

Prevalence and Antibiotic Resistance Profiles of *Campylobacter jejuni* Isolated from Poultry Meat and Related Samples at Retail Shops in Northern India

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Abstract

Campylobacteriosis is the common gastrointestinal disease worldwide. However, in many parts of the world, including India, the impact of campylobacteriosis is less commonly investigated. This study aimed to determine the prevalence and antibiotic susceptibility profiles of *Campylobacter jejuni* in raw poultry meat and poultry-related samples at retail shops in a region of Northern India. A total of 400 samples of chicken meat (150), chicken intestine (150), feathers (50), and chopping boards and knives (50) samples were screened for the presence of *C. jejuni* by selective enrichment culture followed by selective plating on mCCDA and also by polymerase chain reaction (PCR) after selective enrichment. The highest prevalence of *Campylobacter* contamination (38.6%) was observed in chicken meat followed by chicken intestine (24.0%). *C. jejuni* was detected in 14.0% of chopping boards, knives, and feather samples by culturing method. The *hipO* gene based PCR detection yielded 36.0% *C. jejuni* from chicken meat samples; in other samples, however, the prevalence of *C. jejuni* was observed similar to that of cultural method. The antibiotic susceptibility profiles confirmed drug resistance among 97% of *C. jejuni* isolates, with 84.1% of *C. jejuni* isolates resistant to co-trimoxazole followed by cephalothin (81.1%) and tetracycline (59.4%). Low incidence of resistance (6.9–8.9%) was observed against nalidixic acid, ciprofloxacin, erythromycin, gentamicin, and azithromycin. Resistance to multiple drugs (≥ 4) was recorded in 31.6% of the strains. The findings of this study demonstrated high prevalence of drug-resistant *C. jejuni* in raw chicken meat and intestinal isolates. The high occurrence of *C. jejuni* in chicken meat might be due to cross contamination as a result of slaughtering and poor hygienic conditions. Implementation of monitoring/surveillance programs to monitor the prevalence of multidrug-resistant *Campylobacter* spp. in food production animals, particularly, poultry in semiurban regions, as well as main cities in India, is highly required for better public health protection.

Keywords: *Campylobacter jejuni*, chicken meat, *hipO* gene, antimicrobial resistance, PCR

Introduction

CAMPYLOBACTER IS CONSIDERED as one of the leading causes of acute bacterial gastroenteritis in humans in both industrialized and developed countries, as it is believed to be responsible for 400–500 million cases of gastroenteritis worldwide (Kaakoush *et al.*, 2015; Abulreesh *et al.*, 2017). The majority of *Campylobacter*-associated outbreaks are thought to be sporadic cases of food poisoning, while contaminated drinking water is believed to be the vehicle of large *Campylobacter*-associated outbreaks (Abulreesh *et al.*,

2006). *Campylobacter* species that are implicated in food-borne and waterborne human infections are *Campylobacter jejuni* and *Campylobacter coli* (to some extent *Campylobacter lari* and *Campylobacter upsaliensis*). Undercooked meat, raw milk, and contaminated water are the major sources of *Campylobacter* infections (Saint-Cyr *et al.*, 2016); handling of pets (e.g., cats and dogs) or wild birds may also be a source of infection (Abulreesh *et al.*, 2017).

The presence of the *Campylobacter* species in the intestinal tract of poultry is well documented, where campylobacters were isolated from fecal droppings, cloacal swabs, and

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cecal contents of chicken (Ogden *et al.*, 2007; Gormley *et al.*, 2014; Vidal *et al.*, 2016). Poultry are recognized as a major environmental reservoir of *Campylobacter* spp., as colonization of chicken by the bacterium is most likely through contaminated water supply of the broiler and/or campylobacter contamination of the broiler environment (Ogden *et al.*, 2007; Berghaus *et al.*, 2013; Skarp *et al.*, 2016). Once *Campylobacter* colonize the flock, the organism transmits between birds by the fecal–oral route, and *Campylobacter*-positive birds remain colonized until slaughter (Saint-Cyr *et al.*, 2016). Chicken meat obtained from supermarkets and slaughterhouses was found to be heavily contaminated with campylobacters (Wassenaar, 2011; Berghaus *et al.*, 2013; Skarp *et al.*, 2016). The source of meat contamination is the birds themselves, yet the processing (e.g., defeathering; evisceration and packing) could cause cross contamination (Saint-Cyr *et al.*, 2016).

The detection of *Campylobacter* from poultry meat usually involves selective enrichments followed by selective plating on solid media (Seliwiorstow *et al.*, 2016). Various polymerase chain reaction (PCR) protocols have been developed using different sets of primers that target *Campylobacter*-specific genes (e.g., *fla*; *hipO*) for the detection of *Campylobacter* from poultry products (Manzano *et al.*, 1995; Bang *et al.*, 2002), either by direct extraction of target DNA from poultry meat samples (Manzano *et al.*, 1995) or extracting target DNA from selective enrichment culture (Denis *et al.*, 2001).

The detection of antimicrobial-resistant *C. jejuni* in chicken meat is of primary concern as antimicrobial-resistant strains may lead to more prolonged or severe illness than the susceptible strains during the treatment of foodborne campylobacteriosis. It is observed that resistance of *C. jejuni* to fluoroquinolones and macrolides, which are generally used for the treatment of bacterial gastroenteritis, has increased during the past two decades, due to excessive use of this group of antimicrobials in food-producing animals (Grant *et al.*, 2016).

