-PCR	SC	SC-PCR	MF	MF-PCR
Beef	142	20 (14.1)	15 (10.6)	3 (2.1)	3 (2.1)	13 (9.1)	8 (5.6)	4 (2.8)	4 (2.8)
C. jejuni		12[b] (8.4)	13[b] (9.2)	3 (2.1)	3 (2.1)	5[b] (3.5)	6[b] (4.2)	4 (2.8)	4 (2.8)
C. coli		8[c] (5.6)	2[c] (1.4)	0	0	8[c] (5.6)	2[c] (1.4)	0	0
Pork	106	6 (5.7)	6 (5.7)	2 (1.8)	2 (1.8)	3 (2.8)	3 (2.8)	1 (0.9)	1 (0.9)
C. jejuni		3 (2.8)	3 (2.8)	1 (0.9)	1 (0.9)	1 (0.9)	1 (0.9)	1 (0.9)	1 (0.9)
C. coli		3 (2.8)	3 (2.8)	1 (0.9)	1 (0.9)	2 (1.9)	2 (1.9)	0	0
Chicken	104	54 (51.9)	53 (51.0)	33 (31.7)	33 (31.7)	15 (14.4)	14 (13.5)	6 (5.8)	6 (5.8)
C. jejuni		29[b] (27.9)	30[b] (28.8)	17[b] (16.3)	18[b] (17.3)	7 (6.7)	7 (6.7)	5 (4.8)	5 (4.8)
C. coli		25[c] (24.0)	23[c] (22.1)	16[c] (15.4)	15[c] (14.4)	8[c] (7.7)	7[c] (6.7)	1 (0.9)	1 (0.9)
Total	352	80 (22.7)	74 (21.0)	38 (10.8)	38 (10.8)	31 (8.8)	25 (7.1)	11 (3.1)	11 (3.1)
C. jejuni		44[b] (12.5)	46[b] (13.1)	21[b] (5.9)	22[b] (6.2)	13[b] (3.7)	14[b] (3.9)	10 (2.8)	10 (2.8)
C. coli		36[c] (10.2)	28[c] (7.9)	17[c] (4.8)	16[c] (4.5)	18[c] (5.1)	11[c] (3.1)	1 (0.3)	1 (0.3)

[a] BM, *Campylobacter* spp. detected by both microbiological isolation methods (selective culture and membrane filtration); SC, *Campylobacter* spp. detected only by selective culture; MF, *Campylobacter* spp. detected only by membrane filtration.
[b] By PCR, two *C. coli* were speciated by PCR as *C. jejuni*.
[c] By PCR, six *C. coli* were not confirmed as *Campylobacter* spp. and two were speciated as *C. jejuni*.

(13.7%) by membrane filtration alone; hence, 69 (86.2%) samples were positive by selective culture and 49 (61.2%) by membrane filtration. All isolates identified by biochemical tests were analyzed by 16S rRNA and by *mapA* and *ceuE* gene PCRs, and 74 (92.5%) of 80 *Campylobacter* spp. (44 *C. jejuni* and 30 *C. coli,* respectively) were confirmed. The six strains not confirmed by PCR (7.5%), five from beef and one from chicken samples, were all isolated by selective cultures and were biochemically identified as *C. coli*. Phenotypic species identification was confirmed by PCR for 72 (97.3%) of 74 cultures, because two *C. coli* isolates, one from beef and one chicken, were identified by PCR as *C. jejuni*. Therefore, the identification rate discrepancy between molecular and biochemical tests for *C. coli* isolates was 2 (6.7%) of 30. The six strains not confirmed by PCR testing as *Campylobacter* spp. were not identified as *Arcobacter* spp. by PCR either. Hence, the following results for *Campylobacter* spp. are referred only to PCR-confirmed data ($n = 74$).

