Original Article

Campylobacter spp. at Different Stages of the Poultry Slaughtering Line in Algeria: Evaluation of Direct and Indirect Modified ISO 10272:2017 Detection Methods and Characterization of the Isolates

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ABSTRACT

This study aimed to evaluate the effectiveness of direct and indirect modified ISO 10272-1:2017 methods for detecting *Campylobacter* spp. in 10 sites of a poultry slaughterhouse and investigate the relationship between poultry intestinal carriage and carcasses, as well as surfaces contamination during different slaughter steps (scalding, defeathering, evisceration, and rinsing). Antibiotic resistance profiles of the isolates were also determined against 12 antibiotics. A total of 165 intestinal (feces and ceca) and non-intestinal (neck skins and surfaces) samples were collected from 10 different sampling sites before, during, and after the slaughtering of six flocks of broiler chickens. After the isolation and phenotypic identification of the isolates, an antibiotic susceptibility study was performed using the agar diffusion method. Thermotolerant bacteria of the genus *Campylobacter* (TC) were isolated with a prevalence of 47.04% (127/270), and 39.05% (82/210) of the TC isolates were detected in non-intestinal samples. Moreover, 76.19% (80/105) of these microorganisms were detected by a direct isolation method for a sensitivity of 97.56%, while only 1.90% (2/105) of the samples contained TC by an indirect isolation method for a sensitivity of 2.44%. The samples of intestinal origin were positive for TC with a rate of 75.00% (45/60). *C. jejuni* (76.38%; 97/127) was the most isolated bacterial species. Furthermore, 98.43% (125/127) of the TC isolates were multidrug-resistant and 69.29% (88/127) showed simultaneous resistance to ciprofloxacin and erythromycin. Direct isolation seems to be the best method for the detection of *C.* spp. A serious public health problem of multidrug-resistant *C.* spp. isolates with critical resistance profiles can be transmitted to broiler carcasses before, during, and after the evisceration step.

Keywords: *Campylobacter*, ISO method evaluation, slaughterhouse, intestinal origin, non-intestinal origin, antimicrobial resistance

1. Introduction

Diarrheal diseases are the most common illnesses caused by unsafe food. Every year, 550 million people fall ill, including 220 million children under the age of 5 years. Among these diseases, campylobacteriosis is nowadays not only one of the 4 main causes of diarrheal diseases but also the most common bacterial cause of human gastroenteritis in the world (1). This infection is caused by microaerophilic curved or spiral Gram-negative bacilli called thermotolerant *Campylobacter* (TC) (2). The culture of these bacteria is long, fastidious, and expensive because these pathogens have demanding growth requirements involving careful testing procedures to detect them in food samples (3). Furthermore, due to the difficulty of isolating these bacteria in addition to their nutritional and environmental requirements (3), a lot of studies indicate that false-negative results have been observed during the detection of *Campylobacter* spp. in food, notably in poultry meat (3, 4) and human fecal specimens with rates ranging from 24% to 40% (5, 6). These alarming observations indicate that the use of appropriate enrichment broths, selective agar media, and specific conditions that allow regular growth are important for the recovery of *C.* spp. from poultry meat (3, 4) and other types of samples.

This group of microorganisms is generally transmitted to humans by poultry through the ingestion of contaminated foodstuffs, especially raw or undercooked meat because these bacteria massively colonize the gut of this main reservoir (7). Campylobacteriosis can cause diarrhea in humans, which is often bloody and may lead to more serious illnesses, such as Guillain-Barre syndrome (1). On the other hand, WHO classifies TC as a high-priority bacterium for which new treatments are needed. The reason is that this group of microorganisms has become a major public health problem due to the emergence of antibiotic resistance around the world, especially to fluoroquinolones (8).

Due to the widespread colonization of TC in the gut of poultry and the emergence of antibiotic-resistant strains, many studies have been conducted to investigate the prevalence and antibiotic susceptibility of this microorganism in developed countries. However, few studies have been carried out in developing countries, and even fewer in Algeria.

1.2. Objectives

To the best of our knowledge, this study aimed to evaluate for the first time the effectiveness of direct and indirect modified ISO 10272-1: 2017 methods for the detection of *C.* spp. from poultry and environmental samples. This work also contributed to providing the first data in developing countries concerning the study of the relationship between the intestinal carriage and carcasses contamination, as well as some surfaces by *C.* spp. during different slaughter steps. A detailed study of the antibiotic susceptibility of TC isolates to 12 antibiotics was also performed.

