Antimicrobial Susceptibility of *Campylobacter* Strains Isolated from Chicken Carcasses in Senegal

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Key words

Chicken – *Campylobacter* – Antibiotic resistance – Senegal.

Summary

Campylobacter resistance to antimicrobial agents appears as an emerging public health problem in industrialized countries, but, on the other hand, only few data on the subject are available in developing countries. To assess antibiotic susceptibility of *Campylobacter* strains in Senegal, skin samples were collected from 250 chicken carcasses from January 2001 to October 2002. Among 204 *Campylobacter* strains isolated, two species were identified: *C. jejuni* (59%) and *C. coli* (41%). In vitro susceptibility to five antimicrobial drugs (amoxicillin, amoxicillin-clavulanic acid, erythromycin, nalidixic acid and ciprofloxacin) was determined by the E-test method. Minimum inhibitory concentrations (MICs) showed 34% of *Campylobacter* isolates were ciprofloxacin resistant with a high level of resistance (MIC ≥ 32 mg/l) in 25% of both species. Cross-resistance between nalidixic acid and ciprofloxacin was found in 96% of quinolone-resistant strains. The level of amoxicillin resistance was statistically higher for *C. coli* than for *C. jejuni* (20.2 versus 10.8%), but all the strains were susceptible to amoxicillin combined with clavulanic acid. Both species showed low resistance to erythromycin. Multiresistant phenotype to three of the drugs tested was found in 9.8% of the strains: 15.5% of *C. coli* strains and 5.8% of *C. jejuni* strains. No strain was resistant to four or more of the drugs studied. Further studies appear necessary to evaluate antibiotic resistance of *Campylobacter* isolated in human and animal samples in order to control the emergence of new multidrug-resistant strains in Senegal.

■ INTRODUCTION

Thermophilic *Campylobacter* species, especially *C. jejuni* and *C. coli*, are among the main agents of gastrointestinal infections in developed countries (2, 12, 29). *Campylobacter* also poses an emerging public health problem in developing countries (13, 19, 20, 32). Most cases of *Campylobacter* gastrointestinal infections do not require antimicrobial treatment, being brief, clinically mild, and self-limiting. However, complications such as severe cases of enteritis or septicemia may occur and require treatment. Macrolides and fluoroquinolones (FQs) are the most useful antimicrobial drugs for *Campylobacter* infections, but resistance has been reported to be increasing (12, 19, 26, 29).

Contaminated food is the usual source of human infection and poultry products are considered to be the main cause of food-related infection in humans (4). The link between the use of antimicrobial agents as growth promoter or in veterinary medicine and the emergence of resistance among *Campylobacter* from different animal source has been previously reported (2). The spread of antimicrobial-resistant strains in the food chain has raised concerns that treatment of human infections will be compromised.

In Senegal, a modern poultry production sector is expanding around Dakar, its capital, in order to rapidly supply the fast-growing urban

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human population with animal protein. Poultry farmers use antibiotics to fight diseases. Because growing scientific evidence shows that the use of antimicrobial agents in veterinary medicine results in development of resistant pathogenic bacteria (1), the authors' objective was to assess the frequency of antimicrobial resistance of *Campylobacter* strains isolated from "modern" poultry meat.

■ MATERIALS AND METHODS

Specimen collection and isolation

From January 2001 to October 2002, 250 chicken carcasses were collected from slaughterhouses and retail shops in Dakar. The carcasses were immediately brought back to the laboratory in cool boxes (+4°C). Samples of neck skin (10 g) were taken from each carcass and added to 90 ml of Preston broth with Preston antibiotic supplement[1] (Oxoid laboratory, England) and incubated at 42°C during 24 h under microaerophilic conditions (Campygen, Oxoid laboratory, England). Each sample was then streaked onto Virion plates (Mueller Hinton agar, Merck, Germany; Bacto agar, Difco laboratory, USA; with 5% of defibrinated horse blood, AES laboratory, France) and onto Karmali plates (Oxoid laboratory, England). Plates were incubated at 42°C under microaerophilic conditions for 48 h.