The incidence of *C. jejuni* infections, including multidrug-resistant strains, in the community is well documented in various locations within the Indian subcontinent (Bandeekar *et al.*, 2005; Ghosh *et al.*, 2013; Mukherjee *et al.*, 2013, 2014). The consumption of poultry meat is very popular among Indian communities, as poultry meat is more affordable in comparison to livestock (Marinou *et al.*, 2012). Despite the fact that there are few reports documenting the incidence of *C. jejuni* and their susceptibility profiles in poultry meat and other food commodities in India (Kakkar and Dogra, 1990; Sen-Gupta *et al.*, 1991; Khurana and Kumar, 1996; Kumar *et al.*, 2001; Barua and Rathore, 2006; Singh *et al.*, 2009), little is known about incidence of *C. jejuni* on raw meat sold at retail shops and supermarkets in India. Simultaneously, there is no official surveillance program that can monitor the presence of antimicrobial-resistant *C. jejuni* in food animals (Marinou *et al.*, 2012). Therefore, the present study aimed to investigate the prevalence of *C. jejuni* in raw chicken meat samples collected at consumer retail shop in a semiurban community, Bareilly, India. Furthermore, the presence of *C. jejuni* was also investigated in chicken intestine, chopping boards, and knives to study the cross contamination of poultry meat at retail shops.

Materials and Methods

Sample collection

A total of 400 samples comprising raw chicken meat (150), chicken intestine (150), feathers (50), and chopping boards and knives (50) were collected from local retail shops in Bareilly city, India, as described by Andrews and Hammack (2003). Raw poultry meat and intestines (100 g each sample) were collected in screw-cap jar containing 100 mL of buffered peptone water (0.1%; Hi Media Pvt. Ltd., Mumbai, India) aseptically. Chicken feathers (two to three feathers per sample) were collected in sterile test tubes. Most of the time feathers and meat were collected from the same chicken on the same day. Chopping boards and knife samples were collected using sterile swabs (one swab per sample). All samples were transferred to the laboratory on ice away from direct sunlight. Microbiological examinations began at the same day of sampling.

Isolation and identification of *C. jejuni* by cultural methods

Isolation of *C. jejuni* from chicken meat samples was performed according to the U.S. FDA Bacteriological Analytical Manual method described by Hunt *et al.* (2001) with few modifications. Briefly, raw meat (25 g) sample was homogenized for 2 min in 200 mL of Buffered Peptone Water (Hi Media Pvt. Ltd.) and centrifuged at 16,000 × g for 15 min. The supernatant was discarded, and the pellets were resuspended in 10 mL of Buffered Peptone Water. The 3.0 mL of suspension was transferred to 100 mL *Campylobacter* blood-free enrichment broth and incubated at 30°C for 5 h under microaerobic conditions, using Campy Pak, as generating packs (BD-Becton and Dickinson) for pre-enrichment. The incubation was continued for final enrichment at 42°C for 30 h under microaerobic conditions. Enrichment cultures were streaked onto modified *Campylobacter* blood-free agar (mCCDA; Hi Media Pvt. Ltd.) and incubated at 42°C for 48 h. For the detection of *C. jejuni* from intestine, feathers, chopping board, and knife samples, swabs from each sample were immersed in 10 mL of *Campylobacter* enrichment broth and incubated for pre-enrichment (30°C for 2 h) and enrichment (42°C for 30 h) followed by streaking on mCCDA and incubated at 42°C for 48 h under microaerobic conditions.

The confirmation of presumptive *Campylobacter* colonies growing on mCCDA was performed by examination of the morphological features of typical *Campylobacter* growth on mCCDA that appears as gray, moist, flat, and spreading colonies and microscopic examination after Gram staining, where campylobacters appear as Gram-negative, gull-winged shaped. Further confirmations of presumptive *Campylobacter* isolates were carried out by means of biochemical tests determined by catalase, oxidase, glucose fermentation, production of H₂S on Triple Sugar Iron (TSI) agar (Hi Media Pvt. Ltd.), NO₃ reduction, growth at 25°C, 37°C, and 42°C, and hippurate hydrolysis assay as described by Hunt *et al.* (2001).

Detection of *C. jejuni* by PCR

For PCR based detection of *C. jejuni*, DNA was extracted directly from 1.0 mL aliquots taken from *Campylobacter* enrichment cultures. The *hipO* gene based detection for the identification of *C. jejuni* by PCR was performed as described

by Linton *et al.* (1997) with some modifications. The DNA extraction method was adopted as described by Sambrook and Russell (2001), using DNA Extraction Kit (Genei, Bangalore, India). The primers HIP400F (5'-GAA GAG GGT TTG GGT GGT G-3') and HIP1134R (5'-AGC TAG CTT CGC ATA ATA ACT TG-3') used in this study were synthesized by Agile Life Sciences (Mumbai, India). The PCR for amplification of *hipO* gene (735 bp) was optimized as follows: 5.0 μ L 1 \times final concentration PCR buffer (20 mM Tris HCl, pH 8.0 at 25°C, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween 20, and 0.5% Nonidet P-40), 200 μ M of each dATP, dCTP, dGTP, and dTTP, 0.4 μ M of each primer, 0.4 U of *Taq* DNA polymerase, 2.0 mM MgCl₂, and 5.0 μ L of bacterial DNA. Nuclease-free water was added to obtain a final volume of 50 μ L. PCR conditions included an initial denaturation step at 94°C for 5 min, followed by 30 subsequent cycles consisting of heat denaturation at 94°C for 1 min, annealing at 66°C for 1 min, and extension at 72°C for 2 min. A final extension was performed at 72°C for 7 min. PCR products were separated in 1.5% agarose gel electrophoresis, stained by ethidium bromide (Sigma-Aldrich), and visualized under ultraviolet light. The specificity of PCR assay was determined using reference/standard cultures of *C. jejuni* (MTCC 11168), *C. coli* (MTCC 1126), and *Escherichia coli* (MTCC 443), as positive and negative controls.

