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Isolation, Identification and Antimicrobial Susceptibility Profiles of *Campylobacter* Species with Assessment of Their Risk Factors in Broiler Flocks of Bangladesh Agricultural University Poultry Farm

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ABSTRACT

The objective of this study is to isolate, identifies and antibiogram characterizes of the *Campylobacter* species from the cloacal swab and litter samples collected from broiler flocks of BAU poultry farm. Moreover, standard questionnaires were adopted for the exposure assessment of *Campylobacter* spp. in the selected broiler farms. A total of 80 samples were subjected to bacterial isolation and identification by using cultural and biochemical techniques. Furthermore, the isolated *Campylobacter* species were characterized by antimicrobial susceptibility testing. Among the 63 positive *Campylobacter* isolates 74.60% (n = 47) were *Campylobacter jejuni*, and the rest 25.39% isolates (n = 16) were *Campylobacter coli*. *Campylobacter jejuni* were resistant to ampicillin, tetracycline and nalidixic acid and susceptible to gentamicin, chloramphenicol and azithromycin. Furthermore, *Campylobacter coli* were resistant to ampicillin, tetracycline and erythromycin and susceptible to streptomycin and chloramphenicol. Out of 63 *Campylobacter* isolates, 87.23% *Campylobacter jejuni*, and 100% *Campylobacter coli* were detected as multidrug resistant. For the exposure assessment of *Campylobacter* spp. in the selected farms indicated that the maximum farms used electricity power and tubewell water to maintain their farms properly and also contained more than five thousands chicks and used pellet feed for their chicks and followed regular vaccination schedule. For the assessment of biosecurity level in the farms also indicated that highest number of farms removed poultry litter and manure when poultry house is empty and also used disinfectant after cleaning the house and footbath at entrance of poultry house and they also allowed vehicles and poultry cages through a disinfectant spray at farm gate to prevent microorganisms from outsides. On the other hand, many farms allowed visitors walk through a disinfectant footbath and workers change clothes and farm boots when entry into farm. During the study period, it was seemed that most of the farms strictly maintained biosecurity level and unauthorized person should be prohibited.

KEYWORDS: Broiler flocks, *Campylobacter jejuni*, *Campylobacter coli*, risk factors, antimicrobial susceptibility profiles

INTRODUCTION

Campylobacter species are Gram-negative, motile, nonspore-forming, curved-rod shaped bacteria that are approximately 0.2 to 0.5 µm wide and about 0.5 to 5 µm long. The ideal environment for optimal recovery of *Campylobacter* spp. is an atmosphere containing approximately 5% O_2, 10% CO_2, and 85% N_2. Although thermophilic *Campylobacter* is highly prevalent in poultry particularly broilers and turkeys, this organism is rarely detected in commercial broiler flocks under the age of 2 - 3 weeks old (Sahin *et al.*, 2002). Interestingly, once *Campylobacter* organisms are isolated from the flock of around 3 weeks of age, most of the birds in that particular flock will become colonized and environment within or around the poultry house seems to be contaminated with *Campylobacter* spp. (Sahin *et al.*, 2002). Although many studies suggest that horizontal transmission from contaminated farm environment was the major mode of *Campylobacter* colonization in broiler flocks (Jacobs-Reitsma *et al.*, 1995; Newell and Fearnley, 2003),

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several findings indicated that vertical transmission from breeders might also play a role on *Campylobacter* infections in broilers (Shanker *et al.*, 1986; Chuma *et al.*, 1994).

Poultry and poultry products were considered to be the primary source of infection. It is now accepted that *Campylobacter*iosis is predominantly acquired through the consumption of contaminated foods (Humphrey *et al.*, 2007). The indiscriminate use of antimicrobial agents in food animals had resulted in the emergence and dissemination of antimicrobial resistant bacteria, including antimicrobial-resistant *Campylobacter* (Aarestrup and Engberg, 2001), which has potentially serious impact on food safety in both Veterinary and Human health. Although *Campylobacter* with resistance to antimicrobial agents has been reported worldwide (Isenbarger *et al.*, 2002), the situation seems to deteriorate more rapidly in developing countries, where there is widespread and uncontrolled use of antibiotics (Englen *et al*,2003). Moreover, *Campylobacter* infections pose a serious public health problem for which many countries have monitored their infection and antimicrobial resistance patterns (Gaudreau and Gilbert, 1998; Ge *et al.*, 2003; Chen *et al.*, 2010; Kabir *et al.*, 2011; Kabir, 2011).