Of the 74 isolates from meat samples, 46 (62.2%) were identified as *C. jejuni* and 28 (37.8%) as *C. coli*. Chicken meat was more frequently contaminated by *Campylobacter* spp. (51.0%) than were other meats; prevalence of *C. jejuni* and *C. coli* isolates was 56.6 and 43.4%, respectively. Only 10.6% of beef samples (86.7% *C. jejuni*, 13.3% *C. coli*) and 5.7% of pork samples (50.0% *C. jejuni*, 50% *C. coli*) were contaminated. Biotype 1 of both *C. jejuni* (80.4%) and *C. coli* (96.4%) isolates was the most frequently detected biochemical profile, irrespective of the source of isolation; biotype 2 was found in 17.4 and 3.6% of *C. jejuni* and *C. coli* isolates, respectively; only one *C. jejuni* isolate, from chicken meat, was of biotype 3.

Specificity, sensitivity, and positive and negative predictive values of microbiological isolation methods. The effectiveness of microbiological isolation tests and their ability to correctly identify the presence of *Campylobacter* spp. was evaluated for both methods. The microbiological techniques, used together, were assumed able to detect all the positive samples, and results confirmed by PCR were used as the ''gold standard.'' Selective culture was more sensitive than membrane filtration (85 versus 66%), while the specificity of the methods was 98 and 100%, respectively. Hence, positive and negative predictive values, which assess the ability of the method to provide exact results without any confirmation step, were respectively, 91 and 96% for selective culture and 100 and 92% for membrane filtration.

Antimicrobial susceptibility and plasmid carriage. Antimicrobial R-types of isolates are presented in Table 2; resistance profiles (R-types) and plasmid carriage of *C. jejuni* and *C. coli* isolates are reported in Tables 3 and 4, respectively.

Campylobacter jejuni isolates generated 41 different antimicrobial R-types; *C. coli* generated 26. The most prevalent antimicrobial R-type was Kf (three isolates). Two *C. jejuni* and one *C. coli* isolates from chicken meat were sensitive to all antimicrobial drugs tested, and 64 (86.5%) of

TABLE 2. *Antimicrobial resistance of thermophilic* Campylobacter *spp. isolated from meat samples* (n = 74)

Antimicrobial drugs	All meat samples, no. (%)			Beef, no. (%)		Pork, no. (%)		Chicken, no. (%)	
	Campylobacter spp.	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*
Nalidixic acid	53 (71.6)	32 (69.6)	21 (75.0)	6 (46.2)	0	2 (66.7)	2 (66.7)	24 (80.0)	19 (82.6)
Ampicillin	27 (36.5)	16 (34.8)	11 (39.3)	1 (7.7)	1 (50.0)	2 (66.7)	1 (33.3)	13 (43.3)	9 (39.1)
Cephalothin	58 (78.4)	35 (76.1)	23 (82.1)	9 (69.2)	2 (100)	3 (100)	2 (66.7)	23 (76.7)	19 (82.6)
Cefotaxime	20 (27.0)	10 (21.7)	10 (35.7)	1 (7.7)	0	1 (33.3)	1 (33.3)	8 (26.7)	9 (39.1)
Ciprofloxacin	38 (51.3)	23 (50.0)	15 (53.6)	5 (38.5)	0	2 (66.7)	1 (33.3)	16 (53.3)	14 (60.9)
Chloramphenicol	11 (14.9)	7 (15.2)	4 (14.3)	1 (7.7)	0	0	1 (33.3)	6 (20.0)	3 (13.0)
Enrofloxacin	39 (52.7)	24 (52.2)	15 (53.6)	6 (46.2)	0	2 (66.7)	1 (33.3)	16 (53.3)	14 (60.9)
Erythromycin	21 (28.4)	10 (21.7)	11 (39.3)	0	0	1 (33.3)	1 (33.3)	9 (30.0)	10 (43.5)
Gentamicin	5 (6.8)	3 (6.5)	2 (7.1)	1 (7.7)	0	0	1 (33.3)	2 (6.7)	1 (4.3)
Sulphonamides	28 (37.8)	17 (36.9)	11 (39.3)	2 (15.4)	1 (50.0)	1 (33.3)	2 (66.7)	14 (46.7)	8 (34.8)
Sxt[a]	50 (67.6)	34 (73.9)	16 (57.1)	8 (61.5)	2 (100)	3 (100)	1 (33.3)	23 (76.7)	13 (56.5)
Tetracycline	30 (40.5)	14 (30.4)	16 (57.1)	2 (15.4)	0	1 (33.3)	2 (66.7)	11 (36.7)	14 (60.9)

[a] Sxt, trimethoprim-sulfamethoxazole.