Statistical analysis

The *t*-test for comparing the means of two samples was used to determine if there is a difference between the detection of *C. jejuni* by conventional cultural methods and molecular PCR detection. To test the null hypothesis this states that there should be no difference in the detection rates of *C. jejuni* using conventional cultural technique and PCR protocol after selective enrichment.

Determination of antibiotic resistance

Antibiotic resistance profiles of *C. jejuni* isolates were determined by disc diffusion method as described by Giacomelli *et al.* (2014). Nine commercial sensitivity discs (Hi Media Pvt. Ltd.) that belong to six classes of drugs were used as follows: Macrolides: Azithromycin (15 μ g/mL) and Erythromycin (15 μ g/mL), Cephalosporins: Cephalothin (30 μ g/mL), Fluoroquinolones: Ciprofloxacin (5 μ g/mL), Quinolones: Nalidixic acid (30 μ g/mL), Sulfonamides: co-trimoxazole (25 μ g/mL), Aminoglycosides: Gentamicin (10 μ g/mL), Tetracyclines: Tetracycline (30 μ g/mL), and Phenicol: Chloramphenicol (30 μ g/mL). Briefly, 0.1 mL of test bacterial suspension (10⁸ colony forming unit [CFU]/mL) was prepared in brain-heart infusion broth (BHI; Pronadisa) and was spread onto mCCDA plates (Hi Media Pvt. Ltd.). The plates were incubated at 37°C for 18 h microaerobically. Inhibition bacterial growth diameter around the antibiotic disc was measured.

Results

Prevalence of *C. jejuni* in poultry and poultry-related samples

The prevalence of *C. jejuni* in all types of samples is presented in Table 1. Both conventional and PCR based detection methods were applied. Using conventional cultural methods, *C. jejuni* was detected in 58 of raw meat samples

TABLE 1. PREVALENCE OF *CAMPYLOBACTER JEJUNI* IN POULTRY CARCASS, CHOPPING BOARDS, KNIVES, AND FEATHERS

Sample	Prevalence of <i>C. jejuni</i> (%) by cultural methods ^a	Prevalence of <i>C. jejuni</i> (%) by PCR ^b
Raw chicken meat (150)	58 (38.7)	54 (36.0)
Chicken intestine (150)	36 (24.0)	36 (24.0)
Chopping boards and knives (50)	7 (14.0)	7 (14.0)
Feathers (50)	4 (8.0)	4 (8.0)
Total (400)	105 (26.3)	101 (25.3)

No statistical significant difference (p =NS) at 5% (*t*-test for comparing the means of two samples).

^aCultural methods were composed of selective enrichment followed by selective plating.

^bPCR based detection was performed after selective enrichment step.

PCR, polymerase chain reaction.

(38.7%, n =150) followed by intestinal samples (24.0%, n =150). The detection of *C. jejuni* by PCR, targeting the *hipO* gene (735 bp), after selective enrichment showed that 54 of raw meat (36.0%, n =150) and 36 of intestine samples (24.0%, n =150) were positive for *C. jejuni*, respectively. The detection rate of *C. jejuni* in other samples is listed in Table 1 and Figure 1. Overall, 105 presumptive campylobacters were recovered, of which only 101 isolates were confirmed as *C. jejuni* by both conventional cultural and PCR method and 4 isolates by cultural methods only. Overall among 400 samples were collected; 26.3% and 25.3% samples were positive for the presence of *C. jejuni* by cultural and PCR based methods, respectively. No statistical significant differences (*t*-test) were found in the detection rates of *C. jejuni* in all samples using conventional cultural technique and PCR after selective enrichment (Table 1).

Antibiotic resistance profiles of *C. jejuni*

The antibacterial resistance profiles of a total of 101 isolates of *C. jejuni* were determined against 9 antibiotics. The antibiotic susceptibility profiles confirmed drug resistance among 97% of *C. jejuni* isolates, with 84.1% of *C. jejuni* isolates resistant to co-trimoxazole followed by cephalothin (81.1%) and tetracycline (59.4%). Low incidence of resistance (6.9–8.9%) was observed against nalidixic acid, ciprofloxacin, erythromycin, gentamicin, and azithromycin. Only 3 isolates out of 101 (2.9%) were susceptible to all antibiotics tested (Table 2). There were 27 resistance patterns exhibited among the 101 isolates of *C. jejuni*. The most common resistance pattern was detected in 26 (25.7%) of the isolates, which were resistant to cephalothin, co-trimoxazole, and tetracycline. Resistance to 2 or more antibiotics was found in 95 (94.0%) of the isolates. It was found that 10 (9.9%) isolates were resistant to 5–6 antibiotics. Overall resistance to multiple drugs (≥ 4) was recorded in 31.6% isolated strains (Fig. 2 and Table 3). The criterion for multiresistance is defined by resistance to three or more classes of antimicrobial agents (Schwarz *et al.*, 2010); thus, 25.7% of the isolates were exhibiting multi-resistance pattern to three classes of antimicrobials, namely