A few studies from Bangladesh have documented the isolation of *Campylobacter* from patients with diarrhea (Blaser *et al.*, 1980; Alam *et al.*, 2006); however, no documented reports exist yet on the prevalence, antimicrobial resistance and risk factors of *Campylobacter* species in broiler flocks associated with their environments in Bangladesh. Therefore, the aim of the present study is to isolate, identify, determine antimicrobial resistance patterns and the associated potential risk factors of *Campylobacter* species colonized in broiler flocks raised in farm environments in Mymensingh.

MATERIALS AND METHODS

Study area

Cloacal swab and litter samples were collected from the broiler flocks of BAU poultry farm, Mymensingh and transported through ice flasks to the laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh for isolation, identification and antibiogram study of *Campylobacter* spp. along with risk factors analysis for *Campylobacter* colonization in the broiler flocks.

Collection and transportation of samples

A total of 80 samples (cloacal swab and Litter samples) were collected and immediately brought to Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh through cool chain maintaining. After that, the samples were processed immediately for the isolation and identification of *Campylobacter* spp.

Isolation of *Campylobacter* spp.

Isolation of *Campylobacter* spp. were carried out by filtration method (0.45 µm filter) as described by Shiramaru *et al.* (2012). The collected samples were allowed to prepare suspension into eppandorf tube with PBS and then 100µlof suspensions were spread on the filter papers that were placed on the surface of Blood base agar no.2 and allowed to stand for 30 min at room temperature. After 30 minutes just removed the filter from the BBA and then incubated the plates at 37ºC for 48 hrs in microaerobic condition (5% O2, 10% CO2 and 85% N2). After 48h the incubated media were then examined for growth of bacteria. Grey, flat and irregularly spreading colonies were observed on BBA. The colony was then subjected to Gram's Method of staining and observed under microscope for Gram negative curve. The organisms from the agar media were then sub-cultured into Blood agar with the help of inoculating loop in case of gram negative curve in the smears. In case of Blood agar grey, flat and irregularly spreading colony were observed. Thus, single pure colony was obtained. These pure isolates obtaining in this way were used for the further study.

Gram's staining

The *Campylobacter* colonies were characterized morphologically using Gram's stain according to the method described by Khachatourians, G. G. (1998). Briefly, a small colony was picked up from Blood agar plates with a bacteriological loop, smeared on separate glass slide with a drop of distilled water and fixed by gentle heating. Crystal violate was then applied on each smear to stain for two minutes followed by washing with running water. Few drops of Gram's Iodine was then added which acted as

mordant for one minute and then washed with running water. Acetone alcohol was then added (acts as decolorizer) for few seconds. After washing with water, 0.5% carbol fuchsin was added as counter stain and allowed to stain for two minutes. The slides were then washed with water, blotted, dried in air and then examined under microscope with high power objective (100X) using immersion oil.

Biochemical Tests

For this study isolated organisms with supporting growth characteristics of *Campylobacter* were subjected to various tests (catalase test, oxidase test, hippurate hydrolysis test, TSI reaction and hydrolysis of indoxyl acetate) according to the procedures as described by Nachamkin (2003) and Foster *et al.*, (2004).

Antimicrobial susceptibility test

All *Campylobacter* strains were tested against ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), streptomycin (10 µg), gentamicin (10 µg), erythromycin (15 µg), azithromycin (15 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg) and norfloxacin (10 µg) by disk diffusion method as described by Luangtongkum *et al.* (2007) with some modifications. All antimicrobial disks were obtained from Hi Media Laboratories Pvt Ltd, India. Briefly, within 15 minutes after adjusting the turbidity of the inoculum suspension (equivalent to 0.5 McFarland turbidity), a sterile cotton swab was dipped into the adjusted suspension and then, the swab was rotated several times followed by pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. Thereafter, the dried surface of a Muller-Hinton agar supplemented with 5% defibrinated sheep blood was inoculated by streaking the swab over the entire sterile agar surface and this procedure was repeated two more times, and rotated the plate 60^{o} each time to ensure a confluent lawn of bacterial growth. After the inoculates were dry, five antimicrobial disks were applied per plate and incubated in the inverted position at 37^{o}C for 48 hr under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2). The zone diameter breakpoints of each antimicrobial agent were determined according to the breakpoints used by the National Antimicrobial Resistance Monitoring System (NARMS) and the CLSI-established guideline for bacteria isolated from animals (CDC, 2003; National Committee for Clinical Laboratory Standards 2002a; National Committee for Clinical Laboratory Standards 2002b).