74 strains were multidrug resistant (Tables 3 and 4). Resistance of *Campylobacter* spp. to ciprofloxacin (51.3%) and enrofloxacin (52.7%) was similar but lower than to nalidixic acid (71.6%). *C. coli* strains isolated from chicken meat showed the highest cross-resistance for both quinolones (82.6%) and fluoroquinolones (60.9%). Resistance to tetracycline was particularly high for *C. coli* isolated from chicken (60.9%) and pork (66.7%); erythromycin showed a similar trend, although the resistance rates were slightly lower (43.5% from chicken). Coresistance to ciprofloxacin and erythromycin, and to ciprofloxacin, erythromycin, and tetracycline, was found in 18 (24.3%) and 14 (18.9%) *Campylobacter* isolates, respectively (data not shown). Resistance to trimethoprim plus sulfamethoxazole and to cephalothin was high, particularly for *C. jejuni* (73.9%) and *C. coli* (82.1%) isolates, respectively. On the other hand, very low resistance to gentamicin (6.8%) was observed. Plasmids were isolated from six *C. coli* (five from chicken meat and one from beef) and two *C. jejuni* (from beef and chicken meat) isolates (Tables 3 and 4). The molecular mass of plasmid DNA ranged between 1.3 and 50.1 MDa; all strains carried one plasmid, with the exception of one *C. coli* isolated from chicken meat, which carried two plasmids. However, no association was detected between antimicrobial resistance and the presence of plasmid DNA.

DISCUSSION

***Campylobacter* spp. prevalence.** *Campylobacter* spp. prevalence was higher in chicken meat (51%), while beef showed a lower contamination level (10.6%). A previous study carried out in northeastern Italy *(38)* found a much higher contamination for chicken meat (81.3%) and a lower level for beef (1.3%). Recently, Parisi et al. *(36)* found 73% of chicken meat samples to be contaminated by thermophilic *Campylobacter*. Pork meat contamination was the lowest, in agreement with the data of Stern et al. *(41)* and Zanetti et al. *(53).* This study confirms the potential epidemiological role of chicken meat as a principal risk factor for *Campylobacter* spp. transmission and human infection, and the need for more effective management of poultry farming and consumer information, with a ''farm to fork'' approach. The unusual prevalence of *C. jejuni* in pork meat and *C. coli* in chicken meat and the general high contamination level of all meat samples suggest the probability of cross contamination during handling and packaging at markets and butcher shops. Moreover, Denis et al. *(13)* showed a selective effect of Preston broth that favored the growth of *C. coli* after 24 h of incubation, although in our study the enriched broths were subcultured after 48 h. However, the risk for consumer health is essentially related to meat contamination and not to the production phase in which contamination happens. Hence, correct risk management practices during the food handling and packaging process could help reduce foodborne infections in humans.