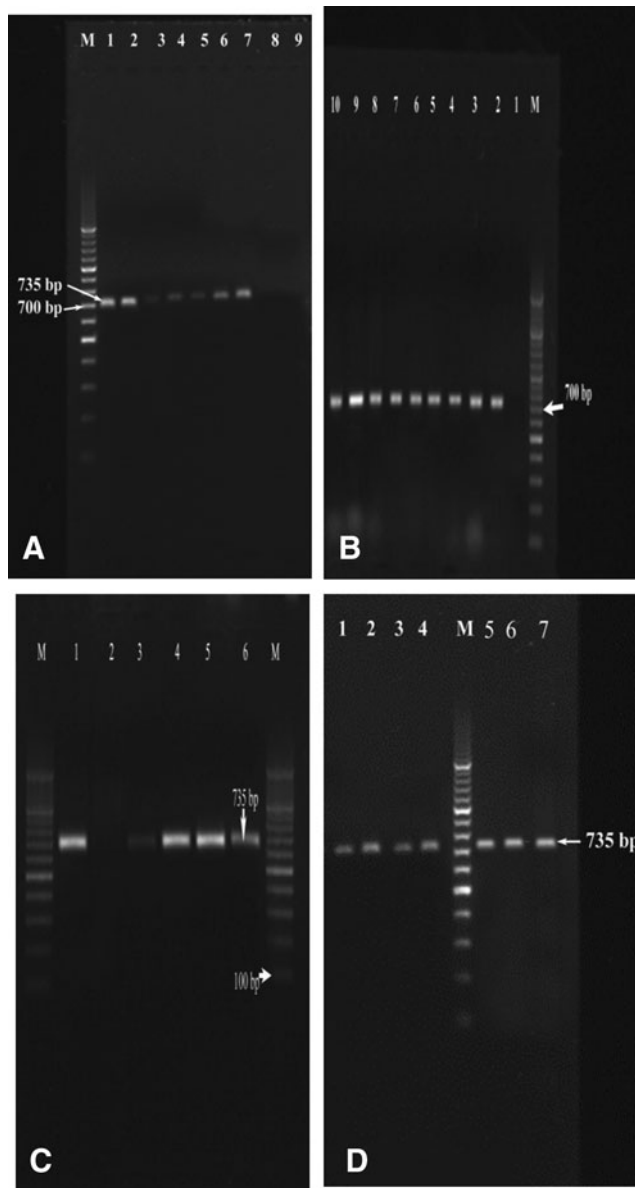


FIG. 1. *hipO* gene (735 bp) based identification of *Campylobacter jejuni* isolates by PCR. Lane M: DNA ladder (100 bp), (A) chicken meat samples: Lanes 1–7: isolates positive for *C. jejuni*, Lanes 8 and 9: isolates negative for *C. jejuni*, (B) intestinal samples: Lane 1: blank (negative control), Lanes 2–10: isolates positive for *C. jejuni*, (C) feather samples: Lane 1: *C. jejuni* (NCTC-11168), Lane 2: blank (negative control), Lanes 3–6: isolates positive for *C. jejuni*, (D) chopping board and knife samples: Lanes 1–7: isolates positive for *C. jejuni*. PCR, polymerase chain reaction.

cephalosporins, sulfonamides, and tetracyclines. A notable multiresistance pattern to five different classes of antimicrobial agents (cephalosporins, sulfonamides, quinolones, macrolides, and phenicols) was observed in four isolates (3.96%, $n = 101$) (Table 3).

Discussion

C. jejuni accounts for the majority of campylobacteriosis in humans, by which half of these cases originate from the

TABLE 2. PREVALENCE OF ANTIBIOTIC RESISTANCE IN *CAMPYLOBACTER JEJUNI* ISOLATED FROM RAW MEAT, INTESTINE, BOARDS, KNIVES, AND FEATHERS

Antibiotics	Drug class	No. of resistant (%)
		<i>C. jejuni</i> ($n = 101$)
Azithromycin	Macrolides	9 (8.9)
Erythromycin		7 (6.9)
Cephalothin	Cephalosporins	82 (81.1)
Ciprofloxacin	Fluoroquinolones	7 (6.9)
Nalidixic acid	Quinolones	7 (6.9)
Co-trimoxazole	Sulfonamides	85 (84.1)
Gentamicin	Aminoglycosides	7 (6.9)
Tetracycline	Tetracyclines	60 (59.4)
Chloramphenicol	Phenicols	51 (50.5)

Three isolates (2.9%) were sensitive to all antibacterial drugs tested.

consumption of undercooked contaminated meat, unpasteurized dairy products, and contaminated water (Jones, 2001). Of these different sources of *Campylobacter*-associated infections, poultry is considered as the most important source of human campylobacteriosis (Josefsen *et al.*, 2015).

Detection of campylobacters in food and environmental samples by PCR after selective enrichment step has provided more accurate accounts of the true incidence of *Campylobacter* spp., as well as reduces the time for detection and eliminates the need for conventional confirmatory tests (Abulreesh *et al.*, 2017). We report 25.3% detection of *C. jejuni* in samples ($n = 400$) examined, by PCR after selective enrichment step, compared to 26.3% by conventional cultural methods; however, no statistical significant differences in the detection rates of *C. jejuni* were found between conventional and molecular techniques as determined by *t*-test. Few samples of the presumptive positive isolates recovered by cultural methods were not confirmed as *C. jejuni*, by PCR method, which requires further detailed investigation by 16S rRNA gene sequence analysis. The detection of campylobacters by PCR after selective enrichment step has been recommended as a standardized method for the detection of *Campylobacter* spp. in poultry products, dairy products, drinking water, and aquatic environments (Denis *et al.*, 2001; Abulreesh *et al.*, 2006, 2014; Gharst *et al.*, 2013), as well in fecal samples where campylobacters are present in relatively higher numbers than in food or environmental samples (Rasmussen *et al.*, 1996; Vanniasinkam *et al.*, 1999).

