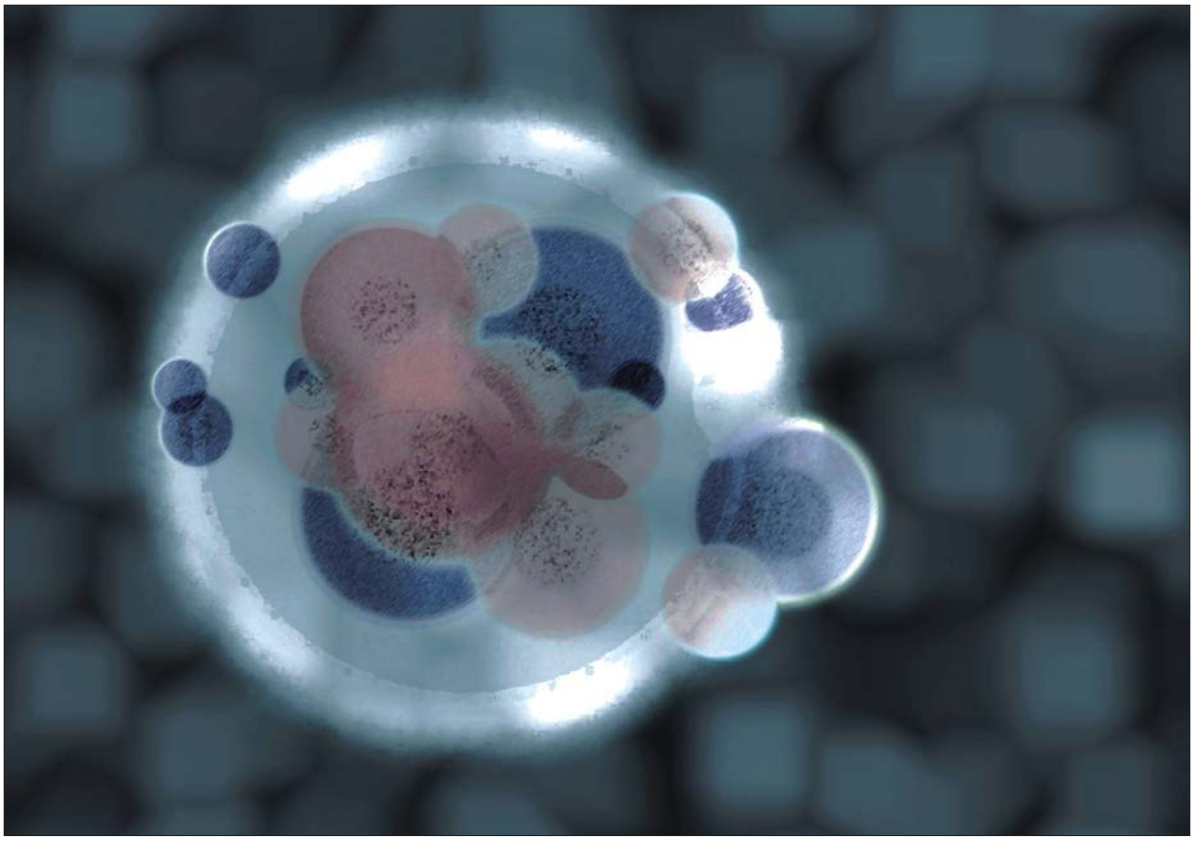

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Antimicrobial Susceptibility of *Campylobacter jejuni* and *Campylobacter coli* Strains Isolated from Humans and Poultry in North of Spain

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Abstract: This study was undertaken to evaluate the antimicrobial susceptibility of *Campylobacter* spp. strains isolated from poultry and human faeces in the Basque Country area, in the North of Spain. Poultry samples at the retail level were randomly purchased in supermarkets and human isolates were obtained from diarrhoea-infected patients. *Campylobacter* identification was achieved by biochemical tests and species-specific PCR. Susceptibility to seven antibiotics was determined by the agar dilution method following the NCCLS recommendations. Genotyping was carried out by PCR-RFLP of the *flaA* gene and PFGE profiling. Highest resistance was found for tetracycline (74%) and quinolones: nalidixic acid (76%), ciprofloxacin (72%) and enrofloxacin (61.5%). Decreasing trends in erythromycin resistance was detected in both food and human isolates. Multiresistance to three or more of the drugs was found in 4.8% strains, being more frequent in *C. coli* (12.5%) than in *C. jejuni* (2.5%).

