



Frequency of contamination and antimicrobial resistance of thermotolerant *Campylobacter* isolated from some broiler farms and slaughterhouses in the region of Algiers

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ABSTRACT

Campylobacteriosis in humans is caused by thermotolerant *Campylobacter* spp, following consumption of contaminated poultry, most commonly broiler.

The aim of this study was to assess the frequency of contamination by thermotolerant *Campylobacter* and to characterize antimicrobial resistance of the strains isolated from broilers in some farms and slaughterhouses in the region of Algiers.

One hundred droppings samples, 100 contents of ceaca and 100 neck skins were taken from six poultry farms and five slaughterhouses, than analyzed according to NF. ISO 10272-1/1995 norm and the OIE recommendations. Susceptibility to antibiotics was determined according to the guidelines of the CA-SFM/2010 by disc diffusion method.

Thermotolerant *Campylobacter* strains were isolated from 85%, 98%, and 80% of droppings, caecal content and neck skin, respectively. All the strains (100%) were resistant to nalidixic acid and sensitive to gentamicin and to chloramphenicol. 83.7% of them were resistant to tetracycline and to ciprofloxacin, 75.3% to ampicillin, 46.8% to amoxicillin/clavulanic acid and 21.7% were resistant to erythromycin. All the isolates showed a multi-drug resistance. Nineteen different profiles were identified with “AM, AMC, NA, CIP, TE” combination as the most common profile identified for 27% ($n = 74$) of isolated strains. In addition, 15% of the strains were resistant to both erythromycin and ciprofloxacin, which are systematically used in treatment of human *Campylobacter* infections.

Our results showed a high prevalence of thermotolerant *Campylobacter* with multidrug resistance profiles in poultry farms and slaughterhouses of Algiers. These results stress that the risk of human contamination throughout the food chain is very high, which may generate: i) a danger of food poisoning by ingestion of chicken meat and chicken meat products and, ii) a cross-resistance to antibiotics between human and avian strains.

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1. Introduction

In human health, *Campylobacter* is considered as the major cause of diarrheal illnesses and, the most common bacterial cause of gastroenteritis in the world (Bolla & Garnotel, 2008), causing up to 14% of diarrhea worldwide. It is estimated that 2.5 million of the diarrhea worldwide occur each year, resulting in 13,000 hospitalizations and 124 deaths (Braam, 2004).

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In western Algeria, a case–control study in infants showed that *Campylobacter* were isolated in 17.7% of the 411 patients and in 14.9% of the 247 controls (Megraud et al., 1990). However, no official data concerning contamination of poultry or other food products in Algeria is available.

Compared to *Salmonella*, *Campylobacter* isolation frequency from human diarrheal stool samples is generally similar or higher; thus they represent a major problem in microbiological food safety. *Campylobacter*'s ubiquitous nature and extent of adaptation to wild, farm animals and pets, contribute to the introduction and persistence of this bacteria in different biotopes and to contamination of the foodstuffs (Federighi, 2005, pp. 145–167). While numerous potential vehicles of transmission exist, commercial chicken meat

has been identified as one of the most important food vehicles for these organisms. Preparation of raw poultry and consumption of contaminated chicken meat or chicken products in general have been identified as the main risk factor for human infection with *Campylobacter* (Hartnett et al., 2009, 33 p.). Sporadic human cases have been associated with consumption of under-cooked poultry meat, while larger outbreaks are associated with raw milk (Denis et al., 2011; Karagiannis et al., 2010). Acute self-limited gastrointestinal illness, characterized by diarrhea, fever and abdominal cramps, is the most common presentation of *Campylobacter* infection; fresh blood may appear in the stools. Local complications such as cholecystitis, pancreatitis and peritonitis occur rarely. It has been recognized that Guillain–Barré syndrome is the most serious complication of *Campylobacter* infection, with an incidence of 1/1000 infections (Butzler, 2004).