The prevalence of *C. jejuni* on raw poultry meat and other poultry products that are available for consumers at points of sale (e.g., supermarkets, retail shops) is of great concern from a public health standpoint; therefore, various studies worldwide investigated the incidence of *C. jejuni* on raw chicken meat. In this study, we found that 36% ($n = 150$) of raw chicken meat sold at local retail shops is contaminated with *C. jejuni*; this is higher than the prevalence rate reported in Argentina (14.5%, $n = 60$) (Zbrun *et al.*, 2015), also higher than 29.3% ($n = 33$) prevalence rate reported in Japan (Kojima *et al.*, 2015). In Europe, the prevalence was even lower in countries like Spain, Italy, and Sweden (30%, 11%, and 8%, respectively) as reported by Mozina *et al.* (2011) in survey conducted between 2003 and 2007; lower prevalence rate of 17.2% ($n = 302$) was also reported in China (Zhang

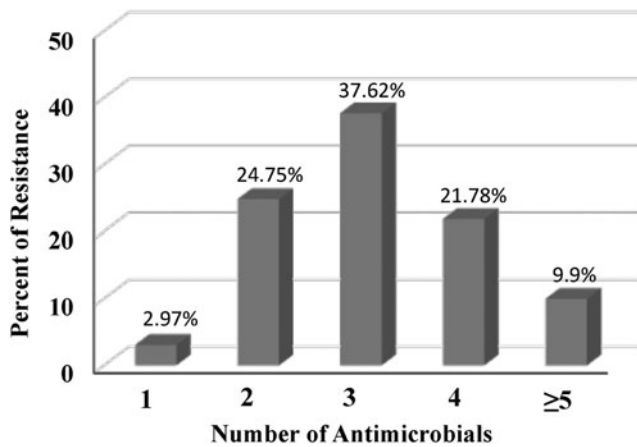


FIG. 2. Antimicrobial resistance to different combinations of antibacterial drugs among 101 *C. jejuni* isolates.

et al., 2016). Conversely, higher prevalence of *Campylobacter* spp. on raw poultry meat at supermarkets or retail shops, higher than what we reported in this study, was found in Qatar with 48% ($n=400$) (Abu-Madi *et al.*, 2016). In a 7-year study in the United States, the average prevalence of *Campylobacter* spp. on raw poultry meat was found to be 41% ($n=755$), with higher prevalence recorded in 2005 (47%), followed by gradual reduction over the years to reach 34% in 2011 (Williams and Oyarzabal, 2012). High prevalence of *C. jejuni* in poultry also observed in various locations in India (Baserisalehi *et al.*, 2007; Rajendran *et al.*, 2012), together with the results reported in this study, may shed some light on the incrimination of poultry as a possible reservoir of human campylobacteriosis in northern India.

Although the differences of sampling design and testing methods may play a role in the variation of the results reported in these studies, nonetheless, the strict surveillance/monitoring programs of hygiene practices that are applied to slaughterhouses and processing plants may, in part, contribute to the low prevalence of *Campylobacter* contamination in raw poultry meat, particularly in the European Union (EU), where monitoring of *Campylobacter* in poultry is mandatory (Josefsen *et al.*, 2015; Mezher *et al.*, 2016). This was observed in some EU countries like France and Germany with higher *Campylobacter*-contaminated poultry meat at retail shops (85% and 40%, respectively); however, these high prevalence rates showed sharp decrease over the years (<30%) due to improved slaughtering practices that reduce cross contamination (Mozina *et al.*, 2011). In India no such surveillance programs are implemented; thus, cross contamination is highly likely to occur, and therefore, high prevalence of *C. jejuni* on raw poultry meat is highly likely to occur.

Furthermore, the local retail shops that were surveyed in this study were performing slaughtering, defeathering, and rinsing of chicken carcass before sale. Common knives, chopping boards, defeathering boards, and common washing tanks are all used throughout the day at these premises; this was obvious in the contamination of the equipment used in these shops by *C. jejuni* (14%, $n=50$). Given the fact that the colonization of *Campylobacter* in chicken is usually high (10^{10} CFU/g cecum content), chicken carcass can be easily contaminated during

TABLE 3. ANTIBIOTIC RESISTANCE PATTERNS AMONG 101 ISOLATES OF *CAMPYLOBACTER JEJUNI*

Isolate origin	Antibiotic resistance pattern	No. of strains
C (2)	Ch, Cl, Ct, T, Cf, G	02
C (1)	Ch, Cl, Ct, T, Na, Cf	01
C (2), I (2)	Az, Ch, Cl, Ct, Na, Er	04
C (1)	Ch, Cl, Na, Cf, Er	01
I (1)	Ch, Ct, T, Na, Cf	01
I (1)	Ch, Cl, Ct, T, G	01
C (30), I (1)	Az, Cl, Ct, T	04
C (5), I (8), F (1)	Ch, Cl, Ct, T	14
C (1)	Ch, Cl, T, G	01
C (1)	Cl, Ct, T, Er	01
I (1)	Ch, Ct, T, Er	01
I (1)	Ch, Cl, Ct, G	01
C (17), I (8), F (1)	Ch, Ct, T	26
C (5), I (1), CK (1), F (1)	Ch, Cl, Ct	08
I (1)	Ch, Cl, T	01
I (1)	Ch, Ct, G	01
C (1)	Cl, Ct, G	01
CK (1)	Az, Ch, Cl	01
C (7), I (4), CK (2)	Ch, Ct	13
C (3)	Ch, T	03
C (3)	Cl, Ct	03
C (2), I (2)	Ct, T	04
C (1)	Ch, Cf	01
I (1)	Ct, Cf	01
CK (1)	Ch	01
I (2)	Ct	02
I (1), F (1), CK (1)	Not resistant to any drug tested	03

C (n) = from chicken; CK (n) = from chopping boards and knives; F (n) = from feathers; I (n) = from intestine.

Az, azithromycin; Cf, ciprofloxacin; Ch, cephalothin; Cl, chloramphenicol; Ct, co-trimoxazole; Er, erythromycin; G, gentamicin; Na, nalidixic acid; T, tetracycline.

slaughtering, defeathering, and processing, either directly from the intestinal content or indirectly through washing water and other equipments; even *Campylobacter*-negative flocks can be contaminated during and after slaughtering process, as a result, the load of the bacterium on the meat ready for sale is usually high (Cason *et al.*, 1999; Josefsen *et al.*, 2015). However, proper hygiene practices after slaughtering (e.g., defeathering, washing, and packaging) resulted in sharp decrease in the loads of campylobacters on poultry meat and other poultry products sold at supermarkets and retail shops. This was observed in Italy, with decreasing rates of *C. jejuni* on poultry carcass noted among slaughterhouses (80%), deboning (49%), butcheries (34%), and finally, large-scale retailers (25%) (Stella *et al.*, 2017). With lack of surveillance/monitoring of retail shops that practice slaughtering and processing of poultry before sale in India, cross contamination is unavoidable, and therefore, the risk of campylobacteriosis in the community is inevitable.