Maintenance of stock culture

During the experiment it was necessary to preserve the isolated *Campylobacter* spp. for longer period. For this purpose pure culture of isolated *Campylobacter* spp. were kept in stock culture. The isolated *Campylobacter* were preserved in 15% glycerol with nutrient broth. In this case colonies of *Campylobacter* spp. from pure culture were dissolved with 1 ml of 15% glycerol with nutrient broth and kept at −80ºC for further used.

Questionnaires

Primary questionnaire for exposure assessment of *Campylobacter* spp. in broiler farm and secondary questionnaire for the assessment of biosecurity level in the selected poultry farms were developed (questionnaires not shown).

RESULTS AND DISCUSSION

The present study was conducted for the isolation and identification of *Campylobacter* spp. from the samples (cloacal swab and poultry litter) which were collected from BAU poultry farm, Mymensingh and antibiogram characterization of the isolated *Campylobacter* strains were also accomplished. A total of 80 samples [Cloacal swabs (n=50), Poultry litter (n=30)] were subjected to isolation of *Campylobacter* strains by filtration method. *Campylobacter* like organisms were presumptively identified in 63 samples of which 39 samples from cloacal swab and 24 samples from litter as shown in Table 1. Since the isolation and correct identification of *Campylobacter* are very crucial, the colonies having typical cultural characteristics were selected as presumptive for *Campylobacter* serotypes. For this blood agar base no.2 was used to culture the organism and filtration method (Allos, 1998) and biochemical tests were used for the isolation and identification of *Campylobacter* that were also used by a number of researchers (Shiramaru *et al.* 2012). The colony characteristics of *Campylobacter* spp. exhibited grey or light pink color (Doyle, 1990; Rowe and Madden, 2000). In Gram's staining, the morphology of the isolated *Campylobacter* from

samples exhibited Gram negative, small curve shaped, single or paired in arrangement under microscope which was analogously reported by other researchers (Doyle, 1990).

Results of percentages (%) of *Campylobacter* spp. are presented in Table 2. 47 (74.60%) were detected as *Campylobacter jejuni* and 16 (25.39%) were detected as *Campylobacter coli*. In catalase test, all the isolates (n = 63) produced bubbles those indicated positive for *Campylobacter*. In oxidase test a purple color change was observed in all the isolates (n=63). In hippurate hydrolysis test some of the isolates (n=16) did not develop any purple color that indicated the isolates were *C. coli* and some of the test isolates (n=47) developed purple color that indicated the isolates were *C. jejuni*. In indoxyl acetate test, 1% glycerine and nitrate reduction test all the isolates (n=63) showed positive result. In TSI *C. jejuni* did not produce H_2S but in case of *C. coli* variable results were seen. These results strongly correlate with the observations of Jacobs-Reitsma *et al*. (1995).

The results of antimicrobial susceptibility testing by disc diffusion method with 10 chosen antimicrobial agents are presented in Tables 3 and 4. In antimicrobial susceptibility assessment, out of 47 *Campylobacter jejuni* isolates, 47 (100%) were resistant to ampicillin, 34 (72.34%) were resistant to tetracycline, 5 (10.64%) were resistant to streptomycin, 28 (59.57%) were resistant to erythromycin, 7 (14.89%) were resistant to azithromycin, 35(74.47%) were resistant to nalidixic acid, 22 (46.81%) were resistant to ciprofloxacin and 25 (53.19%) were resistant to norfloxacin. On the other hand, out of 16 *Campylobacter coli* isolates, 16 (100%) were resistant to ampicillin, 11 (68.75%) were resistant to tetracyclin, 4 (25.00%) were resistant to gentamycin, 13 (81.25%) were resistant to erythromycin, 2 (12.50%) were resistant to azithromycin, 7 (43.17%) were resistant to nalidixic acid, 4 (25.00%) were resistant to ciprofloxacin, 11 (68.75%) were resistant to norfloxacin. These findings are also very much close to the findings of several other researchers (Allos, 1998; Blaser, 2000; Allos, 2001; Butzler, 2004; Suman, 2013).