Campylobacter diseases are primarily treated with fluoroquinolones and macrolides. However, an increase of acquired resistance to these same antibiotics has been reported by the European food safety authority (EFSA, 2010a). Because of their ability to be transmitted to humans through food chain, the zoonotic enteropathogenic bacteria resistant to antibiotics, mainly *Campylobacter*, are more dangerous in terms of human health. In the U.S.A, consumption of poultry has been identified as a risk factor for human infection by fluoroquinolone-resistant *Campylobacter* species (Luangtongkum et al., 2009).

Our study aims to determine the thermotolerant *Campylobacter* prevalence in broiler in some poultry farms and slaughterhouses of Algiers and the susceptibility of isolated strains to several antibiotics.

2. Material and methods

2.1. Samples collection

Our study was conducted from January to December, 2010. 300 samples were collected from six poultry farms representing about the tenth of poultry buildings in this region, and from five slaughterhouses representing the quarter of the abattoirs of this region, randomly chosen in the Algiers region. The poultry farms have livestock capacity between 4000 and 6000 subjects, while the processing capacity of the slaughterhouses are between 600 and 1200 subjects per hour. First, at the end of rearing period, from six poultry farms, 100 freshly emitted droppings samples were collected during the last week preceding removal; second, on five slaughterhouses, after chickens evisceration, 100 samples of caeca were taken and, 100 fresh broiler chicken neck skins were collected at the end of slaughtering chain. Both cecal contents and neck skin samples were collected from the same slaughter batch (animals had the same origin and were sampled randomly in the slaughterhouse line). For each farm and slaughterhouse, a single visit was performed early in the morning; the samples were placed in sterile plastic pots (droppings and cecal contents) and sterile plastic bags (neck skins) inside an isothermal cool-box at +4 °C and transferred immediately to the laboratory in order to be analyzed on the same day.

2.2. Isolation and identification

The isolation and identification of thermotolerant *Campylobacter* were performed according to World Organisation for Animal Health recommendations (OIE, 2008) and to French Agency for Standardization: NF ISO 10272-1: 1995 norm (AFNOR, 2004, 15 p.) using the following procedures.

All cultures and incubations were performed under microaerobic condition which was generated by using an anaerobic jar containing a gas generating CampyGen™ reagents (Oxoid, Dardilly, France).

Culture of *Campylobacter* took place within 4 h after samples collection. From droppings and caecal contents the research of thermotolerant *Campylobacter* was performed by direct isolation on Butzler selective agar medium (Oxoid, Dardilly, France) and incubated at 42 °C for 48 h.

From neck skin samples, isolation on Butzler agar was preceded by a selective enrichment step of sample: 10 g of sample in 90 mL of Preston broth (Oxoid, Dardilly, France) and incubated for 24 h at 42 °C. The culture dishes were then inoculated with a few drops of Preston broth, incubated at 42 °C and read every 24 h up to fifth day.

The control strains were: *Campylobacter jejuni* (CIP 70.2), *Campylobacter coli* (CIP 70.80) and *C. fetus* (CIP 53.96) (Institut Pasteur Paris Collection, France).

Curved Gram-negative bacilli, with typical motility (twirling and rapid darting movements), oxidase positive, catalase positive and which did not show growth at 25 °C were presumed thermotolerant *Campylobacter* in the preliminary identification.

Suspect colonies of *Campylobacter* were subsequently purified on Columbia agar (Bio-Rad, Marnes la coquette, France) with 5% horse blood (IPA: Institut Pasteur d'Algérie, Algiers, Algeria), and confirmed both by using biochemical test on Triple Sugar Iron agar (IPA; Algiers, Algeria) and sensitivity to cephalothin (30 µg) (Bio-Rad, Marnes La Coquette, France).

