International Journal of Food Microbiology 157 (2012) 102–107

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Prevalence, quantification and antimicrobial resistance of *Campylobacter* spp. on chicken neck-skins at points of slaughter in 5 major cities located on 4 continents

Benoit Garin [a,*], Malika Gouali [b], Marguerite Wouafo [c], Anne-Marie Perchec [d], Pham Minh Thu [e], Noro Ravaonindrina [b], Florence Urbès [d], Manu Gay [b], Abdoulaye Diawara [f], Alexandre Leclercq [g], Jocelyne Rocourt [c], Régis Pouillot [c]

[a] *Institut Pasteur de Dakar, BP 220, Dakar, Senegal*
[b] *Institut Pasteur de Madagascar, BP 1274, Antananarivo, Madagascar*
[c] *Centre Pasteur du Cameroun, BP 1274, Yaoundé, Cameroon*
[d] *Institut Pasteur de Nouvelle Calédonie, BP 61, Nouméa, 98845, New Caledonia*
[e] *Institut Pasteur d'Ho-Chi-Minh-Ville, 167, rue Pasteur, Q3 Ho-Chi-Minh-Ville, Vietnam*
[f] *Direction de l'élevage. Services Vétérinaires. Avenue Pasteur, Dakar, Senegal*
[g] *Institut Pasteur, Microbes and host barriers Group, WHO Collaborating Centre for Listeria, Paris, France*

ARTICLE INFO

Article history:
Received 25 July 2011
Received in revised form 22 February 2012
Accepted 21 April 2012
Available online 1 May 2012

Keywords:
Developing countries
Enumeration
Campylobacter

ABSTRACT

Quantitative data on *Campylobacter* contamination of food are lacking, notably in developing countries. We assessed *Campylobacter* contamination of chicken neck-skins at points of slaughter in 5 major cities in Africa (Dakar in Senegal, Yaounde in Cameroon), Oceania (Noumea in New Caledonia), the Indian Ocean (Antananarivo in Madagascar) and Asia (Ho Chi Minh City (HCMC) in Vietnam.

One hundred and fifty slaughtered chickens were collected in each of the 5 major cities from semi-industrial abattoirs or markets (direct slaughter by the seller), and 65.5% (491/750) were found to be *Campylobacter*-positive. Two cities, Yaounde and Noumea, demonstrated high prevalence *Campylobacter* detection rates (92.7% and 96.7% respectively) in contrast with HCMC (15.3%).

Four species were identified among 633 isolates, namely C. *jejuni* (48.3%), C. *coli* (37.3%), C. *lari* (11.7%) and C. *upsaliensis* (1%). HCMC was the only city with *C. lari* isolation as was Antananarivo for *C. upsaliensis*. *C. coli* was highly prevalent only in Yaounde (69.5%).

Among the 491 samples positive in *Campylobacter* detection, 329 were also positive with the enumeration method. The number of *Campylobacter* colony-forming units (CFU) per gram of neck-skin in samples positive in enumeration was high (mean of the $\log_{10}$: 3.2 $\log_{10}$ CFU/g, arithmetic mean: 7900 CFU/g). All the cities showed close enumeration means except HCMC with a 1.81 $\log_{10}$ CFU/g mean for positive samples. Semi-industrial abattoir was linked to a significant lower count of *Campylobacter* contamination than direct slaughter by the seller ($p = 0.006$).

On 546 isolates (546/633, 86.3%) tested for antibiotic susceptibility, resistance to erythromycin, ampicillin and ciprofloxacin was observed for respectively 11%, 19% and 50%. HCMC was the city where antibiotic resistant rates were the highest (95%, $p = 0.014$).

Considering the 329 positive chickens in *Campylobacter* enumeration, the mean number of resistant isolates to at least 2 different antibiotic families (19.8%), may be estimated *ca.* 1500 CFU/g; the corresponding mean of the $\log_{10}$ would be 2.5 $\log_{10}$CFU/g.

As chickens are sold at slaughter and brought directly at home to be cooked, these data suggest a high probability of cross-contamination. A substantial proportion of isolates are drug-resistant, which could lead to potential public health issues. Health authorities should consider measures to reduce *Campylobacter* contamination of chicken during farming and at slaughter, and to provide appropriate food hygiene education. Further studies are needed in particular to investigate food-handling practices in domestic kitchens.

1. Introduction

Campylobacter is an ubiquitous bacterium found in birds, mammals, insects, farms, production lines and various food items (Humphrey et al., 2007). The main source of human infection is undercooked chicken, raw

* Corresponding author at: Institut Pasteur de Madagascar, Laboratoire de Bactériologie Expérimentale, BP 1274, Tananarive, Madagascar. Tel.: +261 20 22 590 19; fax: +261 20 22 415 34.
E-mail address: benoitgarin@gmail.com (B. Garin).

0168-1605/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2012.04.020

or unpasteurized milk, and cross-contamination from the environment, notably in kitchens (Friedman et al., 2004; Humphrey et al., 2007). An estimated 20–40% of human *Campylobacter* infections are linked to the handling or consumption of chicken products (EFSA, 2010). The proportion of foodborne illness cases due to cross-contamination is still unclear (ECDC, 2009; Gorman et al., 2002).