Campylobacteriosis is a self-limiting disease that does not require antibiotic treatment in the majority of cases, unless in those immunocompromised patients (Kaakoush *et al.*, 2015). However, the incidence of multidrug-resistant *Campylobacter* spp. in food production animals and the food chain in general is of great importance to public health authorities.

In this study, we found that 97% ($n = 101$) of *C. jejuni* isolated from raw poultry meat and slaughtering equipments at retail shops are resistant to different antimicrobials. We recorded a common multiresistant pattern in 25.7% of the isolates to three different classes of antimicrobial agents; however, multiresistance to more than three classes was less common, for example, 3.96% were multiresistant to five different classes of antimicrobials. Similar resistant trends were found in central Europe where nearly half of *Campylobacter* spp. originates from poultry meat at retail shops were resistant to at least two unrelated antimicrobial drugs used in human and veterinary medicine (Mozina *et al.*, 2011).

Unlike the resistant trend that has been reported in various studies (Haruna *et al.*, 2012; Agunos *et al.*, 2014; Giacomelli *et al.*, 2014; Wiczorek and Osek, 2015), we found that the prevalence of *C. jejuni* isolates resistant to fluoroquinolones in this study was low; however, prevalence of tetracycline-resistant *C. jejuni* was shown to be similar to the trends reported elsewhere (Jore *et al.*, 2010; Kovalenko *et al.*, 2014; Wiczorek and Osek, 2015). The use of fluoroquinolones and tetracyclines as therapeutic agents for food-producing animal may result in the emergence of resistant strains of *C. jejuni* that can be transmitted to humans (Wiczorek and Osek, 2015).

Low incidence of resistance was observed in this study against macrolides, that is, azithromycin (8.9%) and erythromycin (6.9%) in the strains of *C. jejuni*. This is in agreement with other studies reported by many workers (Haruna *et al.*, 2012; Kovalenko *et al.*, 2014; Wiczorek and Osek, 2015). Erythromycin was suggested to be the drug of choice for treating *Campylobacter*-associated infections, as *C. jejuni* appears to be predominantly susceptible to erythromycin with low levels of resistance (Wiczorek and Osek, 2015). The results reported in this study reinforce the recommendation that the indiscriminate use of antibiotic in human and veterinary medicine in India should be controlled, and the implementation of surveillance of antibiotic susceptibility patterns of *Campylobacter* in poultry and other food production animals, which can be transmitted to human through the food chain, is highly recommended.

Conclusion

The overall prevalence of *C. jejuni*, particularly multidrug-resistance strains on raw poultry meat and slaughtering equipments isolated from local retail shops in semiurban areas in India, is of great concern to public health, since the consumption of poultry meat is very popular in Indian communities and poultry meat is the major source of human campylobacteriosis; thus, antimicrobial-resistant *C. jejuni* can be easily transmitted to humans that increase the burden of *Campylobacter* infections in the community. Therefore, implementation of monitoring/surveillance programs to monitor the prevalence of *C. jejuni*, particularly multidrug-resistant campylobacters in food production animals, particularly raw poultry meat that is sold in markets of semiurban regions, as well as main cities in India, is highly required for better public health protection.

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Authors' Contributions

J.A.K. performed experimental work and prepared a rough draft of the article, R.S.R. experiment designing and supervision, H.H.A. article write up, F.A.Q. data analysis, and I.A. experiment designing, supervision, and editing the final draft of the article.

Disclosure Statement

No competing financial interests exist.

References

- Abulreesh HH, Organji SR, Elbanna K, Osman GEH, Almalki MHK, Ahmad I. *Campylobacter* in the environment: A major threat to public health. *Asian Pac J Trop Dis* 2017;7:374–384.
- Abulreesh HH, Paget TA, Goulder R. *Campylobacter* in waterfowl and aquatic environments: Incidence and methods of detection. *Environ Sci Technol* 2006;40:7122–7131.
- Abulreesh HH, Paget TA, Goulder R. A pre-enrichment step is essential for detection of *Campylobacter* sp. in turbid pond water. *Trop Biomed* 2014;31:320–326.
- Abu-Madi M, Behnke JM, Sharma A, Bearden R, Al-Banna N. Prevalence of virulence/stress genes in *Campylobacter jejuni* from chicken meat sold in Qatari retail outlets. *PloS One* 2016;11:e0156938.
- Agunos A, Leger D, Avery BP, Parmley EJ, Deckert A, Carson CA, Dutil L. Ciprofloxacin-resistant *Campylobacter* spp. in retail chicken, Western Canada. *Emerg Infect Dis* 2014;19:1121–1124.
- Andrews WH, Hammack TS. Food sampling and preparation of sample homogenate. In: *Food and Drug Administration Bacteriological Analytical Manual*, 8th ed. (revision A), Chapter 1. Jackson GJ, Merker RI, Bandler R, eds. Gaithersburg, MD: AOAC International, 2003. Available at: www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063335.htm, accessed June 20, 2016.
- Bandekar JR, Raut AD, Kapadnis BP. Occurrence of *Campylobacter* in local poultry meat in Pune and Mumbai. *Indian Convention of Food Scientists & Technologists* 2005;120–123.
- Bang DD, Wedderkopp A, Pedersen K, Madsen M. Rapid PCR using nested primers of the 16S rRNA and the hippuricase (*hipO*) genes to detect *Campylobacter jejuni* and *Campylobacter coli* in environmental samples. *Molec Cell Prob* 2002;16:359–369.
- Barua R, Rathore RS. Development of modified selective media for the isolation of *C. jejuni* from poultry. *J Food Sci Technol* 2006;45:493–496.
- Baserisalehi M, Bahador N, Kapadins BP. A comparison study of antimicrobial susceptibility of *Campylobacter* spp. isolates from faecal samples of domestic animals and poultry in India and Iran. *J Biol Sci* 2007;7:977–980.
- Berghaus RD, Thayer SG, Law BF, Mild RM, Hofacre CL, Singer RS. Enumeration of *Salmonella* and *Campylobacter* spp. in environmental farm samples and processing plant carcass rinses from commercial broiler chicken flocks. *Appl Environ Microbiol* 2013;79:4106–4114.
- Cason JA, Buhr RJ, Dickens JA, Musgrove MT, Stern NJ. Carcass microbiological quality following intermittent scalding and defeathering. *J Appl Poult Res* 1999;8:368–373.
- Denis M, Refregier-Petton J, Laisney MJ, Ermel G, Salvat G. *Campylobacter* contamination in French chicken production from farm to consumers, use of a PCR assay for detection and identification of *Campylobacter jejuni* and *Camp. coli*. *J Appl Microbiol* 2001;91:255–267.

