



CONSUMERS EXPOSURE TO *CAMPYLOBACTER* SPP. RESISTANT TO B-LACTAMS IN CHICKEN GIZZARDS SOLD ON INFORMAL MARKETS IN NORTHERN CÔTE D'IVOIRE

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Abstract: *Chicken, particularly chicken gizzard, is part of the most consumed foods in the world. However, inappropriate use of antibiotics in the farms may induce a transfer of antimicrobial resistance Campylobacter to gizzard consumers. This study aimed to assess the gizzard quality and consumer exposure to antimicrobial resistance Campylobacter spp. A cross-sectional study was conducted from March to June 2023 to assess the level of contamination of chicken gizzard sold on the informal settings in Korhogo (North Cote d'Ivoire) with Campylobacter spp. Thus, one hundred forty-four samples of fresh (n=91), frozen (n=27) and grilled (n=26) chicken gizzards from chicken markets, informal fish shops and informal restaurants were collected. Additionally, the antibiotic resistance profile of Campylobacter spp. was determined and a household survey was conducted with a structured questionnaire in 384 households of the town of Korhogo to assess risk exposure associated with chicken gizzard consumption. Findings consistently showed that frozen gizzards (100%) are more contaminated by Campylobacter spp. compared to fresh gizzards (98.9%) and the fried gizzards (88.5%). Moreover, all the 42 isolates of Campylobacter spp. from gizzards were sensitive to Gentamicin and resistant to Penicillin and Ampicillin. About half of the consumers (46.6%) of fried gizzard present a potential risk to have campylobacteriosis because 88.5% of fried gizzards samples are contaminated by Campylobacter spp. Frozen, fresh and fried gizzards sold in informal settings in Korhogo are highly contaminated by Campylobacter spp. resistant to Beta-lactams. Fried gizzards consumers in Korhogo households are a potential risk to have Campylobacteriosis.*

Keywords: *Antibiotic, Campylobacteriosis, Côte d'Ivoire, Household, Korhogo, Poultry*

1. Introduction

Poultry meat was the most widely produced type of meat in the world with 40.6 percent of total meat production estimated at 337.3 million tons in 2020 [1]. Among poultry meats, chicken meat has higher rates of consumption globally [2]. Chicken meat

and other poultry meats provide essential macronutrients and micronutrients for physiological functioning and human health [3]. However, poultry and poultry meat are considered the most important sources of human diseases such as salmonellosis and campylobacteriosis [4]. Indeed, handling,

preparation, and consumption of poultry meat are the main ways of contracting campylobacteriosis [5]. The consumption of chicken livers contaminated by *Campylobacter* and *Salmonella* was associated with few recent outbreaks in Europe and the United States of America [6]. Campylobacteriosis with about 550 million cases worldwide each year [7], is a public health concern of global importance [8]. In Africa, the situation seems more worrying due to the lack of control and surveillance systems for campylobacteriosis [9]. Campylobacteriosis is a problem and an economic burden to human population worldwide [10]. The incidence rate of this disease remains high in both the developed and developing countries [5].

Campylobacteriosis is caused by species of *Campylobacter* genus belonging to the family of *Campylobacteraceae* [11]. According to WHO, *Campylobacter* contributes to significant health and economic losses and is the key causes of 1 in 4 diarrheal diseases globally [11]. Chickens are generally the natural reservoirs of *Campylobacter* spp. [12]. Contaminated chicken meat and its food products such as raw or undercooked broiler meat, are the most important single sources of *Campylobacter* infection in humans [13]. The prevalence of *Campylobacter* spp. in chickens and chicken meat ranges between 4.9% and 100% worldwide [14].