Campylobacter can cause both gastroenteritis and extra-intestinal disease. *C. jejuni* and *C. coli* are the most frequent *Campylobacter* species isolated from patients with diarrhea. *C. upsaliensis* (Lastovica and Le Roux, 2003; Westgarth et al., 2009) and *C. lari* may also occasionally cause diarrhea (Coker et al., 2002; ECDC, 2008). In both industrialized and developing countries, diarrheal campylobacteriosis is mainly a pediatric disease (<5 years) (Coker et al., 2002; ECDC, 2009; Foodnet, 2010).

Possible interactions between *Campylobacter* and irritable bowel diseases (Marshall, 2009) and immunoproliferative small-intestinal disease (ISID) (Lecuit et al., 2004) need to be confirmed. Intestinal healthy carriage has also been reported (Lindblom et al., 1995).The main extraintestinal disease associated with *Campylobacter* is Guillain-Barre-Syndrome, the most frequently diagnosed form of acute flaccid paralysis worldwide (McGrogan et al., 2009).

Despite its sporadic dissemination (Friedman et al., 2004), non systematic laboratory diagnosis of diarrhea, and underreporting (ECDC, 2009), *Campylobacter* has become a more frequently recognized cause of gastroenteritis than *salmonellae* (ECDC, 2009). In the European Union (EU), the incidence is relatively stable, at 45–50 cases per 100 000 inhabitants. In the United States, the incidence of *Campylobacter* infection was recently estimated to be 13/100 000 inhabitants (Scallan et al., 2011), compared to 15/100 000 for *Salmonella* (Foodnet, 2010). On the other hand, the incidence of *Campylobacter* infection in developing countries is difficult to assess, owing to the absence of specific national surveillance programs (Coker et al., 2002).

Worldwide, poultry products are a common and main source of *Campylobacter* infection (Humphrey et al., 2007; Jorgensen et al., 2002). A world literature survey (Suzuki and Yamamoto, 2009) showed that about 58% of chickens are contaminated by *Campylobacter*. This figure is estimated at 76% in the EU (EFSA, 2010).

Antibiotic resistance among bacterial pathogens is a worldwide concern (EFSA, 2011; Hawkey and Jones, 2009), and has been linked to antibiotic usage in both the clinical setting and in animal husbandry (Wright, 2010). Human *Campylobacter* infection is treated when necessary, with macrolides or fluoroquinolones (Coker et al., 2002), but the use of these drugs in poultry production is contributing to an increasing prevalence of resistance (Alfredson and Korolik, 2007). Ciprofloxacin resistance rates above 50% have been reported in *Campylobacter* isolates from poultry products worldwide (Cokal et al., 2009; de Jong et al., 2009; EFSA, 2011; Habib et al., 2009; Oporto et al., 2009).

The quantitative risk assessment approach is recommended by international organizations such as the World Trade Organization (WTO), the World Health Organization (WHO), the Food and Agricultural Organization of the United Nations (FAO) and the *Codex Alimentarius* (Codex Alimentarius Commission, 2003), as a tool to support risk management and to harmonize international trade in foods (Nauta et al., 2009). Quantitative exposure assessment is generally based on mathematical models simulating changes in the frequency and level of contamination of a given food item, from a given starting point up to consumption (Nauta et al., 2009). Enumeration data has been highlighted as a major hindrance to meaningful risk assessment in developing countries, notably in Africa and Asia (FAO/WHO, 2001, 2002).

The present study was designed to determine the frequency and quantification of *Campylobacter* contamination of broilers, and the antibiotic susceptibility of isolates, at five cities covered by the Food and Environment Study Group of the International Network of Pasteur Institutes (INPI).

2. Materials and methods

2.1. Sample collection

This study was conducted between late 2005 and 2006 in Africa (Dakar in Senegal, Yaounde in Cameroon), Oceania (Noumea in New Caledonia), the Indian Ocean (Antananarivo in Madagascar) and Asia (Ho Chi Minh City [HCMC] in Vietnam). Senegal is a West African country with a Saharan climate. Dakar is its capital city, with 2 million inhabitants. It is a harbor located on the Atlantic Ocean coast. Yaounde, the city with 7 hills, is the capital city of Cameroon in Central Africa. It is a large urban area with 2 million inhabitants. Its climate is tropical. New Caledonia is a French territory located in the Pacific Ocean, North-East of Australia, with 300 000 inhabitants. Its capital city is Noumea. Antananarivo at an altitude of 1500 m with 2 million inhabitants is the capital of Madagascar in the Indian Ocean, separated from Africa by the Mozambican channel. Ho Chi Minh, on the Saigon River is the biggest city in Vietnam, with 7.3 million inhabitants and plays a role of economical leadership in the country.

In each city, points of direct slaughter by the seller and semi-industrial abattoirs were identified. A random sample of these points of slaughter was selected and their chicken production estimated. The number of chickens sampled per point of slaughter was proportional to the level of production, leading to a self-weighted sampling. Three chickens per week were studied in each of these cities over a one-year period until one hundred and fifty chickens were sampled in final. The points of slaughter were classified as "semi-industrial abattoirs" or "direct slaughter by the seller". Semi-industrial abattoirs were characterized by an automated production chain (slaughter, scalding, plucking, evisceration) with manual carving. Direct slaughter by the seller consisted in an all-in-one manual process carried out at the sell-off in markets. A questionnaire was conducted about the environmental conditions of slaughter.

Slaughtered chickens were transported in less than 3 h to the laboratory under less than 8 °C cooled atmosphere.