- Gharst G, Oyarzabal OA, Hussain SK. Review of current methodologies to isolate and identify *Campylobacter* spp. from foods. *J Microbiol Methods* 2013;95:84–92.
- Ghosh R, Uppal B, Aggarwal P, Chakravarti A, Kumar A. Increasing antimicrobial resistance of *Campylobacter jejuni* isolated from paediatric diarrhea cases in a tertiary care hospital of New Delhi, India. *J Clin Diagn Res* 2013;7:247–249.
- Giacomelli M, Salata C, Martini M, Montesissa C, Piccirillo A. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* from poultry in Italy. *Microb Drug Resist* 2014;20:181–188.
- Gormley FJ, Bailey RA, Watson KA, McAdam J, Avendano S, Stanley WA, Koerhuis ANM. *Campylobacter* colonization and proliferation in the broiler chicken upon natural field challenge is not by the bird growth rate or breed. *Appl Environ Microbiol* 2014;80:6733–6738.
- Grant A, Hashem F, Parveen S. *Salmonella* and *Campylobacter*: Antimicrobial resistance and bacteriophage control in poultry. *Food Microbiol* 2016;53:104–109.
- Haruna M, Sasaki Y, Murkami M, Ikeda A, Kusakawa M, Tsujuyama Y, Ito K, Asai T, Yamada Y. Prevalence and antimicrobial susceptibility of *Campylobacter* in broiler flocks in Japan. *Zoon Pub Health* 2012;59:241–245.
- Hunt JM, Abeyta C, Tran T. *Campylobacter*. In: *Food and Drug Administration Bacteriological Analytical Manual*, 8th ed. (revision A), Chapter 7. Jackson GJ, Merker RI, Bandler R, eds. Gaithersburg, MD: AOAC International, 2001. Available at: www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm072616.htm, accessed June 10, 2016.
- Jones K. *Campylobacters* in water, sewage and the environment. *Symp Ser Soc Appl Microbiol* 2001;90:68S–79S.
- Jore S, Viljugrein H, Burn E, Heier BT, Borck B, Ethelberg S, Hakkinen M, Kuusi M, Reiersen J, Hansson I, Engvall O, Lofdahl M, Wagenaar JA, van Pelt W, Hofshagen M. Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997–2007. *Prevent Vet Med* 2010;93:33–41.
- Josefsen MH, Bhunia AK, Engvall EO, Fachmann MSR, Hoorfar J. Monitoring *Campylobacter* in the poultry production chain—From cultures to genes and beyond. *J Microbiol Methods* 2015;112:118–125.
- Kaakoush NO, Castano-Rodriguez N, Mitchell HM, Man SM. Global epidemiology of *Campylobacter* infection. *Clin Microbiol Rev* 2015;28:687–720.
- Kakkar M, Dogra SC. Prevalence of *Campylobacter* infections in animals and children in Haryana, India. *J Diarr Dis Res* 1990;8:34–36.
- Khurana RK, Kumar A. Examination of fresh meat and egg for *C. jejuni* and *C. coli*. *Indian J Comp Microbiol Immunol Infect Dis* 1996;17:41–44.
- Kojima C, Kishimoto M, Ezaki T. Distribution of antimicrobial resistance in *Campylobacter* strains isolated from poultry at a slaughterhouse and supermarkets in Japan. *Biocont Sci Technol* 2015;20:179–184.
- Kovalenko K, Roasto M, Santare S, Berzins A, Horman A. *Campylobacter* species and their antimicrobial resistance in Latvian broiler chicken production. *Food Cont* 2014;46:86–90.
- Kumar A, Aggarwal RK, Bhilegaonkar KN, Shome BR, Bachhil VN. Occurrence of *C. jejuni* in vegetables. *Int J Food Microbiol* 2001;67:153–155.
- Linton D, Lawson AJ, Owen RJ, Stanley J. PCR detection, identification to species level, and finger printing of *Campylobacter jejuni* and *C. coli* direct from diarrheic samples. *J Clin Microbiol* 1997;35:2568–2572.
- Manzano M, Pipan C, Botta G, Comi G. Polymerase chain reaction assay for detection of *Campylobacter coli* and *Campylobacter jejuni* in poultry meat. *Zentralbl Hyg Umweltmed* 1995;197:370–386.
- Marinou I, Bersimis S, Ioannidis A, Nicolaou C, Mitroussia-Ziouva A, Johnlegakia N, Chatzipanagiotou S. Identification and antimicrobial resistance of *Campylobacter species* isolated from animal sources. *Front Microbiol* 2012;3:1–6.
- Mezher Z, Saccare S, Marciano R, De Santis P, Rodas EMF. Occurrence of *Campylobacter* spp. in poultry meat at retail and processing plants’ level in central Italy. *Italian J Food Safety* 2016;5:5495.
- Mozina SS, Kurincic M, Klancnik A, Mavri A. *Campylobacter* and its multi-resistance in the food chain. *Trends Food Sci Technol* 2011;22:91–98.
- Mukherjee P, Ramamurthy T, Bhattacharya MK, Rajendran K, Mukhopadhyay AK. *Campylobacter jejuni* in hospitalized patients with diarrhea, Kolkata, India. *Emerg Infect Dis* 2013;19:1155–1156.
- Mukherjee P, Ramamurthy T, Mitra U, Mukhopadhyay AK. Emergence of high-level azithromycin resistance in *Campylobacter jejuni* isolates from pediatric diarrhea patients in Kolkata, India. *Antimicrob Agents Chemother* 2014;58:4248.
- Ogden ID, MacRae M, Johnston M, Strachan NJC, Cody AJ, Dingle KE, Newell DG. Use of multilocus sequence typing to investigate the association between the presence of *Campylobacter* spp. in broiler drinking water and *Campylobacter* colonization in broiler. *Appl Environ Microbiol* 2007;73:5125–5129.
- Rajendran P, Babji S, George AT, Rajan DP, Kang G, Ajjampur SS. Detection and species identification of stool samples of children and animals from Vellore, south India. *Indian J Med Microbiol* 2012;30:85–88.
- Rasmussen HN, Olsen JE, Jorgensen K, Rasmussen OF. Detection of *Campylobacter jejuni* and *Camp. coli* in chicken faecal samples by PCR. *Lett Appl Microbiol* 1996;23:363–366.
- Saint-Cyr MJ, Guyard-Nicodeme M, Messaoudi S, Chemaly M, Cappelier MJ, Dousset X, Haddad N. Recent advances in screening of anti *Campylobacter* activity in probiotic for use in poultry. *Front Microbiol* 2016;7:Article 553.
- Sambrook J, Russell DW. *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Press, 2001.
- Schwarz S, Silley P, Simjee S, Woodford N, van Duijkeren E, Johnson AP, Gaastra W. Assessing the antimicrobial susceptibility of bacteria obtained from animals. *Vet Microbiol* 2010;141:1–4.
- Seliwiorstow T, De Zutter L, Houf K, Bottledorn N, Bare J, Van Damme I. Comparative performance of isolation methods using Preston broth, Bolton broth and their modifications for the detection of *Campylobacter* spp. from naturally contaminated fresh and frozen raw poultry meat. *Int J Food Microbiol* 2016;234:60–64.
- Sen-Gupta PG, Nair GB, Mondal S, Gupta DN, Sen D, Sikdar SN, Das P, Sarkar RK, Ghosh S, Saha NC, Deb BC, Pal SC. Epidemiology of campylobacteriosis in a cohort of rural population near Calcutta. *Epidemiol Infect* 1991;106:507–512.
- Singh R, Singh PP, Rathore RS, Dhama K, Malik SVS. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in chicken meat and carcasses collected from local poultry