2. Materials and Methods

2.1. Samples Collection

All the analyzed samples were collected from the only modern broiler slaughterhouse in Algiers. A total of 165 samples were collected randomly from 10 sampling sites before, during, and after slaughtering of 6 broiler flocks. Fresh fecal droppings, cecal contents, and neck skins were collected from all the sampled flocks, while surface samples were collected from the last 3 flocks only. For each type of sampling, 5 samples were collected. Fecal droppings were collected before the beginning of the slaughtering process from transport crates, while ceca were removed from the intestines after the evisceration of broiler carcasses (9). For the non-intestinal samples, neck skins were collected per flock after each step of defeathering, evisceration, and carcass rinsing (10). During broiler slaughtering, surface samples were collected (11, 12). They were represented by the scalding tanks, the plucker fingers, the evisceration knives, the evisceration line, and the carcasses transport crates after the rinsing step. Finally, all the samples were placed inside a cool box and transported immediately to the food hygiene laboratory of the High National

Veterinary School of Algiers to carry out their microbiological analysis within 2 hours.

2.2. Isolation of Thermotolerant *Campylobacter* spp.

Each sample of non-intestinal origin (neck skins and surfaces) (n=105) was subjected to direct (n=105) and indirect isolation (n=105). For samples of intestinal origin (fecal droppings and cecal contents) (n=60), only the direct isolation method was performed because the enrichment step is unnecessary for this type of sample (13, 14). For neck skins, 10 g of each sample were collected and aseptically placed into a sterile stomacher bag containing 90 ml of Bolton broth (Oxoid CM0983) with supplement (Oxoid SR0183) and 5% horse blood (PIA: Pasteur Institute of Algeria) (15, 16). Furthermore, each gauze that was used for surface sampling was placed into a stomacher bag including 50 ml of Bolton selective enrichment broth with 5% horse blood supplement (12, 15). The contents of stomacher bags containing neck skin and surface samples were mixed and poured into sterile jars, which were then tightly closed. Instead of being incubated in microaerophilic conditions for 48 hours as recommended by the standard ISO 10272-1 (2017) (15), the jars were either directly inoculated onto agars or incubated aerobically at 42°C for 24 hours. According to WOAH (2005), a special atmosphere is not required during the enrichment step if a small top space (< 2 cm) is left in the enrichment vial, as long as the cap is tightly closed (14). For fecal droppings and cecal contents, 1 g was aseptically collected and inoculated into 9 ml of 0.9% sterile saline and homogenized using a vortex (15, 16). After that, a bacterial suspension of 100 µl was taken from Bolton broth or 0.9% sterile saline and plated onto the surface of modified cefoperazone and charcoal deoxycholate agar (mCCDA) (Oxoid CM0739) with supplement (Oxoid SR0115). Plates were then incubated at 42°C for 24 hours in microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) using CampyGen™ microaerophilic generators (Oxoid CN0025). *Campylobacter* isolates were produced on mCCDA agar grey, moist, flat spreading colonies, with or without a metallic sheen.

2.3. Identification of Thermotolerant *Campylobacter* spp.

One characteristic colony per agar plate (mCCDA) was purified on Columbia agar (Oxoid CM0331) supplemented with 5% horse blood. All culture media were then incubated at 42°C for 24 hours in microaerophilic conditions. Then, Gram staining (Kit Gram-Nicolle Ref.364320), characteristic motion, oxidase reaction (Merck Bactident® Oxidase 113300), sugar fermentation on TSI agar (PIA), growth at 25°C, and aerobic growth on Columbia agar (Oxoid CM0331) supplemented with 5% horse blood were performed to identify TC (14). All the positive isolates were confirmed (detection of *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus*) by studying H_2S reaction on Triple Sugar Iron agar (PIA), catalase reaction, and by testing the sensitivity to nalidixic acid (30µg) (Bio-rad 68618) and cephalothin (30 µg) (Bio-Rad 66218). Once TC colonies were confirmed, a *Campylobacter* Dryspot latex agglutination test (Oxoid DR0150) was realized, and the identification to the species level was established using the API Campy gallery (14, 15).