2.2. Laboratory investigations

Campylobacter detection and enumeration were carried out with 10 g of neck-skin from each broiler carcass, according to International Standard Organization (ISO) norms (6887–1, 1999; 6887–2, 2004; 10272–1, 2006; 10272–2, 2006). *Campylobacter* was detected after an enrichment step in Preston broth at 37 °C for 4 to 6 h followed by 44 to 48 h at 41.5 °C in microaerobic conditions. Karmali and charcoal-cefoperazone-desoxycholate-agar (CCDA, Oxoid) solid media were streaked with the incubated broth and the plates were then incubated at 41.5 °C for 44 to 48 h in microaerobic conditions. *Campylobacter* enumeration was done in all samples. Briefly, 0.1 ml of 10^{-1} and 10^{-2} neck-skin grind dilutions were streaked on CCDA agar, without an enrichment step and incubated for 44 to 48 h at 41.5 °C. Presumptive *Campylobacter* colonies (between 1 and 5, as specified in the ISO standard) obtained through both methods (detection and enumeration) were subcultured on blood agar and identified by microscopic examination, Gram staining, and biochemical tests (oxidase and catalase production, sensitivity to nalidixic acid and cephalothin, hippurate hydrolysis, and growth at 25 °C). *Campylobacter* susceptibility testing was done by disc diffusion method on colonies recovered after enrichment step and followed the recommendations of the French *Comité de l'Antibiogramme de la Société de Microbiologie* (CASFM). Six antimicrobials disks (Biorad laboratories) were tested: ampicillin (10 μg), cephalothin (30 μg), gentamicin (15 μg), erythromycin (15 UI), nalidixic acid (30 μg) and ciprofloxacin (5 μg). *Escherichia coli* ATCC 25922 was used for susceptibility testing quality control.

2.3. Statistical analysis

On a given sample, distinct isolates were defined as isolates belonging to different species or with different antibiotic susceptibilities. Data

were collected in a specifically designed Microsoft Access database (Microsoft Corp, Redmond, USA) and were analyzed with Microsoft Excel (Microsoft Corp, Redmond, USA) and R software (v.2.11.1, The R Core Team, Vienna). Proportions were compared using Fisher's exact test, and CFU data were compared by using the non parametric Kruskal–Wallis test.

3. Results

3.1. Points of slaughter

Slaughtered chickens were collected from 82 points of slaughter, 77 (93.9%) of which were classified as "direct slaughter by the seller", and 5 (6.1%) as "Semi-industrial abattoirs" (present in Noumea, HCMC and Antananarivo only). Amongst the 750 chickens sampled, 455 (60.7%) were slaughtered by the seller, and 295 (39.3%) in semi-industrial abattoirs.

Two cities, Dakar and Yaounde, had predominant direct slaughter by the seller, 95% and 93%, respectively. Noumea was almost exclusively using semi-industrial abattoirs (97%). The two other cities showed more balanced types of points of slaughter, 60% direct slaughter by the seller in HCMC and 59% semi-industrial abattoirs in Antananarivo.

3.2. Prevalence rates

The prevalence of *Campylobacter* carriage was 65.5% (491/750) overall, and ranged from 15.3% in HCMC to 96.7% in Noumea (Table 1). A total of 633 distinct isolates were recovered from the 491 positive chickens. *C. jejuni* (56.6% of positive chickens and 48.3% of all the isolates) was slightly more frequent than *C. coli* (44.6% of positive chickens and 37.3% of all the isolates) (Table 1). *C. coli* was predominant only in Yaounde (69.5%). As expected, *C. lari* was far less prevalent (4.3% of chickens) as was *C. upsaliensis* (1.2% of chickens). These latter two species were found at only two of the five cities (HCMC and Antananarivo). Surprisingly, *C. lari* was the main species identified in HCMC (76.1% of isolates). Two different species were found on 18.5% (66/357) of positive chickens from which multiple colonies (up to 5) underwent species identification, and at least two distinct isolates were detected on 20.7% (74/357) of the same chickens.

3.3. Enumeration

Campylobacter was never detected by the enumeration method when the detection method was negative. The enumeration method was positive (≥1 confirmed colony on the two enumeration plates) for 43.9% of chickens (329/750) overall, and for 67% of the 491 chickens positive in the detection method. The mean of the $\log_{10}$ transformed number of *Campylobacter* colony-forming units (CFU) per gram of neck-skin, was 3.2 for samples positive in enumeration. All the cities had close mean values except HCMC. The arithmetic mean on positive samples was 7864 CFU/g. Most positive chickens had values of 2–4 $\log_{10}$ CFU/g (79%, 260/329), while 14% (46/329) were above 4 $\log_{10}$ CFU/g (Table 2). Thirty out of the 46 chickens (65.2%) with more than 4 $\log_{10}$ CFU/g were from Yaounde. Yaounde was the city where the mean $\log_{10}$ CFU/g of *Campylobacter* contamination was the highest (3.5) but high rates of maximum $\log_{10}$ *Campylobacter* enumeration were also detected in Noumea (5.2) and Antananarivo (5.4) (Table 2). Both the prevalence and level of *Campylobacter* contamination were low in HCMC (mean 1.81 $\log_{10}$ CFU/g for positive samples).

When all the cities were considered together, counts were higher after direct slaughter by the seller than after slaughter in semi-industrial abattoirs (Kruskal–Wallis test, $p = 0.006$). Considering the cities individually, however, the difference was never significant.