- farms and retail shops of Bareilly, Uttar Pradesh, India. Indian J Comp Microbiol Immunol Infect Dis 2009;30:35–38.
- Skarp CPA, Hänninen ML, Rautelin HIK. Campylobacteriosis: The role of poultry meat. Clin Microbiol Infect 2016;22:103–109.
- Stella S, Soncini G, Ziino G, Panebianco A, Pedonese F, Nuvoloni R, Di Giannatale E, Colavita G, Alberghini L, Giaccone V. Prevalence and quantification of thermophilic *Campylobacter* spp. in Italian retail poultry meat: Analysis of influencing factors. Food Microbiol 2017;62:232–238.
- Vanniasinkam T, Lanser JA, Barton MD. PCR for the detection of *Campylobacter* spp. in clinical specimens. Lett Appl Microbiol 1999;28:52–56.
- Vidal AB, Colles FM, Rodgers JD, McCarthy ND, Davis RH, Maiden MCJ, Clifton-Hadley FA. Genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* isolates from conventional broiler flocks and the impacts of sampling strategy and laboratory methods. Appl Environ Microbiol 2016;82:2347–2355.
- Wassenaar TM. Following an imaginary *Campylobacter* population from farm to fork and beyond: A bacterial perspective. Lett Appl Microbiol 2011;53:253–263.
- Weiczorek K, Osek J. A five-year study on prevalence and antimicrobial resistance of *Campylobacter* from poultry carcasses in Poland. Food Microbiol 2015;49:151–156.
- Williams A, Oyarzabal O. Prevalence of *Campylobacter* spp. in skinless, boneless retail broiler meat from 2005 through 2011 in Alabama, USA. BMC Microbiol 2012;12:184.
- Zbrun MV, Olivero C, Romero-Sharp A, Rossler E, Soto LP, Astesana DM, Blajman JE, Bersivil A, Signorini ML, Frizzo LS. Antimicrobial resistant in thermotolerant *Campylobacter* isolated from different stages of the poultry meat supply chain in Argentina. Food Cont 2015;57:136–141.
- Zhang T, Luo Q, Li T, Wen G, Zhang R, Luo L, Lu Q, Ai D, Wang H, Shao H. Molecular epidemiology, virulence determinants, and antimicrobial resistance of *Campylobacter* spreading in retail chicken meat in central China. Gut Pathogens 2016;8:48.

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