Chicken gizzard, a part of the digestive system of the chicken which makes up 2.5 percent of the chicken carcass [15], is consumed in several countries especially Asian countries [16]. The consumption of gizzard is beneficial for human health because it contains higher total monounsaturated fatty acid and lower total saturated fatty acid levels [17]. However,

the microbiological analysis of 7920 raw gizzards taken from slaughter sites in 11 municipalities of Abidjan administrative district, shows a portage rate of *Salmonella* sp. of 57 % [18]. Given that the gizzard is highly prized by consumers in Côte d'Ivoire, due to its high fat content, that makes it tasty with pleasant flavour [16], it is therefore important to know if the consumption of gizzards represents a risk of having campylobacteriosis for the Ivorian consumers. There is a paucity of information on *Campylobacter* spp. in Côte d'Ivoire due to difficulties related to the isolation and identification of this fastidious bacteria in general and in chicken gizzard in particular. Thus, studies investigating *Campylobacter* spp. in gizzards are very limited. In Côte d'Ivoire, the analysis of 120 samples of chicken cesspools collected on the market of Adjamé (Abidjan), showed a prevalence of *Campylobacter* spp. of 66.66% [19]. The study implemented on evolution of antibiotic resistance in *Campylobacter* sp. isolated from broilers in Abidjan showed an increase in antibiotic resistance level in *Campylobacter* sp. Indeed, a high rate of multiple drug resistance was detected in the strains tested in 2019 (74.41%) compared to those tested in 2010 (14.11%), [20].

The purpose of the study was to contribute in assessing the gizzard quality and consumer exposure to antimicrobial resistance *Campylobacter* spp. The aim of the current study was three-fold. Firstly, to assess the presence of *Campylobacter* spp. in chicken gizzard sold in informal urban settings of Korhogo in northern Côte d'Ivoire. Secondly, to determine the antibiotic resistance profile of *Campylobacter* spp., isolated from chicken gizzard. Finally, to assess the risk exposure considering the forms and frequency of

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chicken gizzard consumption in households of Korhogo according to sex and age group.

2. Materials and Methods

2.1. Study site

The study was conducted in Korhogo (9°27.4818" N; 5°37.7766" W) located in the Poro region in northern Côte d'Ivoire. The figure 1 shows the sampling households in urban neighbourhoods of Korhogo. Many breeders in the region

resort to traditional poultry farming, due to the reduction in grazing areas for domestic ruminants [21]. Family poultry farming is concentrated in northern part of the country, with on average more than 1000 poultry per village. The Savanes administrative District (North) with Korhogo as capital city provides 40% of traditional poultry farming in the country [22].