3.4. Antibiotic susceptibility

Five hundred and forty six (86.3%%) of the 633 isolates were tested for antimicrobial susceptibility, comprising 290 *C. jejuni* (53.1%), 229 *C. coli* (41.9%), 21 *C. lari* (3.9%) and 6 *C. upsaliensis* (1.1%) (Table 3). Overall, 11% of tested isolates were resistant to erythromycin, 19% to ampicillin and 52.1% to all quinolones tested.

Marked differences in antimicrobial resistance were observed across the cities. HCMC was the city with the highest rate of resistant isolates ($p = 0.014$), with 25% of isolates resistant to erythromycin, 40% to ampicillin and 95% to quinolones. Ciprofloxacin resistance was most

Table 1
Distribution of 750 *Campylobacter*-positive chickens and 633 isolates on chicken neck-skin according to the city: Dakar (Senegal), Yaounde (Cameroon), Antananarivo (Madagascar), Noumea (New Caledonia) and Ho-Chi-Minh City (HCMC, Vietnam).

	Dakar	Yaounde	Antananarivo	Noumea	HCMC	Total (95% CI)
Chickens						
Total	49% (73/149)	92.7% (139 /150)	72.7% (109/150)	96.7% (147/151)	15.3% (23/150)	65.5% (61.9–68.9) (491/750)
C. jejuni	64.4% (47/73)	30.9% (43/139)	74.3% (81/109)	72.1% (106/147)	4.3% (1/23)	56.6% (52.1–61.1) (278/491)
C. coli	57.5% (42/73)	83.5% (116/ 139)	16.5% (18/109)	27.9% (41/147)	8.7% (2/23)	44.6% (40.2–49.1) (219/491)
C. lari	0% (0/73)	0% (0/139)	3.7% (4/109)	0% (0/147)	73.9% (17/23)	4.3% (2.7–6.5) (21/491)
C. upsaliensis	0% (0/73)	0% (0/139)	5.5% (6/109)	0% (0/147)	0% (0/23)	1.2% (0.45–2.6) (6/491)
C. spp.	0% (0/73)	0% (0/139)	0% (0/109)	0% (0/147)	13% (3/23)	0.6% (0.13–1.8) (3/491)
Isolates						
C. jejuni	54.3% (57/105)	30.5% (53/174)	74.3% (81/109)	72.5% (111/153)	4.3% (4/92)	48.3% (44.4–52.3) (306/633)
C. coli	45.7% (48/105)	69.5% (121/ 174)	16.5% (18/109)	27.5% (42/153)	7.6% (7/92)	37.3% (33.5–41.2) (236/633)
C. lari	0% (0/105)	0% (0/174)	3.7% (4/109)	0% (0/153)	76.1% (70/92)	11.7% (9.3–14.5) (74/633)
C. upsaliensis	0% (0/105)	0% (0/174)	5.5% (6/109)	0% (0/153)	0% (0/92)	1% (0.35–2.05) (6/633)
C. spp.	0% (0/105)	0% (0/174)	0% (0/109)	0% (0/153)	10.9% (11/92)	1.7% (0.87–3.09) (11/633)

Table 2
Summary of *Campylobacter* enumeration data obtained from chicken neck-skin collected in Dakar (Senegal), Yaounde (Cameroon), Antananarivo (Madagascar), Noumea (New Caledonia) and Ho-Chi-Minh City (HCMC, Vietnam).

	Dakar	Yaounde	Antananarivo	Noumea	HCMC
Negative detection	76	11	41	4	127
Positive detection and negative enumeration	53	7	89	13	0
Positive enumeration/ total number of chickens	20/149 (13.3%)	132/150 (88.0%)	20/150 (13.3%)	134/151 (89.3%)	23/150 (15.4%)
[0–1) $\log_{10}$ CFU/g	0	0	0	0	0
[1–2) $\log_{10}$ CFU/g	0	0	0	3	15
[2–3) $\log_{10}$ CFU/g	4	32	10	54	8
[3–4) $\log_{10}$ CFU/g	15	70	4	68	0
[4–5) $\log_{10}$ CFU/g	1	28	4	8	0
[5–6) $\log_{10}$ CFU/g	0	2	2	1	0
Quantiles (samples with positive detection only) ($\log_{10}$ CFU/g)					
25th percentile	$-\infty$	2.90	$-\infty$	2.54	1.70
Median	$-\infty$	3.45	$-\infty$	3.00	1.70
75th percentile	2.90	3.91	$-\infty$	3.44	2.00
Maximum	4.58	5.18	5.38	5.18	2.18
Mean $\log_{10}$ (CFU/g) (samples with positive enumeration only)	3.36	3.46	3.35	3.06	1.81

frequent at Yaounde (83.5%) and least frequent at Antananarivo (5.5%). Ciprofloxacin was more frequent in *C. coli* (60.7%) than in *C. jejuni* (39%) ($p<0.0001$), while resistance rates to the other groups of antibiotic were not significantly different between these two species (Table 3). *C. lari* isolates were resistant to erythromycin, ampicillin and quinolones in 28.6%, 47.6% and 100% of cases, respectively. *C. upsaliensis* was mainly resistant to ampicillin (83.3%) and erythromycin (100%). Overall, among the 546 isolates tested, 33% (180/546) were susceptible to all the antibiotics tested, 47.2% (258/546) resistant to one group of antibiotics, 17.4% (95/546) to two groups, and 2.4% (13/546) to three groups. Considering the 329 positive chickens in *Campylobacter* enumeration, and assuming independence between the level of bacteria and the antibiotic resistance, the mean number of resistant isolates to at least 2 different antibiotic families (19.8%), may be estimated *ca.* 1500 CFU/g. The corresponding mean of the $\log_{10}$ would be estimated 2.5 $\log_{10}$ cfu/g.