2.4. Antimicrobial Susceptibility Testing

Antibiotic susceptibility of *Campylobacter* isolates was investigated using the agar diffusion method according to the instructions of the Antibiogram Committee of the French Society of Microbiology/European Committee on Antimicrobial Susceptibility Testing (EUCAST) (17). The tested antibiotics (n=12) were: ampicillin (AM) (10 µg) (Bio-Rad 66128), amoxicillin + clavulanic acid (AMC) (20+10 µg) (Bio-rad 66178), cefotaxime (CTX) (30 µg) (Bio-rad 66368), streptomycin (S) (10 UI) (Bio-rad 67418), gentamicin (GM) (15 µg) (Bio-rad 66548), kanamycin (K) (30 UI) (Bio-rad 66618), tobramycin (TM) (10 µg) (Bio-rad 67488), erythromycin (E) (15 UI) (Bio-rad 66448), nalidixic acid (NA) (30 µg) (Bio-rad 68618), ciprofloxacin (CIP) (5 µg) (Bio-rad 68648), tetracycline (TE) (30 UI) (Bio-rad 67448), and chloramphenicol (C) (30 µg) (Bio-rad 66278).

2.5. Statistical Analysis

A 5% confidence interval and Chi-square tests were performed using the software Anastat. The difference was significant when the *P*-value was less than 0.05 ($P<0.05$).

3. Results

Table 1 provides the details of the different percentages recorded. Out of 270 samples analyzed, *C.* spp. and TC were isolated with rates of 52.59% (n=142) and 47.04% (n=127), respectively ($P>0.05$). TCs were directly and indirectly isolated from non-intestinal samples (neck skin and surfaces) with a rate of 39.05% (82/210). The detection rates of *C.* spp. from direct isolation were higher than those from indirect isolation ($P<0.05$) (Table 1). Regarding the results of direct isolation of TC from non-intestinal samples, these microorganisms were isolated with a prevalence of 76.19% (80/105) for a sensitivity of 97.56% and a specificity of 100%. Neck skins were positive for TC isolates with a prevalence of 75.56% (68/90). They were isolated with similar rates ($P>0.05$) from neck skins collected after defeathering (66.67%; 20/30), evisceration (83.33%; 25/30), and rinsing carcasses (76.67%; 23/30). Most of the sampled surfaces (80.0%; 12/15) were also positive for TC (Table 2).

On the other hand, 75.00% (45/60) of samples of intestinal origin were contaminated with TC. This high isolation rate was distributed between fecal droppings (76.67%, 23/30) and cecal (73.33%, 22/30) samples ($P>0.05$). Results of indirect isolation of TC from non-intestinal samples revealed that only 1.90% (2/105) of the samples contained TC for a sensitivity of 2.44% and a specificity of 100%. It should be noted that the TC rate decreased from 74.29% (78/105) after indirect isolation ($P<0.05$). Furthermore, 75.56% (204/270) of the samples were contaminated with microorganisms other than *C.* spp. For non-intestinal samples, the rate of contaminants recorded after indirect isolation (97.14%, 102/105) was higher than that recorded after direct isolation (65.71%, 69/105) ($P<0.05$). Thus, the rate of contaminants (microorganisms other than TC) increased by 31.43% (33/105) after indirect isolation ($P<0.05$). However, samples of intestinal origin had the lowest level of contaminants (55.00%, 33/60).

To investigate the relationship between intestinal carriage of poultry and TC contamination of neck skins and surfaces, a study by flock (Table 3) was performed for TC isolates obtained after direct isolation. Intestinal samples (feces droppings and cecal contents) of flock 6 were negative for TC, which is not the case for the other flocks (flocks 1, 2, 3, 4 and 5). Rates of TC isolated from neck skins increased after the evisceration step for most of the collected flocks (flocks 1, 2, 3, and 5), compared to the recorded rates after the defeathering step. Thereafter, they remained unchanged (flocks 2, 3, and 5) or decreased slightly (flocks 4 and 6) after the rinsing step (Table 3). Finally, all the surfaces of the collected flocks during evisceration (100%; 6/6) of carcasses were positive for TC in contrast to the collected flocks before (83.33%; 5/6) and after evisceration (33.33%; 1/3) of carcasses (Table 3). With an isolation rate of 76.38% (97/127), the most isolated species by far were *C. jejuni* (*C.j*). and *C. coli* (*C.c*) which represented only 23.62% (30/127) of the isolation rate ($P<0.05$) (Table 2).