Campylobacter species other than *C. jejuni* and *C. coli* were not isolated. For selective microbiological analysis, a

TABLE 3. *Plasmid carriage and resistance patterns (R-types) detected in* Campylobacter jejuni *strains isolated from meat samples*[a]

Beef		Pork	Chicken	
R-types	Plasmids (MDa)	R-types	R-types	Plasmids (MDa)
NaKfCipEnrS3SxtTe		NaAmpKfCtxCipEnrESxtTe	NaAmpKfCtxCipEnrS3SxtTe	1.3
NaKfCipCEnrSxt	42	NaAmpKfCipEnrSxt	NaKfCtxCipCEnrES3SxtTe	
NaCipEnrS3Sxt		KfS3Sxt	NaAmpKfCtxCipEnrESxt	
NaKfEnrSxtTe			NaAmpKfCipEnrS3SxtTe	
AmpKfCtxSxt			NaKfCtxCipEnrCnSxtTe	
NaKfCipEnr			NaKfCipCEnrES3SxtTe	
KfCipEnr			NaAmpCtxEnrS3Sxt	
CnSxt			NaCtxCipEnrES3Sxt	
NaSxt			NaAmpKfCipEnrSxt	
Kf[b]			NaAmpKfCtxCSxtTe	
Sxt			NaAmpKfCipES3Sxt	
			NaKfCipEnrESxtTe	
			NaAmpKfES3Sxt	
			NaKfCipES3SxtTe	
			NaKfCipEnrSxtTe[c]	
			NaKfCipEnrS3Sxt	
			NaCtxCipCEnrE	
			NaKfCipEnrSxt	
			NaCEnrCnTe	
			NaKfCS3Sxt	
			NaAmpKfSxt	
			AmpKfS3Sxt[c]	
			NaAmpKf	
			KfS3Sxt	
			AmpKf	
			Na	
			—[d]	

[a] Na, nalidixic acid; Kf, cephalothin; Cip, ciprofloxacin; Enr, enrofloxacin; S3, sulphonamides; Sxt, trimethoprim-sulfamethoxazole; Te, tetracycline; Amp, ampicillin; Ctx, cefotaxime; E, erythromycin; C, chloramphenicol; Cn, gentamicin.
[b] Three strains showed this R-type.
[c] Each R-type was shown by two strains.
[d] Two strains were susceptible to all antimicrobial drugs.

normal concentration (32 mg/liter) of cefoperazone was used. It is commonly believed that modified selective medium containing cefoperazone at lower concentrations and/or longer plate incubation may better support the growth of campylobacters other than *C. jejuni* and *C. coli*. On the other hand, membrane filtration, which does not depend on selective antibiotics, could isolate other species (these, however, were not identified in this study). In practice, not all campylobacters can pass through the filter pores *(17, 27).*

Evaluation of selective culture and membrane filtration for food analysis. Selective culture is still considered as the optimal method for the isolation of enteropathogenic campylobacters, particularly from stool samples *(27).* Direct diagnostic use of PCR is more sensitive than microbiological methods, particularly for low prevalence of *Campylobacter* species *(27),* but because it is more expensive and labor-intensive than culture, it is not currently used in most microbiological laboratories. We await the development of easily applicable and reliable methods for molecular resistance testing; in the meantime, PCR cannot be used to assess many of the antimicrobial drugs resistance, because it does not isolate strains. Since, at present, it seems that the advantages of PCR do not compensate for its cost, we evaluated two microbiological approaches to food analysis in this study. Our results showed significant difference ($P < 0.05$) between selective culture and membrane filtration for genus and *C. coli* isolation. Membrane filtration was also less sensitive, had a lower predictive negative value and, therefore, is not appropriate as a reference method of analysis. Although *Campylobacter* blood-free selective agar gave a higher isolation rate than membrane filtration, all six PCR-unconfirmed cultures were from the selective agar plates. The physical selection performed by membranes provided more specific results, although the sensitivity was too low to identify all positive samples. Selective cultures alone were unable to detect all contaminated samples, and 13.7% of positive specimens were detected only by membrane filtration. Indeed, the strength of membrane filtration is its ability to isolate many different *Campylobacter* spp. and campylobacter-like bacteria not detected by selective isolation media or PCR assays designed for specific bacterial species and to overcome some problems related to campylobacter inhibition by antimicrobial drugs used for selective isolation. Hence, it

TABLE 4. *Plasmid carriage and resistance patterns (R-types) detected in* Campylobacter coli *strains isolated from meat samples*[a]