4. Discussion

Campylobacter contamination rates vary widely among countries. In a study of 26 European Union member states, the chicken carcass contamination rate was 75.8% on average, but ranged from 4.9% to 100% (EFSA, 2010). Similarly, a literature survey (Suzuki and Yamamoto, 2009) referencing 73 publications worldwide on retail poultry meat showed a mean contamination rate of 58% and a range of 8% to 100%. Our results are compatible with the literature, showing a mean contamination rate of 65.5% and city-to-city variations from 15.3% to 96.7%. In Senegal, a study conducted in 2000–2001 on fresh droppings of broiler flocks in farms showed a *Campylobacter* prevalence of 63% (Cardinale et al., 2004), a rate slightly higher than what we found in Dakar (49%). In Vietnam, in 2004, 28.3% of raw poultry and 31% of breast fillets were found to be contaminated with *Campylobacter* (Ha and Pham, 2006; Luu et al., 2006), compared to 15% for neck-skin in our study. In Cambodia, a neighboring country, 80.9% of chicken neck-skin samples were reported to be *Campylobacter*-positive at slaughter (Kruy et al., 2011). In our study, automated slaughter processing lines (semi-industrial abattoirs) did not guarantee a lower prevalence of *Campylobacter* detection than manual slaughter (direct slaughter by the seller) even though contamination level was globally lower ($p=0.006$). For instance, while the production chain is mainly automated in Noumea and the whole process is manual in Yaounde, we found similar neck-skin contamination rates (96.7% in Noumea and 92.7% in Yaounde). The high prevalence rates in automated settings could be explained by contamination of the production line by *Campylobacter*-positive broiler flocks (Figueroa et al., 2009; Johannessen et al., 2007).

Campylobacter detection at the species level is more questionable for *C. lari* and *C. upsaliensis* than for the two usual species *C. jejuni* and *C. coli*. Selective media and incubation temperatures are more efficient for isolating the two latter species, but PCR techniques have improved species identification (Tazumi et al., 2009). The species distribution found in this study was in keeping with previous data, *C. jejuni* being predominant (56.6%), followed by *C. coli* (44.6%) and other *Campylobacter* species (6.1%) (EFSA, 2010; Jorgensen et al., 2002). Interestingly, *C. coli* was three times as frequent as *C. jejuni* in Yaounde, as is the case in some European countries (Ireland, Italy and Spain) (EFSA, 2010). *C. upsaliensis* is not frequent poultry contaminant, as its main reservoir is pets (Damborg et al., 2008), as well as *C. lari* which reservoir is however much wider, wild birds, geese, ducks, sheep, pigs, dogs, cats and water (Matsuda and Moore, 2011). Surprisingly, despite the absence of all these animal species around the points of slaughter in HCMC, *C. lari* was the prevalent species (73.9%). In Phnom Penh (Cambodia), 21% of isolates from neck-skin samples were *C. lari* (Kruy et al., 2011). In the 2010 EFSA report (EFSA, 2010), *C. lari* accounted for 0.2% of batches with species identification underlining the specific situation seen in HCMC. In Vietnam, previous studies on children bacterial diarrhea (Hien et al., 2007; Isenbarger et al., 2001) did not observe *C. lari* as a predominant pathogen even though in Hanoi, 29% of *Campylobacter* isolates on chicken meat were of "unknown" species (Luu et al., 2006). These findings would have to be confirmed by further studies and potential risk factors investigated. In Antananarivo, *C. upsaliensis* was isolated from 5.5% of samples. From data collected by questioning, the presence of dogs was recorded at 37% (10/27) of the slaughter sites, raising again questions as to their involvement in poultry contamination (Westgarth et al., 2009). We observed simultaneous carriage of

Table 3
Antimicrobial susceptibility (4/6 antibiotics) of 546 *Campylobacter* isolates collected from chicken neck-skin by cities and by species.

	Ampicillin (10 μg)	Erythromycin (15UI)	Nalidixic acid (30 μg)	Ciprofloxacin (5 μg)
Cities				
Dakar	30.5% (32/105)	1.9% (2/105)	41.9% (44/105)	39% (41/105)
Yaounde	9/8% (16/164)	1.8% (3/164)	89% (146/164)	83.5% (137/164)
Antananarivo	35.8% (39/109)	18.3% (20/109)	3.7% (4/109)	5.5% (6/109)
Noumea	6.1% (9/148)	20.3% (30/148)	45.3% (67/148)	47.3% (70/148)
HCMC	40% (8/20)	25% (5/20)	95% (19/20)	95% (19/20)
Species				
C. jejuni	17.9% (52/290)	9% (26/290)	40% (116/290)	39% (113/290)
C. coli	16.2% (37/229)	9.6% (22/229)	62.4% (143/229)	60.7% (139/229)
C. lari	47.6% (10/21)	28.6% (6/21)	100% (21/21)	100% (21/21)
C. upsaliensis	83.3% (5/6)	100% (6/6)	0% (0/6)	0% (0/6)
Overall	19% (104/546)	11% (60/546)	51.3% (280/546)	50% (273/546)

two different species more frequently than in other studies (Jorgensen et al., 2002; Manfreda et al., 2006). This may have implications for the estimated species distribution if speciation is based on a single colony. In particular, co-contamination by *C. jejuni* and *C. coli* may be more frequent than generally reported, and our findings suggest that more than one colony should be used for speciation.