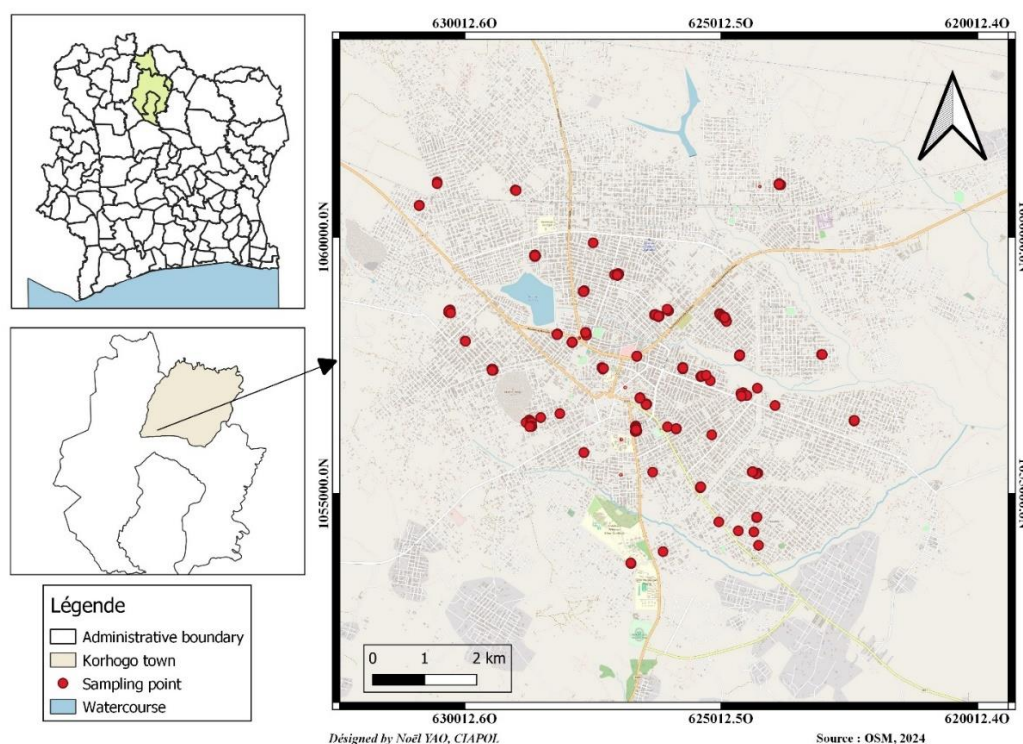


Figure 1. Sampled households within Korhogo town in the Poro region, North Cote d'Ivoire

2.2. Study design

A cross-sectional study was carried out from March to September 2023 to assess the level of contamination of chicken gizzard sold informally in Korhogo by *Campylobacter* spp. Additionally, the antibiotic resistance profile of *Campylobacter* spp. was determined, and a

household survey was conducted with a structured questionnaire in the town of Korhogo to assess the risk exposure associated with chicken gizzard consumption.

2.3. Chicken gizzards and household survey sample size determination

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The size of chicken gizzard samples was determined according to the following formula [23]:

$$n = \frac{1.96^2 * P_{exp} (1 - P_{exp})}{d^2} \quad (1)$$

With n , the sample size, P_{exp} , the expected percentage of chicken meat contaminated by *Campylobacter* spp. set at 90%, 95% confidence interval (CI) ($Z=1.96$) and d , the desired absolute precision (5%), [24].

In total, 144 samples of chicken gizzards were collected from market, informal fish shops and informal restaurants. Using the same formula with P_{exp} , the expected percentage of population of chicken gizzard consumers set at 50%, 384 persons from 384 households were included in the study after obtaining their informed consent.

2.4. Sampling procedure and household selection

A total of 384 households were distributed proportionally within the neighbourhoods of Korhogo. The sampling procedure took into consideration the number of households in each neighbourhood, based on data of the National Institute of Statistics from the 2014 general population census [25]. The pen method combined with the random numbering method [26] was used to select households. The pen method consists of standing at the central crossroads of the urban area under consideration, throwing a pen into the air and rotating it simultaneously. The direction on the ground indicated by the head of the pen corresponds to the urban axis to be investigated. If the direction of the pen is between two axes, the interviewer opts for the axis on the left of the pen head (the left being determined by the observer "behind the pen"). Once a direction has been chosen using the "pen" method, the operator takes a table of random numbers and, with eyes closed,

randomly points a pen at the table: the number indicated corresponds to the rank of the house from the crossroads from which the survey should be started. From that first house, the investigator will visit all the following houses until he/she leaves the investigation zone. In each household, a structured questionnaire was administered to household head (or representative) using a tablet equipped with Open Data Kit (ODK) software. The survey topics were around: (i) Socio-demographic characteristics (sex, age group, level of education and marital status) and (ii) chicken gizzard form and frequency of consumption. The age group was categorized in young ([15; 24]), adults ([25; 59]) and elders (≥ 60) according United Nations definition [27]. The minimum age at which an individual is deemed capable of criminal responsibility under Ivorian law is 13 years old [28]. We considered that at 15 years old, every young person is able to easily answer to questions on gizzard consumption within a household.

2.5. Exposure assessment

The exposure assessment is the estimation of the amount of a particular agent that reaches the target population, the route of exposure, the duration of exposure, its magnitude and frequency [29]. In the study, the risk exposure was assessed considering the forms and frequency of chicken gizzard consumption in Korhogo household according sex and age group.