Identification of isolates

Isolates were identified using a commercial identification method (API Campy®, bioMérieux, France). Identification of every isolate was confirmed by a multiplex PCR, using specific primers of *Campylobacter* genus (MD16S1, MD16S2), *C. jejuni* species (MDMapA1, MDMapA2) and *C. coli* species (COL3, MDCOL2) (10). Briefly, *Campylobacter* colonies from blood agar plate were suspended in 0.2 ml TE buffer. Cells were lysed by heating at 95°C for 10 min and cellular debris were removed by centrifugation at 5000 g for 10 min. The supernatant was used as source of template for DNA amplification. Each multiplex PCR tube contained 200 µM deoxynucleoside triphosphate, 2.5 µl of 10X reaction buffer, 20 mM MgCl2, 0.11 µM *Campylobacter* genus primers, 0.42 µM *C. jejuni* primers and 0.42 µM *C. coli* primers. Template DNA (3 µl) was added and the volume adjusted with sterile water to obtain 30 µl. DNA amplification was carried out in a Perkin-Elmer 9600® thermocycler using an initial denaturation step at 95°C for 10 min, followed by 35 cycles. Cycling conditions were as follows: denaturation, 95°C for 30 s; annealing, 59°C for 90 s; extension, 72°C for 1 min. After the last cycle, a final extension step at 72°C for 10 min was added. Ten microliters of PCR product were analyzed by gel electrophoresis (1.5% gel agarose). Gels were stained with ethidium bromide at 0.5 µl/ml and visualized by UV transillumination. A 100-bp DNA ladder (Amersham Biosciences, France) was used as size marker. Negative controls were added in each run. Positive PCR controls consisted of *C. jejuni* subsp. *jejuni* ATCC 49943 and *C. coli* ATCC 49941.

Susceptibility testing

Several colonies of each strain were suspended in 5 ml of Mueller-Hinton broth to achieve turbidity equal to 0.5 MacFarland standard. The suspensions were inoculated with sterile swabs onto Mueller-Hinton agar with 5% sheep blood. After application of E-test® strips (AB Biodisk, Sweden) plates were incubated at 37°C for 48 h under microaerophilic atmosphere. Minimum inhibitory concentrations (MICs) were read according to the recommendations of the manufacturer by two different readers. Breakpoints were those recommended by the Antimicrobial Committee of the French Society for Microbiology (33). Breakpoints for resistance susceptibility were higher than 16 mg/l for amoxicillin, than 16/2 mg/l for amoxicillin-clavulanic acid, than 4 mg/l for erythromycin, than 16 mg/l for nalidixic acid, and than 2 mg/l for ciprofloxacin.

Statistical analysis

Data were entered and analyzed with SPSS, version 10 (SPSS, Chicago, USA). The χ^2 test and Fisher's exact two-tailed test were used for statistical analysis of the significant difference of resistance rates. An α of 0.05 was used for statistical significance.

■ RESULTS

Campylobacter was isolated from 81.6% of the samples. *C. jejuni* was the most prevalent species (59% *C. jejuni* versus 41% *C. coli*).

Susceptibility testing

The results of antimicrobial susceptibility testing for *C. coli* and *C. jejuni* are shown in Table I. The resistance rate to amoxicillin in *C. coli* and *C. jejuni* was 20.2 and 10.8%, respectively. Among

[1] Polymyxine B (2500 UI); Rifampicine (5 mg), Trimethoprim (5 mg), Cycloheximide (50 mg)

Table I

Antimicrobial susceptibility of *Campylobacter coli* and *C. jejuni* strains isolated from chicken carcasses in Senegal

	***Campylobacter coli* (N = 84)**				***Campylobacter jejuni* (N = 120)**			
Antibiotic	**MIC_{50}***	**MIC_{90}**	**Range**	**Resistant (%)**	**MIC_{50}**	**MIC_{90}**	**Range**	**Resistant (%)**
Amoxicillin	8	256	0.5–256	20.2	4	32	0.125–256	10.8
Amoxicillin + clavulanic acid	2	8	0.5–8		1	2	0.094–8	
Erythromycin	0.5	1	0.064–>256	4.7	0.5	2	0.094–>256	3.3
Nalidixic acid	2	>256	0.5–>256	34.5	2	>256	0.75–>256	31.6
Ciprofloxacin	0.094	>32	0.032–>32	34.5	0.125	>32	0.045–>32	30.8