Beef		Pork	Chicken	
R-types	Plasmids (MDa)	R-types	R-types	Plasmids (MDa)
AmpKfSxt	2.5	NaAmpKfCipEnrS3Te	NaAmpKfCtxCipEnrES3SxtTe	
KfS3Sxt		NaCtxCECn	NaAmpKfCtxCipCEnrES3Sxt	
		KfS3SxtTe	NaAmpKfCtxCipEnrS3SxtTe	50.1
			NaAmpKfCipEnrES3SxtTe	
			NaAmpKfCtxCipEnrSxtTe	44.7
			NaKfCipCEnrECnSxtTe	1.3
			NaKfCtxCipEnrESxtTe	
			NaAmpKfCipEnrSxtTe	
			NaKfCipEnrES3SxtTe	
			NaKfCtxCipCEnrETe	
			NaAmpKfCipEnrETe	
			NaKfCipEnrS3SxtTe	
			NaKfCtxCipEnrETe	
			KfCtxS3SxtTe	
			AmpKfS3Sxt	
			AmpKfSxtTe	
			NaKfCipEnr	
			NaCtxE	
			NaKf[b]	1.6
			Na[b]	31.6–23.9
			—[c]	

[a] Amp, ampicillin; Kf, cephalothin; Sxt, trimethoprim-sulfamethoxazole; Na, nalidixic acid; Cip, ciprofloxacin; Enr, enrofloxacin; S3, sulphonamides; Te, tetracycline; Ctx, cefotaxime; C, chloramphenicol; E, erythromycin; Cn, gentamicin.
[b] Each R-type was shown by two strains.
[c] One strain was susceptible to all antimicrobial drugs.

is suitable to detect false-negative samples, misidentified by traditional microbiological methods. A cost-effective combination of both methods could offer the best results and should be considered when highly sensitive molecular techniques cannot be routinely used in diagnostic laboratories. Thus, at present, when using only one microbiological method, selective culture remains the best technique for detecting campylobacters from potentially contaminated samples; in the future, increased automation of molecular methods may make them the best choice for prevalence estimation.

The low biochemical activity and frequent variability in results make the identification of *Campylobacter* spp. difficult. In fact, even kits, such as the API Campy system, cannot clearly differentiate among some species *(34).* The biochemically based misidentification of several strains in this study was also seen in Diergaardt et al. *(14),* who confirmed only 3 of 22 *Campylobacter* spp. isolates when using 16S rRNA gene sequencing. Molecular methods have marked important steps forward in bacterial diagnostics; PCR and rRNA gene sequencing are used very frequently for species identification. Problems may be encountered during biochemical differentiation of *C. jejuni* and *C. coli* isolates. In fact, the main phenotype discrimination test is the hippurate hydrolysis, which is positive only for *C. jejuni*. A false-negative result will lead to misidentification; therefore, for epidemiological purposes, a reliable identification of *Campylobacter* spp. should be supplemented with a molecular method. With respect to biochemical typing, a high prevalence of biotype 1, common in human infection *(15, 51),* was found in all isolates, reinforcing the role of the animal products, particularly poultry meat, in human disease transmission.

Antimicrobial resistance and plasmid profiling. In the absence of standardized protocols and interpretive criteria for disk diffusion test, the agar dilution technique has recently been considered a standard antimicrobial susceptibility testing method for thermophilic campylobacters *(32).* Although this method is reliable and highly reproducible and provides quantitative MICs, it is a labor-intensive, time-consuming, and costly test *(8).* Hence, disk diffusion can be still considered a consistent antimicrobial screening test for thermophilic *Campylobacter*, particularly for aminoglycosides and quinolones and fluoroquinolones, as well as for erythromycin and tetracycline *(31).* Moreover, the disk diffusion test is still the method of choice in most clinical laboratories for microorganisms with fastidious growth requirements; nevertheless, it is very useful, especially when several antimicrobial agents need to be tested against a few isolates and qualitative data are required. If quantitative data are necessary, other methods such as the agar dilution or the E-test should be used, although the concordance between these two techniques has not yet been fully elucidated *(50).*