All the isolates showed resistance rates to all the tested antibiotics, except for gentamicin (Table 4). In decreasing order of frequency, TC, *C.j*, and *C.c* isolates showed very high rates of resistance to ciprofloxacin (TC: 98.43%; 125/127 vs. *C.j*: 98. 97%; 96/97 vs. *C.c*: 96.67%; 29/30), nalidixic acid (TC: 94.49%; 120/127 vs. *C.j*: 94.85%; 92/97 vs. *C.c*: 93.33%; 28/30), and tetracycline (TC: 89.76%; 114/127 vs. *C.j*: 89.69%; 87/97 vs. *C.c*: 90.00%; 27/30) ($P>0.05$). In contrast, a low rate of resistance to chloramphenicol (4.72%; 6/127 vs. 4.12%; 4/97 vs. 6.67%; 2/30) was recorded. Table 5 shows that all the tested isolates (n=127) were resistant to at least two antibiotics, while 125 (98.43%) were multidrug-resistant and 88 (69.29%) showed simultaneous resistance to ciprofloxacin and erythromycin. In total, 11 different resistance profiles were recorded. The most common resistance pattern was AMC-TE-TM-NA-AM-CTX-S-K-CIP-E, and it was noted 30 times for the *C. j* and *C. c* entity.

Table 1. Prevalence of *Campylobacter* spp., thermotolerant *Campylobacter* and contaminants in different samples after direct and indirect isolation

Steps	*C.* spp.				TC				Contaminants			
	DI		II		DI		II		DI		II	
	n	%	n	%	n	%	n	%	n	%	n	%
Neck skins-defeathering[(a)] (N=30)	23	76.67	1	3.33	20	66.67	1	3.33	19	63.33	29	96.67
Neck skins-evisceration[(a)] (N=30)	27	90.00	0	0.00	25	83.33	0	0.00	20	66.67	30	100.00
Neck skins-Rinssing[(a)] (N=30)	26	86.67	0	0,00	23	76.67	0	0,00	16	53.33	28	93.33
Total[1] (N=90)	76	84.44	1	1.11	68	75.56	1	1.11	55	61.11	87	96.67
Surfaces (N=15)	12	80.00	1	6.67	12	80.00	1	6.67	14	93.33	15	100.00
Total[2] [(b)] (N=105)	88	83.81	02	1.90	80	76.19	02	1.90	69	65.71	102	97.14
Sensitivity					80	97.56	02	2.44				
Specificity					80	100	02	100				
FD (N=30) [(a)]	23	76.67	-	-	23	76.67	-	-	17	56.67	-	-
CC (N=30) [(a)]	29	96.67	-	-	22	73.33	-	-	16	53.33	-	-
Total[3] (N=60)	52	86.67	-	-	45	75.00	-	-	33	55.00	-	-
Total[4] (N^{DI}=165 / N^{II}=105)	140	84.85	02	1.90	125	75.76	02	1.90	102	61.82	102	97.14

N: number of samples; n: number of positive isolates; *C.* spp.: *Campylobacter* spp.; TC: thermotolerant *Campylobacter*; DI: direct isolation; II: indirect isolation; -: not carried out; FD: fecal dropping; CC: cecal content; Total1: Neck skins-slaughter steps; Total2: Neck skins-slaughter steps + surfaces; Total3: IC = FD + CC; Total4: Neck skins-slaughter steps + surfaces + IC; (a) : no significant difference ($P > 0.05$) between the results of DI and II for each type of microorganisms ; (b) : significant difference ($P < 0.05$) for each type of microorganisms and isolations

Table 2. Prevalence of thermotolerant *Campylobacter* species by sampling site after direct isolation

Sites	*C. j*		*C. c*		TC	
	n	%	n	%	n	%
Neck skin-defeathering (N=30)	14	46.67	6	20.00	20	66.67
Neck skin-evisceration (N=30)	18	60.00	7	23.33	25	83.33
Neck skin-rinsing (N=30)	16	53.33	7	23.33	23	76.67
Total (neck skins) (N=90)	48	53.33	20	22.22	68	75.56
Scalding tank (N=3)	1	33.33	1	33.33	2	66.67
Plucker fingers (N=3)	3	100.00	0	0.00	3	100.00
Total (before evisceration) (N=6)	4	66.67	1	16.67	5	83.33
Evisceration line (N=3)	3	100.00	0	0.00	3	100.00
Evisceration knife (N=3)	2	66.67	1	33.33	3	100.00
Total (during evisceration) (N=6)	5	83.33	1	16.67	6	100.00
Carcasses transport crates (N=3)	1	33.33	0	0.00	1	33.33
Total (after evisceration) (N=3)	1	33.33	0	0.00	1	33.33
Total (surfaces) (N=15)	10	66.67	2	13.33	12	80.00
Fecal dropping (N=30)	23	76.67	0	0.00	23	76.67
Cecal Content (N=30)	15	50.00	7	23.33	22	73.33
Total (intestinal content) (N=60)	38	63.33	7	11.67	45	75.00
Total (N=165)[(a)]	96	58.18	29	17.58	125	75.76