* Minimum inhibitory concentrations (mg/l)

C. coli, MICs of all resistant strains were higher than 256 mg/l. Among *C. jejuni*, MICs of 46% of amoxicillin-resistant strains were higher than 256 mg/l. In addition, amoxicillin MIC_{90} was statistically higher for *C. coli* than for *C. jejuni*. No strains resistant to amoxicillin-clavulanic acid were observed. For amoxicillin high level resistant strains (MIC > 256 mg/l), amoxicillin-clavulanic acid MIC was between 2/1 and 8/4 mg/l in both species. The overall resistance rate to erythromycin was 4.7% for *C. coli* and 3.3% for *C. jejuni* without any significant difference between the two species.

Resistance rates to nalidixic acid in *C. coli* and *C. jejuni* were 34.5 and 31.6%, respectively. Among these resistant strains, 55.2% of *C. coli* and 68.5% of *C. jejuni* exhibited MICs higher than 256 mg/l. For both species, MIC_{90} was higher than 256 mg/l. Compared to nalidixic acid, quite similar resistance rates to ciprofloxacin were observed with 34.5 and 30.8% for *C. coli* and *C. jejuni*, respectively. Ciprofloxacin MIC was higher than 32 mg/l for 25% isolates in both species. Among *Campylobacter*-resistant strains, 75.8% of *C. coli* and 78.3% of *C. jejuni* had ciprofloxacin MICs higher than 32 mg/l. Furthermore, 90% of the isolates were inhibited with MICs higher than 32 mg/l for *C. coli* and *C. jejuni*. For both species, MIC_{90} was higher than 32 mg/l.

Coresistant and multiresistant isolates

Drug resistance to one or more drugs was detected in over 38% of the strains (Table II). Among them, *C. coli* and *C. jejuni*, 41.7 and 36.7% of the strains, respectively, exhibited resistance to the tested antibiotics. Among *C. coli* and *C. jejuni*, 33.4 and 30%, respectively, were resistant to both quinolones (nalidixic acid and ciprofloxacin). A cross-resistance between nalidixic acid and ciprofloxacin was found for all isolates (96%) except for one strain of *C. jejuni* and one strain of *C. coli*, which showed a nalidixic acid-resistant ciprofloxacin-susceptible phenotype. Table III shows a comparison between nalidixic acid MICs and ciprofloxacin MICs for cross-resistant isolates: 46.5% of *C. coli* strains and 63.8% of *C. jejuni* strains exhibited a high level of resistance to both quinolones with MICs higher than 256 mg/l for nalidixic acid and 32 mg/l for ciprofloxacin. Multiresistance, defined as resistance to three or more of the drugs tested, was found in 9.8% of *Campylobacter* strains: 15.5% were *C. coli* strains and 5.8% were *C. jejuni* strains. No strain was resistant to four or five drugs. The only multidrug-resistant phenotype was amoxicillin, nalidixic acid and ciprofloxacin in both species.

Table II

Distribution of *Campylobacter* strains depending on their resistance to antibiotics

		Number of resistant strains (%)	
Phenotype	**Num.**	***C. coli* (n = 84)**	***C. jejuni* (n = 120)**
	0	49 (58.3)	76 (63.3)
AMX	1	2 (2.4)	3 (2.5)
CIP	1	1 (1.2)	0
ERY	1	1 (1.2)	0
AMX ERY	2	2 (2.4)	3 (2.5)
ERY NAL	2	1 (1.2)	1 (0.8)
NAL CIP	2	15 (17.9)	30 (25.0)
AMX NAL CIP	3	13 (15.5)	7 (5.8)

AMX = amoxicillin; CIP = ciprofloxacin; ERY = erythromycin; NAL = nalidixic acid

Table III

Comparison between nalidixic acid MICs and ciprofloxacin MICs for cross-resistant *Campylobacter coli* and *C. jejuni* isolates