2.6. Gizzard sampling

One hundred forty-four ($n=144$) fresh ($n=91$), frozen ($n=27$) and grilled ($n=26$) chicken gizzards from chicken market, informal fish shops and informal restaurants respectively were collected into sterile container (100 mL) (Biosigna, Italy) and

transported into a coolbox containing cold accumulators to the headquarter of the National Laboratory for Support to Agricultural Development (LANADA) in Korhogo. Chicken gizzards were collected using an empirical accidental sampling approach. This is a non-probabilistic method in which individuals are retained when encountered until the desired number of individuals is obtained. This method was chosen because of the difficulty to find chicken gizzards on market.

2.7. Research of *Campylobacter* spp. in chicken gizzards

For enrichment, ten (10) grams of shred chicken gizzard were transferred into 90 ml of Brain Heart Infusion Broth (BHIB) with added *Campylobacter* growth factor (SR 0232E Oxoid, OXOID LTD., Basingstoke, Hampshire, United Kingdom) and 7% (v/v) of lacquered blood sheep (frozen and defrosted abruptly). Incubation was carried out in an anaerobic jar containing microaerobic gas generator bags (5% oxygen, 10% carbon dioxide, 85% nitrogen) of the CAMPYGEN type (CN0025A Oxoid, Basingstoke, Hampshire, UK) for 24 hours at 37 °C [30, 31]. The isolation was done after enrichment by spreading 50 µL of the Brain Heart Infusion Broth (BHIB) with *Campylobacter* growth factor and the blood sheep on the surface of the Charcoal Cefoperozone Deoxycholate Agar (CCDA) with the supplement previously poured in a Petri dish. *Campylobacter* was isolated at 37 °C for 48 h under microaerophilic atmosphere. Isolates of *Campylobacter* spp. were confirmed by biochemical tests (oxidase test, catalase test and Gram type) [32].

2.7.1. Determination of Gram type with Potassium hydroxide (KOH)

The Gram type was determined by the potash test (3% aqueous KOH solution) instead of the usual Gram stain. A droplet of 3% KOH solution was placed on a clean slide. A portion of pure *Campylobacter* colony was collected with a sterile toothpick and then dipped into the KOH droplet. After 20 to 30 seconds of observation, the tip of the toothpick was slowly removed from the droplet for production of filamentous material [33, 34].

2.7.2. Search for catalase

Catalase activity of isolates *Campylobacter* was determined using hydrogen peroxide (H₂O₂). A drop of H₂O₂ was placed on a clean slide, in which a suspicious colony obtained on agar was emulsified. The presence of the catalase is manifested by the release of gas bubbles [33, 34].

2.7.3. Oxidase test

An oxidase disk impregnated with reagent (N-tetramethyl paraphenylene diamine oxalate) was placed on a slide, then soaked with a drop of sterile distilled water using a Pasteur pipette. An isolated colony of *Campylobacter* spp. was overwritten on a disk for one to ten seconds. The change of the disk from white color to purple indicates that the isolate is oxidase positive [35].

2.8. Antimicrobial resistance profiles of *Campylobacter* spp. isolates

Three (3) antibiotics belonging to two families (Beta-lactams and Aminocyclitol) were used for the determination of antibiotic resistance profiles of *Campylobacter* spp. isolates. The antibiotics were Penicillin (1µg, Beta-lactams), Ampicillin (30 µg, Beta-lactams) and Gentamicin (30 µg, Aminocyclitol). Prior

to inoculation, the inoculum was prepared from a 24 hour culture of a pure isolate obtained on nutrient agar. One or two colonies were emulsified in a tube containing 2 mL of 0.85% NaCl physiological water (BioMérieux, reference 08026E). The density of the mixture was then adjusted using a densimeter to obtain 0.5 Mac-Farland (108 CFU/mL).