N: number of samples; n: number of positive isolates; *C. j*; *Campylobacter jejuni* ; *C. c* : *Campylobacter coli* ; (a) : significant difference ($P < 0.05$) between the results of *C. j* and *C. c*

Table 3. Prevalence of thermotolerant *Campylobacter* by flock after direct isolation

Sample step	Flock 1 (N=5)		Flock 2 (N=5)		Flock 3 (N=5)		Flock 4 (N=5)		Flock 5 (N=5)		Flock 6 (N=5)		Total (N=30)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
a	2	40.0	4	80.0	4	80.0	5	100.0	1	20.0	4	80.0	20	66.7
b	4	80.0	5	100.0	5	100.0	5	100.0	3	60.0	3	60.0	25	83.3
c	3	60.0	5	100.0	5	100.0	5	100.0	3	60.0	2	40.0	23	76.7
d	5	100.0	3	60.0	5	100.0	5	100.0	5	100.0	0	0.0	23	76.7
e	5	100.0	3	60.0	4	80.0	5	100.0	5	100.0	0	0.0	22	73.3

N: number of samples; n: number of positive isolates; -: not carried out; a : neck skin-defeathering ; b : neck skin-evisceration; c : neck skin-rinsing; i : fecal dropping; j : cecal content

Table 4. Antibiotic susceptibility rates of isolates

ATB	*C. j* (n=97)		*C. c* (n=30)		CT (n=127)		IC 95%*
	n'	%	n'	%	n'	%	
AMC	53	54.64	20	66.67	73	57.48	[48.9%-66.1%]
TE[(a)]	87	89.69	27	90.00	114	89.76	[84.5%-95.0%]
TM	62	63.92	15	50.00	77	60.63	[52.1%-69.1%]
NA[(a)]	92	94.85	28	93.33	120	94.49	[90.5%-98.5%]
C	4	4.12	2	6.67	6	4.72	[1.0%-8.4%]
AM[(b)]	68	70.10	27	90.00	95	74.80	[67.3%-82.4%]
GM	0	0.00	0	0.00	0	0.00	[0.0%-0.0%]
CTX	67	69.07	22	73.33	89	70.08	[62.1%-78.0%]
S	50	51.55	23	76.67	73	57.48	[48.9%-66.1%]
K	44	45.36	8	26.67	52	40.94	[32.4%-49.5%]
CIP[(a)]	96	98.97	29	96.67	125	98.43	[96.3%-100.6%]
E[(b)]	62	63.92	25	83.33	87	68.50	[60.4%-76.6%]

TC : thermotolerant *Campylobacter*; *C. j* : *Campylobacter jejuni* ; *C. c* : *Campylobacter coli* ; ATB : antibiotic ; n: number of positive isolates ; n' : number of isolates resistant to ATB; AMC: Amoxicillin/Clavulanic acid; TE: Tetracycline; TM: Tobramycin; NA: Nalidixic Acid; C: Chloramphenicol; AM: Ampicillin; GM: Gentamicin; CTX: Cefotaxime; S: Streptomycin; K: Kanamycin; CIP: Ciprofloxacin; E: Erythromycin; * : Confidence Interval (CI 95%) of TC ; no significant difference ($P > 0.05$) for each category of microorganisms