2.3. Antibiotic sensitivity analysis

Antimicrobial sensitivity of *Campylobacter* strains was assessed using the disk diffusion assay. All isolates were tested with the following antibiotics: ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), gentamicin (10 µg), erythromycin (15 IU), nalidixic acid (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg) and chloramphenicol (30 µg) (Bio-Rad, Marnes La Coquette, France), the choice of tested antibiotics was carried out according to the guidelines of the CA-SFM/2010 and according to the standardization of the antibiogram in human medicine on a national scale (WHO, 2008b, 102 p.).

Bacterial suspension of 0.5 McFarland was prepared from a pure 18 h culture, than seeded by swabbing. The isolated strains were inoculated on Mueller-Hinton agar (Bio-Rad, Marnes La Coquette, France) supplemented with 5% defibrinated horse blood. After deposition of antibiotics disks, the plates were incubated at 42 °C for 24–48 h in microaerophilic atmosphere. Inhibition zones were measured by a caliper and diameters were interpreted as recommended by the Antibiogram committee of the French Society of Microbiology (CA-SFM, 2010, 50 p.). For quality control reason, reference strains *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were tested concomitantly with samples.

2.4. Statistical analysis

Chi-square test was used for comparisons in order to determine if there were statistically significant differences at 95% level in the prevalence of thermotolerant *Campylobacter* samples between the farms and between the slaughterhouses and to compare antimicrobial resistance rates. Significant differences were considered when probability (p) was equal to or less than α risk ($p \leq 0.05$).

3. Results

3.1. Thermotolerant *Campylobacter* Prevalence in tested poultry farms and slaughterhouses

Among 300 tested samples, 263 were positive to thermotolerant *Campylobacter*, which represent an overall prevalence of 87.7%.

Campylobacter were isolated in 85% ($n = 85$) of droppings samples, 98% ($n = 98$) of ceecal contents and 80% ($n = 80$) of neck skins; indeed no statistically difference was identified between rates ($p > 0.05$). All samples issued from five farms were positive to thermotolerant *Campylobacter*, whereas no strain has been isolated from the sixth one. *Campylobacter* was isolated from both types of samples in all slaughterhouses (Table 1).

3.2. Antibiotic susceptibility of isolated strains

All isolated strains were resistant to nalidixic acid, and susceptible to gentamycin and to chloramphenicol. 83.7% ($n = 220$) were resistant to tetracycline and ciprofloxacin, 75.3% ($n = 198$) to ampicillin, 46.8% ($n = 123$) to amoxicillin/clavulanic acid, and 21.7% ($n = 57$) to erythromycin (Table 2). Resistance rates among antibiotics used for the same type of sample showed a significant difference ($P < 0.05$) but there was no significant difference between the strains isolated from feces, ceecal contents and those isolated from neck skins to the same antibiotic.

Moreover, all isolates showed a multidrug resistance to antibiotics (resistance to two antibiotics or more), 5.7% ($n = 15$) resistant to two antibiotics, 27.4% ($n = 72$) to three antibiotics, 24.7% ($n = 65$) to four antibiotics, 36.9% ($n = 97$) to five antibiotics and 5.3% ($n = 14$) to six antibiotics. Indeed, nineteen different resistance profiles were noted, with “ampicillin, amoxicillin/clavulanic acid, nalidixic acid, ciprofloxacin, tetracycline” combination as the most common profile identified for 27% ($n = 74$) of isolated strains, 15% ($n = 38$) of isolates were resistant to both ciprofloxacin and erythromycin (Table 3).

4. Discussion

4.1. Thermotolerant *Campylobacter* prevalence

Bacteriological analysis of samples showed in a part, high intestinal carriage of *Campylobacter* in broiler (98%) with a high rate of contaminated carcasses (80%). The intestinal carriage is due to the fact that these enteric bacteria are adapted to live in digestive tract mucus (Megraud & Bultel, 2004). The prevalence of positive broiler flocks in the European Community was estimated to 75% (EFSA, 2010a, 2011, 2012). According to the Swiss report of zoonoses, *Campylobacter* were recovered from all tested chicken flocks in Switzerland (Luginbühl, Marthaler, Geiser, Lutz, & Danuser, 2010).