Müller Hinton agar poured into Petri dishes was inoculated using the flooding technique. After a contact time of 5 minutes, the excess inoculum was removed with a micropipette. Using a swab, excess inoculum was removed by pressing on the edges of the dish, tilting it at approximately 60 °C. The dish was dried at 37 °C for 5 minutes [36].

The antibiotic discs were applied to the agar surface with forceps, taking care to space the discs 2 cm apart. Once applied, the antibiotic disc is not moved. The plates were incubated for 18 to 24 hours at 37 °C for all strains. Critical diameters and interpretive reading rules were done according the French Society of Microbiology [37].

2.9. Data analysis

Quantitative data was recorded and analysed using SPSS version 20 software. Chi-square (χ^2) or Fischer's exact test when appropriate was performed to compare the percentage of gizzard consumers considering the form and the frequency of consumption according to sex and age group. As well, a Chi-square (χ^2) or Fischer's exact test when appropriate was performed to compare prevalence gizzard contamination by *Campylobacter* spp. according to place of purchase and type of gizzard. An association was declared statistically significant when the p-value was less than 0.05.

3. Results and discussion

The purpose of the study was to contribute in assessing the gizzard quality and consumer exposure to antimicrobial resistance *Campylobacter* spp. This was the first study to focus on contamination of fresh, frozen and fried gizzards by *Campylobacter* spp. sold informally in Côte d'Ivoire.

The study showed that out of the 91 fresh gizzards sampled in the market, 90 (98.9%) were contaminated by *Campylobacter* spp. All the 27 frozen gizzards (100%) sampled in fish shops were contaminated by *Campylobacter* spp. Concerning fried gizzards, out of the 26 samples from informal restaurants, 23 (88.5%) were contaminated by *Campylobacter* spp. The frozen gizzard samples from fish shops are more contaminated by *Campylobacter* spp. compared to the fresh gizzards sampled in fish shops and the fried gizzards sampled in informal restaurants. The difference in the contamination of the gizzards by *Campylobacter* spp. regarding the type of gizzards and the place of purchase was statistically significant (Table 1).

Worldwide, there are very limited studies conducted on the prevalence of *Campylobacter* spp. in frozen chicken meat [38]. Generally, refrigeration and freezing are used to control bacterial growth in foods but the study rather shows that frozen gizzards are contaminated by *Campylobacter* spp. This result could be explained by the fact that a significant portion of *Campylobacter* spp. on gizzards survived during the freezing process. Indeed, the optimum growth temperature for thermophilic *Campylobacter* spp. is estimated to range between 37 °C (humans) -42 °C (chicken) but some strains even surviving for weeks at -20 °C on chicken

meat and skin [39]. This finding suggests that frozen gizzards will not add a significant margin of safety with respect to *Campylobacter* and cannot replace sanitary production and handling. If fresh gizzards are already contaminated somewhere along the process before storage at low temperatures, the chances of *Campylobacter* to survive are still high. Moreover, table 2 shows that all the forty-two (42) isolates (i.e., 100%) of *Campylobacter* spp. from gizzards were sensitive to Gentamicin (Aminosides) and resistant to Penicillin (Beta-lactams) and Ampicillin (Beta-lactams). *Campylobacter* spp. isolated from humans and poultry has been widely reported to be resistant to ampicillin and other β -lactam agents [40]. Resistance to Beta-lactam is a major problem due to the fact that beta-lactamase genes can be transferred between bacteria [41]. The development of *Campylobacter* spp. resistance against antibiotics can be explained by the drastic increase in use of antibiotics as a mandatory requirement for the production of poultry [42]. The indiscriminate use of these antibiotics in veterinary medicine and as growth stimulators in the poultry production can also explain this resistance of *Campylobacter* spp. to antibiotics [41]. In Ouagadougou, antibiotic residues were found in chicken gizzards certainly due to the unsuitable use of these antibiotics in veterinary medicine [43]. Breeders are increasingly aware of the resistance of microorganisms to antibiotics. According to sociological studies, different personal contexts such as succession, health problem in breeding, health problem in the family lead breeders to better knowledge and practices about resistance of microorganisms to antibiotics [44]. The use