Table 5. Antibiotic resistance profiles of isolates

N.ATB	Resistance profile	n. TC	Species	Flock	Site (n.)
2	NA-CIP	2	*C. j*	2	a(1)-b(1)
3	TE-NA-CIP	12	*C. j*	2	a(3)-b(2)
			C. j	3	a(2)-b(2)-c(1)-e(1)-f(1)
		1	*C. c*	2	b(1)
	NA-AM-E	1	*C. j*	2	c(1)
	NA-AM-CIP	1	*C. c*	2	f(1)
	C-S-E	1	*C. c*	1	b(1)
4	AMC-NA-AM-CIP	1	*C. j*	2	b(1)
	TE-NA-AM-CIP	3	*C. j*	3	c(1)-e(1)-f(1)
	TE-NA-CTX-CIP	5	*C. j*	3	a(2)-b(2)-c(1)
	TE-NA-CIP-E	2	*C. j*	1	f(2)
5	AMC-TE-NA-AM-CIP	1	*C. j*	2	c(1)
	AMC-TE-NA-CIP-E	1	*C. j*	1	f(1)
	TE-TM-NA-CTX-CIP	2	*C. j*	3	b(1)-c(1)
	TE-TM-NA-K-CIP	1	*C. j*	2	e(1)
	TE-NA-AM-CTX-CIP	1	*C. j*	3	f(1)
	TE-NA-AM-CIP-E	4	*C. j*	1	f(1)
			C. c		f(1)
			C. c	2	c(2)
	NA-C-AM-CIP-E	1	*C. j*	2	c(1)
	NA-CTX-S-CIP-E	1	*C. c*	1	c(1)
6	AMC-TE-NA-AM-CIP-E	1	*C. j*	2	f(1)
	TE-TM-NA-C-K-CIP	1	*C. j*	2	e(1)
	TE-TM-NA-AM-K-CIP	1	*C. j*	2	e(1)
	TE-TM-NA-CTX-S-CIP	1	*C. j*	3	c(1)
	TE-NA-AM-CTX-CIP-E	3	*C. j*	1	e(1)
	TE-NA-AM-S-CIP-E	2	*C. c*	1	b(2)
	TE-NA-CTX-S-CIP-E	1	*C. j*	1	c(1)

N.ATB	Resistance profile	n. TC	Species	Flock	Site (n.)
7	AMC-TE-NA-AM-CTX-CIP-E	2	*C. j*	1	e(1)
			C. j	6	c(1)
		1	*C. c*	6	c(1)
	AMC-TE-AM-CTX-S-CIP-E	1	*C. c*	1	b(1)
	TE-TM-NA-AM-CTX-K-CIP	2	*C. j*	3	e(1)-f(1)
	TE-TM-NA-CTX-S-K-CIP	1	*C. j*	3	e(1)
	TE-NA-AM-CTX-S-K-CIP	1	*C. j*	3	e(1)
	TM-NA-AM-CTX-S-K-CIP	1	*C. j*	2	f(1)
8	AMC-TE-TM-NA-AM-CTX-CIP-E	2	*C. j*	5	c(2)
		1	*C. c*	5	c(1)
	AMC-TE-NA-AM-CTX-S-CIP-E	2	*C. j*	1	a(1)
			C. j	6	a(1)
		3	*C. c*	6	a(3)
9	AMC-TE-TM-NA-AM-CTX-S-CIP-E	8	*C. j*	5	d(3)-e(5)
		7	*C. c*	1	a(1)
			C. c	5	d(1)-f(5)
	AMC-TE-TM-AM-CTX-S-K-CIP-E	5	*C. j*	4	e(5)
	AMC-TE-NA-AM-CTX-S-K-CIP-E	1	*C. c*	5	a(1)
	AMC-TM-NA-AM-CTX-S-K-CIP-E	4	*C. j*	4	a(3)-a'(1)
	TE-TM-NA-C-AM-CTX-K-CIP-E	1	*C. j*	1	e(1)
	TE-TM-NA-AM-CTX-S-K-CIP-E	1	*C. c*	4	b(1)
10	AMC-TE-TM-NA-AM-CTX-S-K-CIP-E	25	*C. j*	4	a(1)-b(3)-c(4)-d(3)-f(5)
			C. j	5	b(3)
			C. j	6	b(3)-d(3)
		5	*C. c*	1	c(1)
			C. c	4	a(1)-b(1)-d(1),d'(1)
11	AMC-TE-TM-NA-C-AM-CTX-S-K-CIP-E	1	*C. j*	4	d(1)
		1	*C. c*	4	c(1)

TC : thermotolerant *Campylobacter*; *C. j* : *Campylobacter jejuni* ; *C. c* : *Campylobacter coli* ; ATB : antibiotic ; N. : number of antibiotic resistance; n.: number of isolates/profile; a : neck skin-defeathering (direct isolation); a': neck skin- defeathering (indirect isolation); b: neck skin-evisceration (direct isolation); c: neck skin-rinsing (direct isolation); d: surface (direct isolation); d': surface (indirect isolation); e: fecal dropping (direct isolation); f: cecal content (direct isolation)

A study on the flocks revealed the existence of identical resistance profiles among some isolates from cecal contents and other types of samples, namely feces (flocks 3, 4, and 5), neck skins (flocks 3 and 4), and surfaces (flocks 4 and 5). The same observation was noted among the isolates of neck skin samples collected after defeathering and evisceration (flocks 2 and 3) or rinsing carcasses (flock 3). For other flocks, resistance profiles recorded for neck skins isolates were only noted for isolates collected from surface samples (rinsing step of flock 4 and evisceration step of flock 6).