We found that a smaller number of chickens were positive by enumeration than by simple detection (43.9% *vs* 65.5%). This difference was due to a level of *Campylobacter* contamination below the detection limit of the method used. However, it is difficult to compare results across studies, owing to the use of different materials and enumeration methods (Scherer et al., 2006). In the 2010 EFSA report (EFSA, 2010), *Campylobacter* counts between 2 and 6 $\log_{10}$ CFU/g were less frequent on broiler neck-skins (40.9%) than in our study (79%, 260/329). Therefore, compared to a similar study conducted in Phnom Penh in Cambodia, (Kruy et al., 2011), where 54.6% of chickens had C*ampylobacter* counts between 4 and 6 $\log_{10}$ CFU/g, the lower proportion of samples with this range of contamination in our study (14%, 46/329), suggests a lower probability of cross-contamination. The mean of the $\log_{10}$-transformed data obtained in our study (3.2 $\log_{10}$ CFU/g), was between that obtained in a study of raw retail chicken leg-skins in Berlin (2.6 $\log_{10}$ CFU/g) (Scherer et al., 2006), and the one observed in a study conducted in England between 1998 and 2000 on neck-skin of raw chickens from retail outlets (4.1 $\log_{10}$ CFU/g) (Jorgensen et al., 2002). The type of slaughter point did not significantly impact the prevalence of contamination at a given city in our study but influenced the abundance of *Campylobacter* contamination. Semi-industrial abattoirs were associated with contamination rates similar to direct slaughter by the seller, but CFU counts were lower. This could be due to contamination of chickens previously free of *Campylobacter* on the production line (Figueroa et al., 2009; Johannessen et al., 2007).

Antimicrobial resistance rates also varied according to the city. High levels of ciprofloxacin resistance were observed (39% to 95%), except in Antananarivo (5.5%), with an overall mean of 50%. Ciprofloxacin resistance has already been reported worldwide, especially in poultry flocks in the Basque region of Spain (57.9%) (Oporto et al., 2009), chicken meat in Belgium (53.1%) (Habib et al., 2009), chicken intestinal samples collected in the EU (*C. jejuni* in 36.6% and *C. coli* in 60.2%) (de Jong et al., 2009) and cecal samples from broiler chickens in Turkey (74.2% of *C. jejuni* and 65.5% of *C. coli* were ciprofloxacin-resistant) (Cokal et al., 2009). In Phnom-Penh (Cambodia), resistance to ciprofloxacin was 25.9% (Kruy et al., 2011). In Senegal, our results are similar to those published in 2003, with 34% of ciprofloxacin-resistant *C. jejuni* and *C. coli* isolates (Cardinale et al., 2003). In this study, *C. coli* was more resistant to ciprofloxacin than *C. jejuni* (60.7% *vs* 39%, $p<0.0001$) and this has been previously reported (EFSA, 2011; Gallay et al., 2007). But, as generally observed (ECDC, 2008; Luangtongkum et al., 2009), *C. coli* was not more frequently resistant to erythromycin or ampicillin than *C. jejuni*. *C. upsaliensis* was more resistant to antibiotics than the other *Campylobacter* species isolated in Antananarivo, except for quinolones and this contrasts with the low level of antibiotic resistance found in Antananarivo. *C. lari* was the species most frequently resistant to ciprofloxacin in our study (100%), while a rate of 63.3% has been reported in Phnom Penh (Kruy et al., 2011).

Considering the 329 chickens positive in enumeration test and the percentage of isolates resistant to more than two different antibiotic families (19.8%), assuming an independence between the level of bacteria and the antibiotic resistance , it results a *ca.* 1500 CFU/g average of resistant isolates born by these chickens. Very few articles on quantitative food contamination have mentioned such data and thereby, it is difficult to compare our findings across other studies (Jorgensen et al., 2002). However, antimicrobial resistances among zoonotic bacteria is considered by food safety authorities as of major concern and national surveillance systems aimed at collecting these data, have been recently implemented in developed countries (EFSA, 2011; Lahuerta et al., 2011).

In developing countries, as chickens are sold to consumers directly after slaughter, contamination will have direct implications for cross-contamination in domestic kitchens. Food-handling practices in private kitchens should be further studied in order to better understand mishandling and cross-contamination behaviors. Efforts to reduce *Campylobacter* contamination and to improve food hygiene must focus on farms, slaughterhouses, points of direct slaughter, and private kitchens.

Acknowledgments

We thank David Young for the editorial assistance.

This work was supported by a project fund (ACIP) from the Paris Pasteur Institute.

References

6887–1, 1999. Microbiologie des aliments - Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique - Partie 1: règles générales pour la préparation de la suspension mère et des dilutions décimales. AFNOR V08-010-1.

6887–2, 2004. Microbiologie des aliments - Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique - Partie 2 : règles spécifiques pour la préparation des viandes et produits à base de viande. AFNOR V08-010-2.

10272–1, 2006. Microbiology of Food and Animal Feeding Stuffs —Horizontal Method for Detection and Enumeration of *Campylobacter* spp. Part 1: Detection Method. AFNOR V 08-026-1.

10272–2, 2006. Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for Detection and Numeration of *Campylobacter* spp. Part 2: Colony-count Technique. AFNOR V 08-026-2.