Table 1
Prevalence of thermotolerant *Campylobacter* in investigated farms and slaughterhouses.

Farms		Slaughterhouses		
Designation	Droppings no. positive/ total of samples (%)	Designation	Caecal contents no. positive/total of samples (%)	Neck skins no. Positive/total of samples (%)
1	0/15 (0)	1	18/20 (90)	13/20 (65)
2	20/20 (100)	2	20/20 (100)	18/20 (90)
3	15/15 (100)	3	20/20 (100)	15/20 (75)
4	20/20 (100)	4	20/20 (100)	16/20 (80)
5	15/15 (100)	5	20/20 (100)	18/20 (90)
6	15/15 (100)			
Total	85/100 (85)	Total	98/100 (98)	80/100 (80)
P ₁ -Value	$P < 0.05$	P ₂ -Value	$P > 0.05$	$P > 0.05$

Abbreviations: No, Number; AM, ampicillin; AMC, amoxicillin/clavulanic acid; GM, gentamycin; E, erythromycin; C, chloramphenicol; TE, tetracycline; NA, nalidixic acid; CIP, ciprofloxacin.

P-Value: Value for the antimicrobial resistance difference between the strains isolated from feces, caecal content and those isolated from neck skin samples to the same antibiotic.

The prevalence of *Campylobacter* was in agreement with previous studies in Iran (Ansari Lari et al., 2011). However it remains fairly high compared to that reported by Chena et al. in 2010 in China.

In this survey, of the five farms, all samples were contaminated, this suggests a horizontal transmission. It has been reported that all chickens are infected one week after introduction of bacteria in poultry building (Shreeve, Toszeghy, Pattison, & Newell, 2000). Chickens are coprophagous and fecal excretion is probably an important factor in spread of microbes in poultry flocks (Usha et al., 2010). This result can also be related to high animal density, which promotes contact between animals especially those carrying *Campylobacter* (Megraud & Bultel, 2004). In addition to sources such as soiled litter, untreated water, other farm animals, wild birds, insects, as well as the human flow, Viable Non-Cultivable forms were associated to *Campylobacter* transmission in broilers, which would in turn be responsible for transmission of these microorganisms to the environment (Humphrey, O'Brien, & Madsen, 2007).

Although, horizontal transmission of *Campylobacter* in the environment of breeding seems to be the main track, vertical transmission of *Campylobacter* from parent to their chicks is not ruled out (Pearson et al., 1996).

One of the studied farms that received a food supplemented with organic acids was free from *Campylobacter* which suggests that supplementation with organic acids could be indirectly involved in protection as demonstrated by Chaveerach, Keuzenkamp, Urlings, Lipman, and Knapen, in 2002 and Solis de los Santos et al. in 2009. These molecules have been recommended by an expert report of the FAO/WHO (Food and agriculture organization/World Health Organization) as a means of hazard control of *Campylobacter* in chicken meat (Anonymous, 2001, 53 p.).

In slaughterhouses, contamination of neck skin could be related directly to gut contents of the same animal and/or to cross contamination throughout slaughtering equipment. This contamination may occur in the scalding tanks, in feather removal machine or during evisceration (Ghafr & Daube, 2007; Posch et al., 2006). The nature of slaughter processing makes it impossible to prevent cross-contamination of negative batches by positive batches (Frediani & Stephan, 2003; Ono & Yamamoto, 1999). In a previous study in Algeria, 66% of chicken neck skins were contaminated (Mouffok & Lebres, 1992). Our results are similar to those reported in Brazil by Franchin, Ogliari, and Batista, in 2007 with a contamination rate of 84.7%.

4.2. Antimicrobial resistance of isolated strains

High susceptibility to gentamicin and chloramphenicol could be explained by none or moderate use of these antibiotics due to the no registration in Algeria since 2006 (WHO, 2008a, 100 p.).