4. Discussion

The majority of the isolated *C.* spp. were TCs as the difference in prevalence of these microorganisms was not significant (P>0.05). For samples of non-intestinal origin, compared to the results of indirect isolation, the detection rates of *C.* spp. and TC from direct isolation were significantly higher (P<0.05) and had a higher percentage of sensitivity. These data corroborate those of Benetti, Abrahão (4), who isolated these bacteria from poultry meat only where the sensitivity rates of the direct and enrichment methods were 84.8% and 39.4%, respectively. Furthermore, by comparing the two types of isolation, we have observed that the rate of contaminants significantly increased while the rate of TC significantly decreased after indirect isolation. This indicates that direct isolation resulted in false-negative results, even though enrichment is recommended to improve the sensitivity of the culture of

microorganisms that may be stressed by the environment or in cases of low numbers of microorganisms (14). However, the aerobic incubation of Bolton broths as recommended by the WOAH manual (2005) may have contributed to the excessive proliferation of contaminants, which would have prevented the growth of *C.* spp. even though the use of an incubation temperature of 42°C can minimize the growth of contaminants (14). The indirect isolation method would also be the cause of the development of viable but non-cultivable forms of *C.* spp. Indeed, the detection of these bacteria by conventional culture methods is difficult and of limited sensitivity due to the use of selective media, the low number of bacteria in the samples (environmental samples) and possibly also due to the presence of non-culturable or sub-lethally injured stages of the bacteria (18). On the other hand, the proliferation of contaminants in Bolton enrichment broth and mCCDA agar, both during direct and indirect isolations, indicates that the culture media recommended by ISO 10772-1 (2017) should be modified once again. According to (3), Benetti, Abrahão (4) who evaluated the horizontal method ISO 10272-1 (2006), the consecutive use of Bolton broth and mCCDA agar can give false negative results due to the presence of contaminants and the antibiotics used in these media.

The direct isolation method showed a high incidence of TC in the sampled flocks (Table 3). When a flock is positive, the entire slaughter line is contaminated, particularly the scalding, defeathering, and evisceration rooms, which are the main sources of cross-contamination of carcasses in slaughter establishments (7). The evisceration chain would have been contaminated not only by intestinal content but also by any carcass that had previously been contaminated during the scalding and plucking steps. As reported by the literature, it is recommended that each worker use two or more evisceration knives, which should be disinfected once or twice a day in a water bath at over 82°C (19). However, in our study, only one evisceration knife was used for the evisceration of different batches without being changed or sterilized after each use, which contributes to the increased dissemination of *Campylobacter*. Moreover, the gloves worn all day by the personnel who manually eviscerate the carcasses would have also contributed to the contamination of the knives. Compared to the evisceration step, the level of TC generally remained unchanged or slightly decreased after rinsing for the sampled flocks, suggesting that the rinsing step did not contribute to the elimination of these microorganisms that were still present on the carcass transport crates. According to Jeffrey, Tonooka (13), the intestine (cecum) is the only organ that reflects the prevalence of TC in flocks at the slaughterhouse level. Given the similarity of TC rates in fecal droppings and cecal contents (P>0.05), we can assume that freshly emitted droppings collected from slaughterhouses could also represent the rate of TC in farms, which would allow for faster and easier detection of contaminated farms at the slaughterhouses level. Flocks 1 and 5 were the first slaughtered flocks of the day after cleaning and disinfecting the visited establishment.

Therefore, carcasses and surfaces of the slaughterhouse would have been only contaminated by the intestinal contents of their own samples. Nevertheless, flocks 2, 3, 4, and 6 were the second flocks slaughtered of the day. Therefore, carcasses and surfaces could have been contaminated not only by intestinal contents of the own harvested animals but also by those of the previously slaughtered animals, as there is no cleaning and disinfection of the equipment and material of the slaughterhouse between flocks since it is only done at the end of the day.