		Number of isolates (%)	
Nalidixic acid MICs*	**Ciprofloxacin MICs**	***C. coli* (N = 28)**	***C. jejuni* (N = 36)**
>16–≤256	>2–≤32	7 (25)	6 (16.7)
	>32	8 (28.5)	6 (16.7)
>256	>2–≤32	0 (0)	1 (2.8)
	>32	13 (46.5)	23 (63.8)

* Minimum inhibitory concentrations (mg/l)

■ DISCUSSION

In Senegal, beside traditional poultry production, modern poultry production is expanding around Dakar. Senegalese poultry farmers try to minimize the impact of diseases on their production with huge amounts of antibiotics, like in Europe or the United the States. Considering the growing prevalence of resistant strains in industrialized countries, antimicrobial drugs must be used very carefully in Senegal.

In the present survey, 81.6% of chicken carcasses were contaminated with *Campylobacter*. In developed countries, several studies have also reported high levels of *Campylobacter* isolation from chicken carcasses and retailed chickens: 46% in Germany (4), 46% in Japan (23) and from 73% to 100% in the USA (40). Although little information is available from developing countries, the present results are consistent with those from Kenya and China where thermophilic *Campylobacter* has been isolated from 77 and 76% of chicken samples, respectively (24, 31). As reported by several studies, *Campylobacter* isolated from animals may contaminate humans either by direct ingestion of undercooked food or by cross-contamination of raw poultry to other foods caused by non hygienic handling such as unwashed hands or dirty utensils (18, 22). As already reported (28), *C. jejuni* was more frequently isolated than *C. coli* (59 versus 41%). This phenomenon is easily explained by the fact that *C. coli* is mainly associated with pigs (3).

Because no great discrepancies have been observed between double dilution agar and E test according to Baker et al. (5) and Funke et al. (14), the authors used the E test for susceptibility testing. This method is reliable, technically simple, and needs no special equipment (13). In some countries FQ resistance rates were similar between strains isolated from poultry meat and from man (17, 19, 29), highlighting the need to carefully monitor resistance in human and animal samples.

In the present survey, high resistance rates to quinolones were observed in both species isolated from chicken samples. This high resistance rate was similar to those observed in several European countries (26) or in Japan (9). As reported by Saenz et al. in Spain (29), a cross-resistance was observed between nalidixic acid and ciprofloxacin. But unlike Thwaites and Frost in the United Kingdom (37), *Campylobacter* strains with a high level of resistance to both quinolones were predominant. Nevertheless, rates of resistance to ciprofloxacin observed in the present study appeared lower than those described in Belgium (39), Spain (29)

or Lebanon (35), where they ranged from 61 to 100%. By contrast, no *Campylobacter* strains were found ciprofloxacin resistant in Chile (13).

Since 1991, when Endzt et al. (11) identified the first quinolone-resistant *Campylobacter* strains in *C. jejuni* and *C. coli* in the Netherlands, *Campylobacter* resistance to FQ has been on the increase throughout the world (12, 29). The percentage of ciprofloxacin-resistant strains in man increased in Spain from 0-3% in 1989 to 30-50% in 1991 after licensing the use of enrofloxacin in 1990 in animal husbandry (30). During this period, the same evolution was observed in The Netherlands (11), Finland (27) and Canada (15). Likewise in England, before the introduction of FQ in veterinary medicine, a study showed that domestically-bred poultry were less contaminated by ciprofloxacin-resistant strains than those imported from countries where the use of FQ was legal (16). Thus, this major development of FQ-resistant strains in humans and animals seems related to the introduction of FQ in veterinary medicine (11, 29).

The speed at which the level of resistance to nonfluorinated (nalidixic acid) and fluorinated (ciprofloxacin) quinolones (34 versus 31%) has been reached may be related to the mechanisms by which quinolone resistance involves a single chromosomal mutation on the targets of quinolone action (DNA gyrase, DNA topoisomerase IV) (12, 25). In Senegal, FQs (norfloxacin, enrofloxacin) were introduced in veterinary medicine in 1996 in poultry production to treat respiratory and intestinal diseases as salmonellosis or colibacillosis. Since 2000, they became first line molecules because of treatment failures with other antibiotic drugs. According to observations from other reports and despite the absence of previous studies on antibiotic resistance in Senegal, the high prevalence of FQ resistance could thus be related to the introduction of FQs in the country especially for poultry production (21). As in developed countries, FQ resistance may soon become a public health problem.