High levels of resistance were observed towards different antibiotics including nalidixic acid, ciprofloxacin, tetracycline and ampicillin (antibiotics widely used in tested farms) which shows similarity of our results compared to those reported by EFSA (2010b). By contrast, no antibiotic resistance against ampicillin, erythromycin, tetracycline and nalidixic acid have previously been observed with strains isolated from droppings and neck skins of broiler in the region of Algiers in 1992 (Mouffok & Lebres, 1992).

Several authors agree that the use of antibiotics in animals as prophylactic, therapeutic agents or for growth promotion can select for resistance and reduce the effectiveness of these products in veterinary and human medicine, because of emergence of resistant strains, which may occur during or after antimicrobial treatment (Avrain et al., 2003; Rahimi, Momtaz, Ameri, Ghasemian-Safaei, & Ali-Kasemi, 2010; Usha et al., 2010; WHO, 2008c, 5 p.). However, the use of disinfectants or other biocides (Russell, 2002; EFSA,

Table 2Antimicrobial resistance rates of thermotolerant *Campylobacter* isolated strains.

	Profil	AM	AMC	GM	E	C	TE	NA	CIP
Resistant strains from Droppings	No. (%)	62 (72.9)	42 (49.4)	0 (0)	24 (28.2)	0 (0)	72 (84.7)	85 (100)	77 (90.6)
Resistant strains from Caecal contents	No. (%)	73 (74.5)	42 (42.9)	0 (0)	18 (18.4)	0 (0)	79 (80.6)	98 (100)	78 (79.6)
Resistant strains from Neck skins	No. (%)	63 (78.8)	39 (48.8)	0 (0)	15 (18.8)	0 (0)	69 (86.3)	80 (100)	65 (81.3)
Resistant strains	No. (%)	198 (75.3)	123 (46.8)	0 (0)	57 (21.7)	0 (0)	220 (83.7)	263 (100)	220 (83.7)
Total									
P-Value		P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

Abbreviations: No, Number; AM, ampicillin; AMC, amoxicillin/clavulanic acid; GM, gentamicin; E, erythromycin; C, chloramphenicol; TE, tetracycline; NA, nalidixic acid; CIP, ciprofloxacin.

P-Value: Value for the antimicrobial resistance difference between the strains isolated from feces, caecal content and those isolated from neck skin samples to the same antibiotic.

2010b), and the environmental stresses encountered by bacteria during the slaughter process (McMahon, Xu, Moore, Blair, & McDowell, 2007) may also play a role in the phenomenon of antibiotic resistance.

21.7% of isolated strains were resistant to erythromycin, this resistance remains low in industrialized countries, however, resistant strains are frequently observed in developing countries, including Thailand (50%) and Zimbabwe (14%) (WHO, 2003). In another way, Lin et al. (2007) showed that erythromycin used in low doses over a long period (which corresponds to their use as a growth factor) selects for resistant strains of *Campylobacter*.

83.7% of isolated strains were resistant to ciprofloxacin. Several studies have demonstrated the rapid development of mutants resistant to fluoroquinolones in chickens infected with initially fluoroquinolones susceptible *Campylobacter*, and treated with enrofloxacin. Indeed, treatments of chickens do not eradicate the organism but convert initially susceptible population to resistant fluoroquinolones population which emerges as soon as 24 h from start of treatment (Farnell et al., 2005; Han, Sahin, Barton, & Zhang, 2008; McDermott, Bodeis, & English, 2002;).

The resistance to tetracycline was very frequent (83.7%) as reported by other authors (Bester & Essack, 2008; Mansouri-najand, Saleha, & Wai, 2012; Tambur, Miljkovic-Selimovic, Doder, & Kulisic, 2010).

Table 3Resistance pattern profiles of isolated thermotolerant *Campylobacter* strains.