The results of antimicrobial resistance patterns of *C. jejuni* and *C. coli* are summarized in Table 5. Out of 47 *Campylobacter jejuni* isolates, 2 (4.26%) were resistant to 4 antibiotics. Furthermore, 9(19.15%) and 2 (4.26%) were resistant to each of 3 antibiotics respectively. Moreover, 6(12.76%) and 4(8.51%) were resistant to each of 5 antibiotics. Furthermore, 2 (4.26%) and 4 (8.51%) were resistant to each of 1 antibiotic respectively and 9 (19.15%) and 9 (19.15%) were resistant to each of 6 antibiotics. These findings are also in agreement with the observations of several other researchers (Khachatourians, 1998; Kabir *et al.*, 2013; Suman, 2013). Out of 16 *Campylobacter coli* isolates, 2 (12.50%) were resistant to 2 antibiotics, 2 (12.50%), 4(25.00%) and 4 (25.00%) were resistant to each of 4 antibiotics respectively. On the other hand, 41 (87.23%) *Campylobacter jejuni* (n = 47); 16 (100%) *Campylobacter coli* (n = 16) were detected as multidrug resistant isolates as shown in Table 6. Several researchers had the more or less similar observations (Khachatourians, 1998; Kabir *et al*, 2013; Suman, 2013). This study suggested that gentamicin, chloramphenicol and azithromycin might be more effective against *Campylobacter jejuni*. *C. coli*, in particular, displayed significantly higher resistance rates to ampicillin and erythromycin. On the other hand, streptomycin and chloramphenicol are more susceptibile to *C. coli*. It is demonstrated that streptomycin and chloramphenicol might be more effective against *Campylobacter coli.* Multidrug resistance has been observed in most of the *Campylobacter* isolates in the present study. Several investigators have reported the increasing incidence of human *C. jejuni* and *C. coli* infections in many parts of the world for the last decade with higher multidrug resistance (Hakanen *et al.*, 2003; McGill *et al.*, 2006; Moore *et al.*, 2006; Luangtongkum *et al.*, 2009). Since *C. jejuni* and *C. coli* demonstrate different susceptibility profiles, it is important to differentiate *Campylobacter* at the species level, and to provide antimicrobial susceptibility data for each species, in order to monitor the trend of antimicrobial resistance among *Campylobacter* isolates and to ensure effective treatment of *Campylobacter* infections.

Exposure assessment of *Campylobacter* spp. in the selected poultry farms indicated that the maximum (60%) owner of the farms completed primary school, where 40% completed secondary school. In terms of electricity power, the highest numbers (80%) used electric power to maintain their farms properly. Regarding the source of water in the selected farms, the maximum numbers (50%) used tube well water, followed by (30%) used supply water and the lowest 20% used river or pond water for their farm purposes. A total of 60% farms contained more than 5000 chicks per batch and 40% farms contained below 5000 chicks. However, 70% chicks got directly from hatchery and 30% from supplied middle man. During the study period, it was noticed that about 60% farms used pellet feed and 40% used Mesh Feed. Around 90%

of the total farms were maintained vaccination schedule and 10% farms did not follow vaccination schedule. In the selected farm, 60% followed multi-age farming system and 40% followed all-in-all-out farming system. It is beyond question that hygiene is essential to prevent *Campylobacter* colonization in broilers. Water supply to the broiler houses from private water source was associated with increased risk for a broiler flock to test positive for *Campylobacter* spp. compared with broiler houses with official waterworks. This finding is in line with an earlier Norwegian study (Kapperud *et al*., 1993). A recent study from Iceland showed that the use of official water and treating unofficial water sources may assist in reducing colonization (Guerin *et al*., 2007). Variations in climate have been described as having an effect on flock prevalence of *Campylobacter* spp. in broilers (Patrick et al., 2004). In Norway, the broiler farms are clustered in different regions, and both the climatic and geographic conditions vary between these regions, which could have an impact on the risk for a flock to be colonized by *Campylobacter* spp.

The assessment of biosecurity level in the selected poultry farms for the development of strategies in order to reduce *Campylobacter* spp. on poultry farms indicated that 70% farms always removed poultry litter and manure when poultry house is empty and 30% farms sometimes removed to avoid contamination from farm. Among the respondents farms, highest 60% farms used disinfectant to prevent direct contamination from the farms. It was observed that around 80% farms maintained good fencing around poultry house to avoid wild and domestic animals from the farms. Moreover, 90% respondents farms followed footbath at entrance of poultry house to avoid organisms from outsides. During the study period, it was viewed that 80% farms who maintained disinfectant spray at farm gate before vehicles entry into farms for controlling microorganisms from the outside. Among the farms, 70% followed poultry cages/egg trays cleaned and disinfected before entry into farms and 30% did not follow such these. It was observed that 80% farms who allowed visitors walk through a disinfection footbath and 20% did not allow. Again 60% respondents farms allowed farm workers change clothes and farm boots upon entry into farm to avoid contamination.