High levels of amoxicillin resistance were found, particularly in *C. coli* strains. *Campylobacter* resistance to penicillin A has been reported in previous studies with resistance rates never exceeding 39% (3, 9, 13, 29). However, in Senegal, amoxicillin was rarely used for treatment of pasteurellosis or salmonellosis in poultry production. Resistance is usually associated with beta-lactamase production but other mechanisms of resistance could be involved, such as modified penicillin-binding proteins or impermeability (29, 34). As reported in other studies, most of the isolates of the present survey showed high susceptibility to amoxicillin-clavulanic acid (35). Clavulanic acid inhibits some beta-lactamases and has been claimed to have intrinsic antibacterial activity against *Campylobacter* (36).

Among the stains isolated, 4.7% of *C. coli* and 3.3% of *C. jejuni* strains were resistant to erythromycin. This appeared much lower than results from Nigeria, where resistance rate reached 40.4% among animal strains (32). In the present study, erythromycin-resistance rates were similar in both species, contrary to results from surveys conducted in Spain (17 and 83% of resistance in *C. jejuni* and *C. coli*, respectively; 29) or in Vietnam (17 and 50% of resistance in *C. jejuni* and *C. coli*, respectively; 19). This difference may be explained by the introduction in these countries of tylosin, especially used as growth promoter in the pig industry (39). In Senegal, macrolides were not used as growth promoters in poultry production; they were only used for treatment of some respiratory diseases such as chronic respiratory diseases. Resistance to erythromycin is chromosomally mediated and is due to the alteration of the ribosome (12), but natural resistance as efflux pump has been observed too (8).

To the five most relevant antimicrobial drugs selected for campylobacteriosis treatment, seven resistance patterns were observed, but only 9.8% of total strains were resistant to three drugs. None were resistant to four or more drugs. The present results were much lower than those reported from Belgium (39) or Spain (29). However, consistently with these studies, *C. coli* exhibited more multidrug-resistant strains than *C. jejuni* in the present survey.

This study is the first to highlight the decrease of antibiotic susceptibility of *Campylobacter* isolated from chicken in Senegal. Many reasons explain this situation; among others, there is the lack of information among poultry farmers and veterinary surgeons on the use of antibiotics and on drug resistance, the inordinate use of antibiotics due to the high prevalence of infectious diseases among flocks, and the difficulty to perform large antimicrobiological studies.

However, as everywhere else in the world, the increase of *Campylobacter*-resistant strains seems to be related to the amounts of antibiotics used in animals (38). Thus, to prevent transfer of resistant bacteria or resistance genes from animals to humans via the food chain (6) in Senegal, measures to be implemented are the same as the ones in developed countries: reduction of the use of antibiotics (antibiotics should not be available over the counter in order to avoid self medication), restricting their use to encourage narrow-spectrum specific antibiotic therapy instead of broad spectrum antimicrobials (7), and replacement of antibiotics with improvements in hygiene and flock management.

Consequently, it would be useful to set up an epidemiological surveillance network to monitor antimicrobial resistance in bacteria of animal origin. The authors also recommend conducting further antimicrobial-resistance studies among *Campylobacter* isolated from human and animal samples in order to control the emergence of a new public health problem in Senegal.