Except for flock 6 where only neck skins were contaminated with *Campylobacter*, but not the intestinal contents, farms of the other sampled flocks were all contaminated with TC isolates. As reported by the literature, poultry flocks initially negative for *Campylobacter* may become highly positive after passing through a contaminated slaughter line as a result of cross-contamination (20). Indeed, when poultry flocks contaminated with *Campylobacter* are

slaughtered, high numbers of these bacteria can be found during all slaughter processes, as well as on the machines, and in the scalding water when the temperature is below 53°C, which makes it impossible to prevent cross-contamination of negative flocks by positive flocks up to 100% (7). The sampled flocks were frequently contaminated with both *C. j* and *C. c* but all the identified isolates belonged mainly to *C. j* and less often to *C. c*. Our results are in agreement with data from the literature, which indicate that poultry is mostly reservoirs of *C. j*, less often of *C. c*, and rarely of other species (14).

In Algeria, similar rates of resistance have been reported for gentamicin (0%), chloramphenicol (8%), and ampicillin (75.3%) (21,22). On the other hand, cefotaxime and kanamycin have not been tested previously. β-Lactams, aminoglycosides, cyclins, quinolones, and macrolides are among the main families of antibiotics that are used in avian therapy in Algeria (21). However, it should be noted that the use of chloramphenicol, gentamicin (22), and ciprofloxacin (23) is prohibited. This explains the resistance rates recorded for curative antibiotics in farms and the absence of resistance to gentamicin. In addition, most isolates were resistant to 10 antibiotics. This can be related to the common and sometimes uncontrolled use of these same antibiotics in poultry farms to cure bacterial infections. Among the tested antibiotics, kanamycin has the lowest resistance rate, suggesting that this molecule is less frequently used in Algerian farms, compared to other antibacterial agents with very high prevalence, such as tetracycline. Almost all isolated bacteria were resistant to ciprofloxacin. An increase in ciprofloxacin resistance has been reported in *C.* spp. strains in several countries since the authorization of enrofloxacin for veterinary use, particularly in the poultry industry. Indeed, resistance to enrofloxacin is often cross-resistant with ciprofloxacin. Therefore, the widespread use of enrofloxacin in farms could contribute to the increase in ciprofloxacin resistance rates (24). The high rate of resistance to nalidixic acid may be associated with ciprofloxacin due to the existence of cross-resistance between these two antibiotics (25). The same finding was noted for the increase in the resistance rate to erythromycin, which is generally associated with the heavy use of tylosin in the poultry industry, leading to a major public health problem (26).

Furthermore, the majority of TC isolates were multidrug-resistant to the tested antibiotics. Our results corroborate those of several studies, such as Hong, Kim (24) which revealed that meats are often reservoirs of *Campylobacter* strains that are multidrug-resistant. On the other hand, most of the recorded resistance profiles included ciprofloxacin and erythromycin. *Campylobacter* strains that are resistant to both ciprofloxacin and erythromycin have critical profiles because these two antibiotics are the treatment of choice for human campylobacteriosis (27). The fact that there are different resistance profiles among certain isolates of non-intestinal origin (neck skins and surfaces) and intestinal origin, whether for flocks slaughtered just after a previous flock or not, indicates that there would be TC isolates from previously slaughtered flocks in this establishment that have contaminated the surfaces, and consequently, the sampled carcasses. However, it can also be assumed that the environmental stresses endured by *C.* spp. during poultry slaughter could have an effect on the bacterium's antibiotic resistance, as demonstrated by McMahon, Xu (28).

The use of a direct isolation method inspired by modified ISO 10272-1 (2017) could represent a good alternative to the indirect isolation method in developing countries, as it would increase the detection rate of *C.* spp. due to the decrease in detection time, costs (equipment, culture media, and supplements), and contaminant rates. Contamination of carcasses by TC came either from the intestinal content or the slaughterhouse equipment. Furthermore, the situation of antibiotic multi-resistance among *C.* spp. isolates remain concerning. The obtained results suggest that

further studies are necessary to control the risk associated with the presence of *Campylobacter* in food products in Algeria.

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Authors' Contribution

Study concept and design: R. B.
Acquisition of data: R. B.
Analysis and interpretation of data: R. B.
Drafting of the manuscript: R. B.
Critical revision of the manuscript for important intellectual content: H. T. M.
Statistical analysis: R. B.
Administrative, technical, and material support: H. T. M.
Study supervision: H. T. M.

Ethics

Not Applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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