Alfredson, D.A., Korolik, V., 2007. Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli*. FEMS Microbiology Letters 277, 123–132.

Cardinale, E., Dromigny, J.A., Tall, F., Ndiaye, M., Konte, M., Perrier-Gros-Claude, J.D., 2003. Fluoroquinolone susceptibility of *Campylobacter* strains, Senegal. Emerging Infectious Diseases 9, 1479–1481.

Cardinale, E., Tall, F., Gueye, E.F., Cisse, M., Salvat, G., 2004. Risk factors for *Campylobacter* spp. infection in Senegalese broiler-chicken flocks. Preventive Veterinary Medicine 64, 15–25.

Codex Alimentarius Commission, 2003. Principles and guidelines for the conduct of microbiological risk assessment. Codex Alimentarius Commission, Roma.

Cokal, Y., Caner, V., Sen, A., Cetin, C., Karagenc, N., 2009. *Campylobacter* spp. and their antimicrobial resistance patterns in poultry: an epidemiological survey study in Turkey. Zoonoses and Public Health 56, 105–110.

Coker, A.O., Isokpehi, R.D., Thomas, B.N., Amisu, K.O., Obi, C.L., 2002. Human campylobacteriosis in developing countries. Emerging Infectious Diseases 8, 237–244.

Damborg, P., Guardabassi, L., Pedersen, K., Kokotovic, B., 2008. Comparative analysis of human and canine *Campylobacter upsaliensis* isolates by amplified fragment length polymorphism. Journal of Clinical Microbiology 46, 1504–1506.

de Jong, A., Bywater, R., Butty, P., Deroover, E., Godinho, K., Klein, U., Marion, H., Simjee, S., Smets, K., Thomas, V., Valle, M., Wheadon, A., 2009. A pan-European survey of antimicrobial susceptibility towards human-use antimicrobial drugs among zoonotic and commensal enteric bacteria isolated from healthy food-producing animals. Journal of Antimicrobial Chemothererapy 63, 733–744.

ECDC, 2008. Food-and Waterborne diseases and zoonoses surveillance network. http://ecdc.europa.eu/en/publications/surveillance_reports/annual_epidemiological_report/Pages/2008_epi_report.aspx. 2011/03/04.

ECDC, 2009. Annual epidemiological report on communicable diseases in Europe. http://www.ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=580.

EFSA, 2010. Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008, Part A: *Campylobacter* and *Salmonella* prevalence estimates. http://www.efsa.europa.eu/en/efsajournal/pub/2017.htm.

EFSA, 2011. The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2009. EFSA Journal 9 (7), 2154, http://dx.doi.org/10.2903/j.efsa.2011.2154 (321 pp.).

FAO/WHO, 2001. Hazard identification, exposure assessment and hazard characterization of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood, a joint FAO/WHO expert consultation. FAO/WHO, Geneva, Switzerland. 23–27 July 2001.

FAO/WHO, 2002. Risk assessment of *Salmonella* in eggs and broiler chickens - Technical report. Food and Agriculture Organization of the United Nations and World Health Organization, Rome, p. 301.

Figueroa, G., Troncoso, M., Lopez, C., Rivas, P., Toro, M., 2009. Occurrence and enumeration of *Campylobacter* spp. during the processing of Chilean broilers. BMC Microbiology 9, 94.

Foodnet, 2010. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food — 10 states, 2009. MMWR. Morbidity and Mortality Weekly Report 59 (14), 418–422 (April 16).

Friedman, C.R., Hoekstra, R.M., Samuel, M., Marcus, R., Bender, J., Shiferaw, B., Reddy, S., Ahuja, S.D., Helfrick, D.L., Hardnett, F., Carter, M., Anderson, B., Tauxe, R.V., 2004. Risk factors for sporadic *Campylobacter* infection in the United States: a case–control study in FoodNet sites. Clinical Infectious Diseases 38 (Suppl. 3), S285–S296.

Gallay, A., Prouzet-Mauleon, V., Kempf, I., Lehours, P., Labadi, L., Camou, C., Denis, M., de Valk, H., Desenclos, J.C., Megraud, F., 2007. *Campylobacter* antimicrobial drug resistance among humans, broiler chickens, and pigs, France. Emerging Infectious Diseases 13, 259–266.

Gorman, R., Bloomfield, S., Adley, C.C., 2002. A study of cross-contamination of foodborne pathogens in the domestic kitchen in the Republic of Ireland. International Journal of Food Microbiology 76, 143–150.

Ha, T.A., Pham, T.Y., 2006. Study of *Salmonella*, *Campylobacter*, and *Escherichia coli* contamination in raw food available in factories, schools, and hospital canteens in Hanoi, Vietnam. Annals of the New York Academy of Sciences 1081, 262–265.

Habib, I., Miller, W.G., Uyttendaele, M., Houf, K., De Zutter, L., 2009. Clonal population structure and antimicrobial resistance of *Campylobacter jejuni* in chicken meat from Belgium. Applied and Environmental Microbiology 75, 4264–4272.

Hawkey, P.M., Jones, A.M., 2009. The changing epidemiology of resistance. Journal of Antimicrobial Chemotherapy 64 (Suppl. 1), i3–i10.

Hien, B.T., Trang do, T., Scheutz, F., Cam, P.D., Molbak, K., Dalsgaard, A., 2007. Diarrhoeagenic *Escherichia coli* and other causes of childhood diarrhoea: a case–control study in children living in a wastewater-use area in Hanoi, Vietnam. Journal of Medical Microbiology 56, 1086–1096.