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Résumé

Cardinale E., Dromigny J.-A., Tall F., Ndiaye M., Konte M., Perrier Gros-Claude J.-D. Sensibilité aux antibiotiques de souches de *Campylobacter* isolées de carcasses de poulets au Sénégal

La résistance de *Campylobacter* aux antibiotiques constitue aujourd'hui un problème émergent de santé publique dans les pays industrialisés, mais, en revanche, peu d'informations sont disponibles sur le sujet dans les pays en développement. Pour évaluer la sensibilité des souches de *Campylobacter* au Sénégal, des prélèvements de peau ont été réalisés sur 250 carcasses de poulet entre janvier 2001 et octobre 2002. Parmi les 204 souches de *Campylobacter* isolées, deux espèces ont été identifiées : *C. jejuni* (59 p. 100) et *C. coli* (41 p. 100). La sensibilité in vitro à cinq antibiotiques (amoxicilline, amoxicilline et acide clavulanique, erythromycine, acide nalidixique et ciprofloxacine) a été déterminée par la méthode du E-test. L'étude des concentrations minimales inhibitrices (CMI) a montré que 34 p. 100 des isolats étaient résistants à la ciprofloxacine avec un haut niveau de résistance (CMI ≥ 32 mg/l) dans 25 p. 100 des deux espèces. Une résistance croisée entre l'acide nalidixique et la ciprofloxacine a été constatée dans 96 p. 100 des souches résistantes aux quinolones. Le niveau de résistance à l'amoxicilline a été statistiquement plus important pour *C. coli* que pour *C. jejuni* (20,2 p. 100 contre 10,8 p. 100) mais toutes les souches ont été sensibles à l'association amoxicilline-acide clavulanique. Les deux espèces ont présenté une faible résistance à l'érythromycine. Un phénotype de multirésistance à trois des antibiotiques testés a été identifié dans 9,8 p. 100 des souches : 15,5 p. 100 pour *C. coli* et 5,8 p. 100 pour *C. jejuni*. Aucune souche ne s'est avérée résistante à quatre antibiotiques ou plus. Des études complémentaires apparaissent nécessaires pour évaluer la résistance aux antibiotiques des *Campylobacter* isolés chez l'homme et chez l'animal afin de contrôler l'émergence de nouvelles souches multirésistantes au Sénégal.

Mots-clés : Poulet – *Campylobacter* – Résistance aux antibiotiques – Sénégal.

Resumen

Cardinale E., Dromigny J.-A., Tall F., Ndiaye M., Konte M., Perrier Gros-Claude J.-D. Susceptibilidades anti-microbianas de cepas de *Campylobacter* aisladas de carcasas de pollos en Senegal

La resistencia de *Campylobacter* a los antibióticos constituye hoy en día un problema emergente de salud pública en los países industrializados, sin embargo, existe poca información disponible en los países en vías de desarrollo. Para evaluar la sensibilidad de las cepas de *Campylobacter* en Senegal, se obtuvieron muestras de piel en 250 carcasas de pollo, entre enero 2001 y octubre 2002. Entre las 204 cepas de *Campylobacter* aisladas, se identificaron dos especies: *C. jejuni* (59%) y *C. coli* (41%). Se determinó la sensibilidad in vitro a 5 antibióticos (amoxicilina, amoxicilina y ácido clavulánico, eritromicina, ácido nalidíxico y ciprofloxacina), mediante el método de E-test. El estudio de las CMI mostró que 34% de los aislamientos fueron resistentes a la ciprofloxacina con un alto nivel de resistencia (CMI ≥ 32 mg/l), en 25% de las dos especies. Mediante el "E-test", se observó una resistencia cruzada entre el ácido nalidíxico y la ciprofloxacina en 96% de las cepas resistentes a las quinolonas. El grado de resistencia a la amoxicilina fue significativamente más importante para *C. coli* que para *C. jejuni* (20,2% contra 10,8%), pero todas las cepas fueron sensibles a la asociación amoxicilina-ácido clavulánico. Las dos especies presentaron una baja resistencia a la eritromicina. Se identificó un fenotipo multi-resistente a tres de los antibióticos en 9,8% de las cepas: 15,5% para *C. coli* y 5,8% para *C. jejuni*. Ninguna cepa se mostró resistente a más de cuatro antibióticos. Parece necesaria la realización de estudios complementarios, con el fin de evaluar la resistencia a los antibióticos de los *Campylobacter* aislados en el hombre y en el animal, para controlar el surgimiento de nuevas cepas resistentes en Senegal.

Palabras clave: Pollo – *Campylobacter* – Resistencia a las antibióticos – Senegal.