Key words: *Campylobacter*, resistance, antimicrobial susceptibility, poultry

INTRODUCTION

During the last years, the importance of *Campylobacter jejuni* and *Campylobacter coli* as foodborne pathogens has increased throughout the world. In fact, *Campylobacter* spp. are now recognized as the leading cause of bacterial zoonosis in many countries[1]. These organisms can colonize the intestinal tract of animals, including poultry, cattle and swine, without causing illness, but they are responsible of gastrointestinal and extra-intestinal illness in humans[2]. Most *Campylobacter* infections are sporadic but outbreaks may occur[3]. Inadequately cooked meat, particularly poultry, unpasteurised milk and contaminated drinking water are the most common sources for epidemic and sporadic foodborne cases[1,4].

The use of antibiotics in modern intensive animal production for therapy and prevention of diseases is thought to be a contributing factor for the emergence of resistance in zoonotic bacteria as campylobacters[5]. Recently, concern for foodstuffs contaminated with this foodborne pathogen has gained significant attention due to the frequent isolation of antimicrobial resistant strains in humans and animals. These resistant strains can pose a significant health risk for humans if they become part of the food chain[6,7]. This matter applies particularly for strains resistant to fluoroquinolones and erythromycin, widely used for therapy in human systemic infections, inmunocompromised state or in severe or long-lasting cases of enteritis[8]. Furthermore, fluoroquinolones are the prophylactic treatment against traveller's diarrhoea and tetracycline, doxicyclin and chloranphenicol are sometimes listed as alternative drugs for treatment.

The aim of this study was to evaluate the prevalence of antimicrobial resistant strains of *Campylobacter* spp. in raw poultry products at the retail level and from human samples in the Basque Country area, in the North of Spain.

MATERIALS AND METHODS

Bacterial strains: A total of 104 *Campylobacter* spp. strains from human infections and poultry samples were investigated. In addition, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 and *C. jejuni* NCTC 11322 were used as control strains in susceptibility tests.

***Campylobacter* isolation:** All *Campylobacter* cultures were incubated under microaerobic atmosphere containing 5, 10 and 85% of O_2, CO_2 and N_2, respectively

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(CampyPak Plus, BBL). Food samples at the retail level were randomly purchased from different supermarkets at the Basque Country area, in the North of Spain. The sample types were raw poultry products including portioned poultry with and without skin, carcass, liver and gizzards. The *Campylobacter* isolation was based on ISO 10272/1995[9]. Selective Preston broth amended with 5% laked horse blood (SR48; Oxoid), FBP supplement (SR84; Oxoid) and selective supplement (SR117; Oxoid) as enrichment broth and CCDA (Oxoid) with selective supplement (SR 155; Oxoid) as plating medium were used. Enrichment was carried out in Preston broth incubated at 42°C for 24 h. A loop of the enriched culture was streaked in duplicate onto CCDA plates and incubated at 42°C for 48 h.

Human isolates were obtained from faecal samples of diarrhoea-infected patients in the Basque Country area. The samples were collected over a six-month period, January-June 2003. The *Campylobacter* isolation was carried out on Campy BAP selective (Becton Dickinson) plates incubated for 48 h at 42°C under microaerobic atmosphere.

Identification of isolates: Presumptive identification of *C. jejuni* and *C. coli* from both human and food samples was carried out from typical colonies on selective agar plates by testing cell morphology under epifluorescent microscopy and acridine orange stain. *Campylobacter* confirmation was achieved by using biochemical tests: oxidase production, hippurate hydrolysis and sensitivity to 30 μg discs of cephalothin. The specie-specific detection was based on the PCR amplification of the *mapA* gene for *C. jejuni*[10] and *ceuE* gene for *C. coli*[11], as previously described[12].

Antibiotic resistance testing: *Campylobacter* isolates were examined for susceptibility to seven antibiotics enrofloxacin, ciprofloxacin, nalidixic acid, tetracycline, erythromycine, gentamicin and chloramphenicol. Antibiotic susceptibility was determined by the agar dilution method on Mueller-Hinton agar (Oxoid) supplemented with 5% of defibrinated horse blood (Oxoid) following the NCCLS recommendations[13]. Twofold serial dilutions of each antibiotic at the concentration 0.006-32 μg mL^{-1} were used. Inocula were prepared from 48 h cultures in Nutrient Broth (Oxoid) at 42°C and adjusted to 0.5 McFarland determined by a CrystalSpec (Becton Dickinson). The final inoculum of 10^5 cfu per spot was applied with a multipoint inoculator. The agar plates were incubated at 42°C for 24 h in a microaerobic atmosphere.