Humphrey, T., O'Brien, S., Madsen, M., 2007. *Campylobacters* as zoonotic pathogens: a food production perspective. International Journal of Food Microbiology 117, 237–257.

Isenbarger, D.W., Hien, B.T., Ha, H.T., Ha, T.T., Bodhidatta, L., Pang, L.W., Cam, P.D., 2001. Prospective study of the incidence of diarrhoea and prevalence of bacterial pathogens in a cohort of Vietnamese children along the Red River. Epidemiology and Infection 127, 229–236.

Johannessen, G.S., Johnsen, G., Okland, M., Cudjoe, K.S., Hofshagen, M., 2007. Enumeration of thermotolerant *Campylobacter* spp. from poultry carcasses at the end of the slaughter-line. Letters in Applied Microbiology 44, 92–97.

Jorgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D.R., Bolton, F.J., Frost, J.A., Ward, L., Humphrey, T.J., 2002. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. International Journal of Food Microbiology 76, 151–164.

Kruy, S.L., Yith, V., Ping, S., Khem, P., Sarthou, J.L., 2011. Prevalence, numbers and antimicrobial susceptibilities of *Salmonella* serovars and *Campylobacter* spp. in retail poultry in Phnom Penh, Cambodia. The Journal of Veterinary Medical Science 73 (3), 325–329 (Mar).

Lahuerta, A., Westrell, T., Takkinen, J., Boelaert, F., Rizzi, V., Helwigh, B., Borck, B., Korsgaard, H., Ammon, A., Makela, P., 2011. Zoonoses in the European Union: origin, distribution and dynamics — the EFSA-ECDC summary report 2009. Euro Surveillance 16.

Lastovica, A.J., Le Roux, E., 2003. Prevalence and optimal detection of *C. upsaliensis* in stool specimens. Clinical Infectious Diseases 1624–1625 (author reply 1625).

Lecuit, M., Suarez, F., Lortholary, O., 2004. Immunoproliferative small intestinal disease associated with *Campylobacter jejuni*. Medecine Sciences (Paris) 20, 638–640.

Lindblom, G.B., Ahren, C., Changalucha, J., Gabone, R., Kaijser, B., Nilsson, L.A., Sjogren, E., Svennerholm, A.M., Temu, M., 1995. *Campylobacter jejuni/coli* and enterotoxigenic *Escherichia coli* (ETEC) in faeces from children and adults in Tanzania. Scandinavian Journal of Infectious Diseases 27, 589–593.

Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C.M., Zhang, Q., 2009. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiology 4, 189–200.

Luu, Q.H., Tran, T.H., Phung, D.C., Nguyen, T.B., 2006. Study on the prevalence of *Campylobacter* spp. from chicken meat in Hanoi, Vietnam. Annals of the New York Academy of Sciences 1081, 273–275.

Manfreda, G., De Cesare, A., Bondioli, V., Stern, N.J., Franchini, A., 2006. Enumeration and identity of *Campylobacter* spp. in Italian broilers. Poultry Science 85, 556–562.

Marshall, J.K., 2009. Post-infectious irritable bowel syndrome following water contamination. Kidney International Supply S42–S43.

Matsuda, M., Moore, J.E., 2011. The epidemiology and zoonotic transmission of thermophilic *Campylobacter lari*. British Microbiology Research Journal 1, 104–121.

McGrogan, A., Madle, G.C., Seaman, H.E., de Vries, C.S., 2009. The epidemiology of Guillain-Barre syndrome worldwide. A systematic literature review. Neuroepidemiology 32, 150–163.

Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., van der Logt, P., Fazil, A., Christensen, B., Katsma, E., Borck, B., Havelaar, A., 2009. A comparison of risk assessments on *Campylobacter* in broiler meat. International Journal of Food Microbiology 129, 107–123.

Oporto, B., Juste, R.A., Hurtado, A., 2009. Phenotypic and genotypic antimicrobial resistance profiles of *Campylobacter jejuni* isolated from cattle, sheep, and free-range poultry faeces. International Journal of Microbiology 2009, 456573.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States–major pathogens. Emerging Infectious Diseases 17, 7–15.

Scherer, K., Bartelt, E., Sommerfeld, C., Hildebrandt, G., 2006. Comparison of different sampling techniques and enumeration methods for the isolation and quantification of *Campylobacter* spp. in raw retail chicken legs. International Journal of Food Microbiology 108, 115–119.

Suzuki, H., Yamamoto, S., 2009. *Campylobacter* contamination in retail poultry meats and by-products in the world: a literature survey. Journal of Veterinary Medicine and Science 71, 255–261.

Tazumi, A., Kakinuma, Y., Misawa, N., Moore, J.E., Millar, B.C., Matsuda, M., 2009. Identification and characterization of intervening sequences within 23S rRNA genes from more than 200 *Campylobacter* isolates from seven species including atypical *Campylobacters*. BMC Microbiology 9, 256.

Westgarth, C., Porter, C.J., Nicolson, L., Birtles, R.J., Williams, N.J., Hart, C.A., Pinchbeck, G.L., Gaskell, R.M., Christley, R.M., Dawson, S., 2009. Risk factors for the carriage of *Campylobacter upsaliensis* by dogs in a community in Cheshire. Veterinary Records 165, 526–530.

Wright, G.D., 2010. Antibiotic resistance in the environment: a link to the clinic? Current Opinion in Microbiology 13, 589–594.