of plant extracts for animal treatments can be an alternative to antibiotic pressure [45]. The household survey conducted in Korhogo shows that, among the 384 respondents, 138 (35.9%) were men and 246 (64.1%) women. Considering age groups, 270 (70.3%) respondents were between 25 and 59 years whereas, 93 (24.2%) were over 60 years old and 21 (5.5%) were between 15 and 24 years old (Table 3). Gizzards are consumed by 310 (80.7%) respondents including 117 (84.8%) men and 193 (78.5%) women with no statistically significant difference in consumption between men and women. Contrary to some African contexts, there is no gendered restrictions on the consumption of gizzards in Korhogo and there is no male domination regarding this food practice [46]. However, women (47.6%) consume more fried gizzards than men (44.9%). Among gizzard consumers, participants having 60 years old and above (60.2%) consumed more fried gizzards than participants with age group between 25 and 59 years old (42.6%) and those with age group between 15 and 24 (38.1%). The difference in the forms of gizzard consumption between age groups was statistically significant (Table 4). Among the forms of consumption of gizzard in households, the form fried was the most common with 46.6% (Table 4). The consumption of fried gizzards could be explained by the fact that the quality and the type of food consumed depend on people's beliefs and worldviews. Consumer beliefs are important social logics in their choice of foods from animal origin [47]. Moreover, regarding frequency of consumption, chicken gizzards are consumed occasionally in households with 29.4% (Table 4). This result is due to the fact that most of the households in the

country consume mainly beef as compared to chicken meat that is generally consumed during celebrations.

Even, chicken gizzards are consumed occasionally, the risk that can be evaluated in absolute terms by the number of ill persons after consumption of the product and the severity of hazard, should not be neglected. In this perception of the risk, the consumption of fried gizzards represents a potential risk for the consumers to have campylobacteriosis because 88.5% of fried gizzards samples are contaminated by *Campylobacter* spp. Women and consumers aged 60 and over are at risk of campylobacteriosis because they are the most consumers of fried chicken gizzards. This risk can also be assessed by relative terms as representations of a product is

appreciated differently depending on the person and social groups [48].

This study has several limitations. The first one is inherent to the cross-sectional design, which did not allow us to assess the contamination of gizzard by *Campylobacter* spp. over time. The second limitation is related to the fact that only three (3) antibiotics belonging to two families (Beta-lactams and Aminocyclitol) were used to antimicrobial resistance profiles.

Despite these shortcomings, our study sheds new light on the contamination by resistant *Campylobacter* spp. of fresh, frozen and fried gizzards sold informally in Korhogo and the potential risk of campylobacteriosis among fried gizzard consumers in the households in Korhogo.

Table 1.

Percentage of presence of *Campylobacter* spp. in gizzards according to the place of purchase and the type of gizzard

Category	Numbers of samples (n)	Numbers of positive samples (n)	Percentage (%) of positive samples	p-value
Type of gizzard and place of purchase				
Fresh on Market	91	90	98.9	0.031*
Fried on Informal restaurant	26	23	88.5	
Frozen in Fish shops	27	27	100	

* Significant for $p < 0.05$ (Fisher's exact test)

Table 2.

Antimicrobial Resistance Profiles of *Campylobacter* spp. isolated from Gizzard

Antibiotics	Family of antibiotics	Number of sensibles isolates (%)	Number of resistant isolates (%)
Penicillin	β -lactams	00 (0%)	42 (100%)
Ampicillin	β -lactams	00 (0%)	42 (100%)
Gentamicin	Aminosides	42 (100%)	00 (0%)

Sensitive (S), Resistant (R)

Table 3.