The following NCCLS MIC breakpoints for members of the *Enterobacteriaceae* were used as tentative breakpoints for the resistance of *Campylobacter*: gentamicin and tetracyclin MIC ≥16 μg mL^{-1}; erythromycin MIC ≥8 μg mL^{-1}; chloramphenicol and nalidixic acid MIC ≥32 μg mL^{-1} and ciprofloxacin and enrofloxacin MIC ≥4 μg mL^{-1}.

Genotyping: Most of the strains were submitted to PCR RFLP and PFGE analysis. For PCR-RFLP, a 1.7 kb fragment of the *fla*A gene was amplified and analysed after digestion with the restriction enzyme *Dde*I by the procedure described by Nachamkin *et al.*[14]. For PFGE profiling, DNA-agarose samples were prepared from formaldehyde-treated bacterial cells by the protocol of On *et al.*[15]. DNA was digested with *Sma*I and fragments were separated by use of the CHEF-DRIII PFGE system (Bio-Rad Laboratories). In both methods, standardized parameters were as proposed by CAMPYNET (http://www.campynet.vetinst.dk).Profiling was completed by computer-assisted analysis using the software program Gel Compar PC (version 4.0).

RESULTS AND DISCUSSION

In total, 104 strains from human infections (n = 50) and poultry samples (n = 54) were isolated, identified and investigated for antibiotic susceptibility. Identification of the *Campylobacter* isolates confirmed the distribution of species reported in literature. *C. jejuni* was the most prevalent species in either human or poultry samples. Forty-six (92%) of the 50 human isolates and 34 (63%) of the 54 poultry isolates were identified as *C. jejuni* according to the biochemical test and PCR. The remaining *Campylobacter* isolates belonged to the species *C. coli* (4 strains from human samples and 20 strains from poultry samples). These results also confirmed *C. jejuni* as largely predominant human pathogen, while *C. coli* appeared to be less common in causing human disease[16-18].

Overall results of susceptibility testing for *C. jejuni* and *C. coli* strains isolated from humans and poultry are shown in Table 1. Owing to the lack of international standards and breakpoints for *Campylobacter* susceptibility testing, the susceptibility was analysed based on the guidelines provided by the NCCLS for members of the *Enterobacteriaceae*[19]. A high level of resistance was found among the *Campylobacter* isolates regardless of their origin. Highest resistance was found for tetracycline and quinolones with 76% of resistance to nalidixic acid, 72% to ciprofloxacin and 61.5% to enrofloxacin (Table 1). Tetracycline resistance was 74%

Table 1: Percentages of antimicrobial resistance among *Campylobacter* strains isolated from poultry and human samples

			C. jejuni		*C. coli*	
Antimicrobials	Poultry (n = 54)	Human (n = 50)	Poultry (n = 34)	Human (n = 46)	Poultry (n = 20)	Human (n = 4)
Nalidixic acid	74.1	78.0	70.5	80.4	80.0	50.0
Ciprofloxacin	66.7	78.0	61.8	80.4	75.0	50.0
Enrofloxacin	72.2	50.0	70.5	52.2	75.0	25.0
Erythromycin	9.3	8.0	2.9	6.5	20.0	25.0
Tetracycline	83.3	70.0	85.3	71.7	80.0	50.0
Chloramphenicol	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	1.9	0.0	2.9	0.0	0.0	0.0

Table 2: Resistance profiles of *Campylobacter* strains used in this study

Poultry strains (n)	Human strains (n)
QUI (2 *C. jejuni* and 13 *C. coli*)	QUI (24 *C. jejuni* and 1 *C. coli*)
QUI+ERY (1 *C. jejuni* and 3 *C. coli*)	QUI+ERY (1 *C. jejuni*)
QUI+TET (17 *C. jejuni* and 13 *C. coli*)	QUI+TET (18 *C. jejuni*)
ERY+TET (1 *C. jejuni* and 4 *C. coli*)	ERY+TET (3 *C. jejuni*)
QUI+ERY+TET (1 *C. jejuni* and 3 *C. coli*)	ERY+TET+QUI (1 *C. jejuni*)

QUI: Nalidixic acid, Ciprofloxacin and Enrofloxacin; TET: Tetracycline; ERY: Erythromycin

with a similar distribution among species, 77.5% of the *C. jejuni* and 75% of the *C. coli*. Resistance to erythromycin was low, 8.7%, varying from 9.3% in poultry to 8.0% in human isolates. Further, species-specific resistance was found for erythromycin. On average, 20.8% of the *C. coli* isolates were resistant to erythromycin whereas in *C. jejuni* it was 5%. Similar findings were described in Spain and other countries[19-21]. All isolates but one was susceptible to gentamicin. No resistance was found to chloramphenicol.