Associated resistances to	Resistance pattern profiles	No. of strains	No. total (%)
Two antibiotics	NA,CIP	7	15 (5.7)
	NA,AM	2	
	NA,TE	6	
Three antibiotics	NA,CIP,AM	18	72 (27.4)
	NA,CIP,E	6	
	NA,CIP,TE	32	
	NA,AM,AMC	4	
	NA,AM,TE	2	
	NA,TE,E	10	
Four antibiotics	NA,CIP,AM,AMC	9	65 (24.7)
	NA,CIP,AM,TE	41	
	NA,CIP,AMC,TE	1	
	NA,CIP,TE,E	4	
	NA,AM,AMC,TE	8	
Five antibiotics	NA,AM,TE,E	2	97 (36.9)
	NA,CIP,AM,AMC,TE	74	
	NA,CIP,AM,TE,E	14	
	NA,AM,AMC,TE,E	9	
Six antibiotics	NA,CIP,AM,AMC,TE,E	14	14 (5.3)

Abbreviations: No, Number; AM, ampicillin; AMC, amoxicillin/clavulanic acid; GM, gentamicin; E, erythromycin; C, chloramphenicol; TE, tetracycline; NA, nalidixic acid; CIP, ciprofloxacin.

In Malaysia, resistance rates were higher than our results; 86% was recorded to ampicillin, 82% to ciprofloxacin, 92% to tetracycline and 99% to erythromycin and even with gentamicin (35%) (Tang, Mohamad Ghazali, Saleha, Nishibuchi, & Son, 2009).

Roughly, Vandeplas et al. (2008) believe that changes in rates of antimicrobial resistance between countries reflect various veterinary practices in antimicrobial use for treatment and prevention.

All our isolates were multi-drug resistant; we had identified a dominant profile combining resistance to two beta-lactams, two quinolones and tetracycline. In Belgium, the National Reference Laboratory for foodborne disease and antimicrobial resistance of zoonotic agents, announced that the percentages of strains multi-drug resistant of *C. jejuni* and *C. coli* isolated from chickens were 40% and 64% respectively, the dominant profile was "nalidixic acid, ciprofloxacin, tetracycline" (Dierick, Botteldoorn, Denayer, & Naranjo, 2009).

15% of strains isolated in our study were resistant to both ciprofloxacin and erythromycin. These strains represent a critical profiles, since these two antibiotics are the mostly used in *Campylobacter* infections in humans (Engberg, Aarestrup, Gerner-Smidt, & Nachamkin, 2001; Luangtongkum et al. 2009). In addition to clinical troubles, transmission of multi-resistant *Campylobacter* to humans through food chain can also compromise the effectiveness of treatment. Indeed, the EFSA has published a report indicating that antimicrobial resistance phenomenon occurs in most common zoonosis bacteria, particularly *Campylobacter* from animals and food in the European Union (EFSA, 2010b).

5. Conclusion

Unlike other foodborne pathogens, *Campylobacter* are very susceptible to many environmental conditions, as concluded in this survey, our results confirm that *Campylobacter* can survive well from poultry farms to the plate of consumer and are of concern of food poisoning. Indeed, intestinal carriage and neck skins contamination rates in the present study showed high survival of *Campylobacter* in farms, in slaughterhouses and carcasses. It revealed that antibiotic resistant *Campylobacter* is common among chickens which can find their way into the food chain. The recorded multi-drug resistant campylobacters which was also reported to be common in other countries reflect an alarming situation with regard to this phenomenon which may be due to the widespread use of antibiotics in poultry farming. As major source of human campylobacteriosis, prevention should be focused on reducing *Campylobacter* infection at all stages of poultry production chain, as well as for the controlling use of antimicrobials in veterinary medicine in Algeria. Thus, there is an urgent need for prudent use of antibiotics in poultry production to reduce the development and spread of multi-drug resistant *Campylobacter* which must be diligently monitored. Our results also emphasize the

need for a surveillance and monitoring system for the prevalence and antimicrobial resistance of *Campylobacter* in broiler, poultry and other food animals.

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