Campylobacter is increasingly resistant to antibiotics, especially fluoroquinolones and macrolides, which are the drugs of choice for infection treatment when therapy is

necessary *(30)*. Erythromycin is still considered the optimal drug for treatment *(4, 12),* although antimicrobial resistance has emerged during therapy with macrolides *(21)*. Interestingly, our results showed higher percentages of resistant strains isolated from meat samples compared to those reported by Pezzotti et al. *(38)* and Dionisi et al. *(15)*. In addition, the discrepancy found between the resistance rate to erythromycin of *C. coli* and *C. jejuni* isolates from poultry was in agreement with Parisi et al. *(36),* although it was not statistically significant, thus providing further evidence that the two species show different tendencies to become resistant to macrolides *(2, 20)*. The widespread use of these antimicrobial drugs, including erythromycin, in veterinary medicine has accelerated this resistance trend. In fact, this increasing level of resistance in *Campylobacter* spp. is becoming a major public health concern, particularly in the United States and Europe *(21)*. An alternative therapeutic regimen for adults is ciprofloxacin, but resistance to this class of agents as well as to tetracyclines has been increasing *(18)*. Resistance to fluoroquinolone has been frequently found in our study, which also confirmed that resistance to enrofloxacin, used to treat flocks of poultry and other animals, is indicative of resistance to ciprofloxacin *(37, 42)*. Among *Campylobacter* spp. isolates from human cases, resistance to ciprofloxacin, nalidixic acid, and tetracycline was common in the European Union (45.0, 37.6, and 29.1%, respectively) *(18)*. This antimicrobial susceptibility pattern resembled the occurrence of resistance in our isolates from meat samples, which, however, showed higher percentages. Indeed, a high level of ciprofloxacin resistance may limit the treatment options for human infections, although only resistance to tetracycline was statistically significant ($P < 0.05$), particularly for *C. coli* isolates from chicken meat.

Plasmid carriage is not essential for *Campylobacter* spp. virulence *(43)*; however, Tracz et al. *(48)* reported an association between pVir plasmid carriage and patients who developed bloody diarrhea, although these findings were not confirmed elsewhere *(29)*. Isolates containing pVir were also associated with the presence of a tetracycline-resistance plasmid *(48)*. In our study, plasmids with a molecular weight corresponding to pVir (≈37.5 kb) were isolated from two *C. coli*. However, they did not show tetracycline resistance, although they harbored plasmids of a size correlated to the resistance, as indicated by Tenover et al. *(47)*. In a previous study, Zheng et al. *(54)* found that pVir was absent in almost all the *Campylobacter* strains isolated from meat products, suggesting that *C. jejuni* and *C. coli* present in retail meat can show diverse ability to adhere to and invade human intestinal epithelial cells.

Although most of the antimicrobial resistance in *Campylobacter* spp. is of chromosomal origin, it is well known that tetracycline resistance can be plasmid mediated *(45, 46)*. Tenover et al. *(47)* found that plasmids capable of transferring tetracycline resistance via conjugation ranged in size from 27.6 to 65.8 MDa. Our study showed that five strains (one *C. jejuni* and four *C. coli*) carried plasmids of potentially correct sizes, although three of five isolates did not show tetracycline resistance, suggesting a different biological role of harbored plasmids. In general, plasmid carriage was not directly correlated with any antimicrobial resistance pattern found in this study. Similar results were obtained by Aquino et al. *(5)*. Indeed, further study is needed to determine the real pathogenetic ability of *Campylobacter* spp. isolates, which despite the presence of putative virulence genes and plasmid DNA, could not cause diseases in humans *(39)*.

ACKNOWLEDGMENT

We acknowledge the helpful comments from Dr. Jim McLauchlin, Health Protection Agency, Food, Water & Environment Microbiology Network, London.

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