In previous studies[21,22] as in this study alarmingly high resistance rates were observed for ciprofloxacin. However, unlike other researchers, in the present study ciprofloxacin resistance was similar in *Campylobacter* strains isolated from humans and food samples and species-specific resistance was not detected. This is of maximum significance because fluoroquinolones are normally regarded as empirical treatment of both community-acquired acute diarrhoea and traveller's diarrhoea[23]. The percentages of erythromycin resistance observed were substantially lower than those detected previously[21,22], but similar to other recent studies[23] in both food and human isolates. Although erythromycin resistance was low, it is worrying because erythromycin is another commonly used agent for treating *Campylobacter* enteritis[8].

Resistance to one or more drugs was detected in 95% of the strains (Table 2). However, only 4.8% of the 104 strains were multiresistant (resistance to three or more of the drug groups tested). Multiresistance was found on 2.5% of the *C. jejuni* strains but it was more common in *C. coli* (12.5%). Multiresistant isolates always remained susceptible to gentamicin and chloramphenicol.

Pulsed-field gel electrophoresis (PFGE) and PCR-RFLP of the *flaA* gene were used for genotyping *Campylobacter* isolates. Among the *C. jejuni* and *C. coli* isolates, multiple distinct patterns were found by both PFGE and PCR-RFLP profiling. The PCR-RFLP fingerprints of all strains appeared to be highly heterogeneous and no characteristic pattern of strains infecting either poultry or humans was identified. Also, no separate grouping of antibiotic-resistant strains was obtained. The genotyping and antimicrobial susceptibility profiles did not correlate well as *Campylobacter* isolates with identical antimicrobial susceptibility profiles displayed different PFGE and PCR-RFLP patterns.

The emergence and spread of the resistance to quinolones and macrolides among *Campylobacter* strains remain uncertain. However, large numbers of published works have been related the increasing resistance to quinolones and macrolides among *Campylobacter* isolates from humans with the use of these antibiotics in veterinary practices[5,21,22,24,25]. Macrolide-lincosamide group, specially tylosin and spiramycin, have been used for up to 1999 either for therapeutic or for growth promotion in animal production. On turn, enrofloxacin, a derivative of ciprofloxacin, is used as therapeutic drugs in veterinary medicine. As consequence of these trends, several studies have documented an increase in the occurrence of erythromycin and ciprofloxacin resistance among *Campylobacter* strains from food animals[8,23]. Recently, the use of macrolides for growth promotion has been banned in all European Union countries and fluoroquinolones are subjected to a more limited usage in veterinary medicine. Although there is no evidence for changes in the occurrence of fluoroquinolone resistance following these directives, results from this study point out to the occurrence of decreasing resistance to erythromycin.

Results indicated that most *Campylobacter* isolates from retail poultry and from acute diarrhoeal infections were resistant to at least one of the antimicrobials tested. The co-resistance to erythromycin and ciprofloxacin was not alarmingly high. However, it is of interest to regain susceptibility rates existing before the exposure of the *Campylobacter* animal strains to the antibiotics, on the basis of a more restricted or suspended usage of fluoroquinolones and macrolides in food animal

production. In order to protect human health it is recommended that such interventions be implemented on a worldwide scale.