Socio-demographic characteristics of respondents in Korhogo households

Characteristics of population samples	Number	Percentage (%)
Gender		
Men	138	35.9
Women	246	64.1
Age group (years)		
[15; 24]	21	5.5
[25; 59]	270	70.3
≥ 60	93	24.2
Education		
None	211	54.9
Koranic school	12	3.1
Primary	51	13.3
Secondary	73	19.0
University	37	9.63
Marital status		
Single	73	19.0
Married	229	59.6
Widower	76	19.8
Separated	6	1.6

No education refers to no attendance to primary, secondary, university and Koranic school.

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Table 4

Gizzard consumption according to sex and age group								
Consumption of Gizzards	Sex			<i>p</i> value	Age group			<i>p</i> value
	Total N (%)	Male n (%)	Female n (%)		[15; 24] n (%)	[25;59] n (%)	60 and more n (%)	
Yes	310 (80.7)	117 (84.8)	193(78.5)	0.131	18 (85.7)	216 (80.0)	76 (81.7)	0.783
No	74 (19.3)	21 (15.2)	53 (21.5)		3 (14.3)	54 (20.0)	17 (18.3)	
Total	384	138	246		21	270	93	
Form of consumption								
None	74 (19.3)	21(15.2)	53 (21.5)	0.003	3 (14.3)	54 (20.0)	17 (18.3)	0.007
Soup	70 (18.2)	34 (24.6)	36 (14.6)		5 (23.8)	47 (17.4)	18 (19.4)	
Fried	179 (46.6)	62 (44.9)	117 (47.6)		8 (38.1)	115 (42.6)	56 (60.2)	
Braised	27 (7)	15 (10.9)	12 (4.9)		2 (9.5)	23 (8.5)	2 (2.2)	
Sauteed	34 (8.9)	6 (4.3)	28 (11.4)		3 (14.3)	31 (11.5)	0 (0.0)	
Frequency of consumption								
None	74 (19.3)	21 (15.2)	53 (21.5)	0.016	3 (14.3)	54 (20.0)	17 (18.3)	0.001
Every day	4 (1)	1 (0.7)	3 (1.2)		1 (4.8)	3 (1.1)	0 (0.0)	
Once a week	38 (9.9)	20 (14.5)	18 (7.3)		2 (9.5)	34 (12.6)	2 (2.2)	
Once a month	56 (14.6)	17 (12.3)	39 (15.9)		5 (23.8)	41 (15.2)	10 (10.8)	
2 times a week	9 (2.3)	5 (3.6)	4 (1.6)		2 (9.5)	7 (2.6)	0 (0.0)	
2 times a month	8 (2.8)	4 (50.0)	4 (50.0)		0 (0.0)	6 (2.2)	2 (2.2)	
More than 2 times a week	6 (1.6)	2 (1.4)	4 (1.6)		2 (9.5)	4 (1.5)	0 (0.0)	
More than 2 times a month	5 (1.3)	3 (2.2)	2 (0.8)		1 (4.8)	4 (1.5)	0 (0.0)	
More than 2 times a year	1 (0.3)	1 (0.7)	0 (0)		0 (0.0)	1 (0.4)	0 (0.0)	
Occasionally	113 (29.4)	37 (26.8)	76 (30.9)		4 (19.0)	66 (24.4)	43 (46.2)	
Rarely	70 (18.2)	24 (17.4)	46 (18.7)		1 (4.8)	50 (18.5)	19 (20.4)	

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4. Conclusion

The study showed that frozen, fresh and fried gizzards sold in informal settings in Korhogo are highly contaminated by *Campylobacter* spp. resistant to Beta-lactams. Women and consumers aged 60 and over have a potential risk to have campylobacteriosis because they are the most consumers of fried chicken gizzards. Awareness must be raised among farmers on hazards related to inappropriate use of antibiotics in poultry farms and consumers of chicken gizzards on the risk of human campylobacteriosis.

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