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REFERENCES

1. Friedman, C.R., J. Neimann, H.C. Wegener and R.V. Tauxe, 2000. Epidemiology of Campylobacter Jejuni Infections in the United States and Other Industrialized Nations. In: *Campylobacter* 2nd (Eds. Nachamkin, I. and M.J. Blaser), Am. Soc. Microbiol., Washington, D.C., pp: 121-138.
2. Frost, J.A., 2001. Current epidemiological issues in human campylobacteriosis. J. Applied Microbiol. Symp. Suppl., 30: 85S-95S.
3. Llovo, J., E. Mateo, A. Muñoz, M. Urquijo, S.L.W. On and A. Fernandez-Astorga, 2003. Molecular typing of *Campylobacter jejuni* isolates involved in a neonatal outbreak indicates nosocomial transmission. J. Clin. Microbiol., 41: 3926-3928.
4. Hänninen, M.L., H. Haajanen, T. Punimi, K. Wermundsen, M.L. Katila, H. Sarkkinen, I. Miettinen and H. Rautelin, 2003. Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organisms in three waterborne out breaks in Finland. Applied Environ. Microbiol., 69: 1391-139.
5. Aarestrup, F.M. and J. Engberg, 2001. Antimicrobial resistance of thermophilic *Campylobacter*. Vet. Res., 32: 311-321.
6. Khachatourians, G.G., 1998. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Can. Med. Assoc. J., 159: 1129-1136.
7. 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 and the investigation Team, 1999. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. N. Engl. J. Med., 340: 1525-1532.
8. Engberg, J., F.M. Aarestrup, D. Taylor, P. Gerner-Smidt and I. Nachamkin, 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *coli*: Resistance mechanisms and trends in human isolates. Emerg. Infect. Dis., 7: 24-34.
9. International Organization for Standardization, 1995. Microbiology of food and animal feeding stuffs-horizontal method for detection of thermo-tolerant *Campylobacter* International Organization for Standardization, Geneva, Switzerland.
10. Stucki, U., J. Frey, J. Nicolet and A.P. Burnens, 1995. Identification of *Campylobacter jejuni* on the basis of a species-specific gene that encodes a membrane protein. J. Clin. Microbiol., 33: 855-859.
11. Gonzalez, I., K.A. Grant, P.T. Richardson, S.F. Park and M.D. Collins, 1997. Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* using a PCR test based on the *ceu*E gene encoding a putative virulence determinant. J. Clin. Microbiol., 35: 759-763.
12. Mateo, E., J. Carcamo, M. Urquijo, I. Perales and A. Fernandez-Astorga, 2005. Evaluation of a PCR assays for the detection and identification of *Campylobacter jejuni* and *Campylobacter coli* in retail poultry products. Res. Microbiol., 56: 568-574.
13. National Committee for Clinical Laboratory Standards, 2005. Performance standards for antimicrobial susceptibility testing. 15th Informational Supplement. NCCLS Document M100-S15,225. National Committee for Clinical Laboratory Standards, Wayne, Pa., pp: 142-143.
14. Nachamkin, I., K. Bohachick and C.M. Patton, 1993. Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. J. Clin. Microbiol., 31: 1531-1536.
15. On, S.L.W., E.M. Nielsen, J. Engberg and M. Madsen, 1998. Validity of *Sma*I-defined genotypes of *Campylobacter jejuni* examined by *al*I, *Kpn*I and *Bam*HI polymorphisms: Evidence of identical clones infecting humans, poultry and cattle. Epidemiol. Infect., 120: 231-237.
16. Cloak, O.M., G. Duffy, J.J. Sheridan, I.S. Blair and D.A. McDowell, 2001. A survey on the incidence of *Campylobacter* sp. and the development of a surface adhesion polymerase chain reaction (SA-PCR) assay for the detection of *Campylobacter jejuni* in retail meat products. Food Microbiol., 18: 287-298.

17. Fricker, C.R. and R.W.A. Park, 1989. A two-year study of the distribution of thermophilic *Campylobacters* in human, environmental and food samples from the Reading area with a particular reference to toxin production and heat stable serotype. J. Applied Bacteriol., 66: 477-490.
18. Lastovica, A.J. and M.B. Skirrow, 2000. Clinical Significance of *Campylobacter* and Related Species Other than *Campylobacter jejuni* and *C. coli*. In: *Campylobacter* 2nd (Eds. Nachamkin, I. and M.J. Blaser), Am. Soc. Microbiol., Washington, D.C., pp: 89-120.
19. Ge, B., D.J. White, P.F. McDermott, W. Girard, S. Zhao, S. Hubert and J. Meng, 2003. Antimicrobial-resistance *Campylobacter* species from retail raw meats. Applied Environ. Microbiol., 69: 3005-3007.
20. Luber, P.J.W., H. Hahn and E. Bartelt, 2003. Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* strains isolated in 1991 and 2001-2002 from poultry and humans in Berlin, Germany. Antimicrob. Agents Chemother., 47: 3825-3830.
21. Saenz, 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.
22. Prats, G., B. Mirelis, T. Llovet, C. Muñoz, E. Miro and F. Navarro, 2000. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona. Antimicrob. Agents Chemother., 44: 1140-1145.
23. Van Looveren, M., G. Daube, L. De Zuter, J.M. Dumont, C. Lammens, M. Wijdooghe, P. Vandamme, M. Jouret, M. Cornelis and H. Goossens, 2001. Antimicrobial susceptibilities of Campylobacter strains isolated from food animals in Belgium. J. Antimicrob. Chemother., 48: 235-240.
24. Delsol, A.A., J. Sunderland, M.J. Woodward, L. Pumbwe, L.J. Piddock and J.M. Roe, 2004. Emergence of fluoroquinolone resistance in the native *Campylobacter coli* population of pigs exposed to enrofloxacin. J. Antimicrob. Chemother., 53: 872-874.
25. Endtz, H.P., G.J. Ruijs, B. van Klingeren, W.H. Jansen, T. vander 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.