Campylobacter spp. can be isolated from the boots of a broiler farm worker (Gregory *et al.*, 1997; Johnsen *et al.*, 2006), thus indicating a possible route of transmission of *Campylobacter* spp. into the broiler house. Farms on which the animal caretaker was hired had a greater risk for having flocks testing positive for *Campylobacter* spp. than did farms on which the owner, family, or neighbors were caring for the birds. Hired caretakers might be traveling between different farms, and thereby contribute to the risk of introducing pathogens, or they might not be quite so careful in implementing the hygienic procedures as the owners themselves. Farms on which transport personnel delivering day-old chickens passed through the hygiene barrier of the broiler house also had an increased risk of having flocks positive for *Campylobacter* spp. In an earlier Norwegian study (Johnsen *et al.*, 2006), the same *Campylobacter* spp. subtypes were present in broiler flocks as in the outdoor environment close to the broiler houses and also in the broiler flocks on adjacent farms. There is also research indicating that flies play an important role in spreading *Campylobacter* spp. into the broiler house (Hald *et al.*, 2004). These findings stress the importance of having good hygienic practices and strict barriers on the farm.

Campylobacter species were isolated and characterized successfully from cloacal swab and litter samples collected from broiler flocks of BAU poultry farm using different cultural, morphological examination, biochemical and antimicrobial susceptibility test. The findings of the present study revealed the presence of multidrug resistant *C. jejuni* and *C. coli* isolates in cloacal swab and litter samples collected from broiler flocks of BAU poultry farm. Further molecular studies on the isolated *C. jejuni* and *C. coli* strains will be required for better understanding of their clonality and mechanisms of antimicrobial resistance.

Table 1. Isolation of *Campylobacter* spp. by filtration method from cloacal swab and litter samples collected from BAU poultry farm, Mymensingh.

Origin of sample	No. of sample	No. of *Campylobacter* spp.
Cloacal swabs	50	39
Poultry litter	30	24
Total	**80**	**63**

Table 2. Results of percentages (%) of *Campylobacter* spp. available in cloacal swabs and poultry litter samples.

Name of isolates (n=63)	% of the isolates recovered from cloacal swabs and poultry litter
Campylobacter jejuni **(n=47)**	74.60
Campylobacter coli **(n=16)**	25.39

Table 3. Antimicrobial susceptibility pattern of *Campylobacter jejuni* (n=47) identified by the disk diffusion method.

Antimicrobial agents	Number (%) of *Campylobacter* isolates		
	S (%)	I (%)	R (%)
Ampicillin	0(0)	0(0)	47(100)
Tetracycline	8(17.02)	5(10.64)	34(72.34)
Chloramphenicol	34(72.34)	13(27.66)	0(0)
Streptomycin	29(61.70)	13(27.66)	5(10.64)
Gentamicin	38(80.85)	9(19.15)	0(0)
Erythromycin	14(29.78)	5(10.64)	28(59.57)
Azithromycin	30(63.83)	10(21.28)	7(14.89)
Nalidixic acid	5(10.64)	7(14.89)	35(74.47)
Ciprofloxacin	15(31.91)	10(21.28)	22(46.81)
Norfloxacin	17(36.17)	5(10.64)	25(53.19)

Legends:
S = Susceptible
I = Intermediate resistance
R = Resistance

Table 4. Antimicrobial susceptibility pattern of *Campylobacter coli* (n=16) identified by the disk diffusion method.

Antimicrobial agents	Number (%) of *Campylobacter* isolates		
	S (%)	I (%)	R (%)
Ampicillin	0(0)	0(0)	16(100)
Tetracycline	3(18.75)	2(12.50)	11(68.75)
Chloramphenicol	11(68.75)	5(31.25)	0(0)
Streptomycin	11(68.75)	5(31.25)	0(0)
Gentamicin	7(43.75)	5(31.25)	4(25.00)
Erythromycin	3(18.75)	0(0)	13(81.25)
Azithromycin	9(56.25)	5(31.25)	2(12.50)
Nalidixic acid	5(31.25)	4(25.00)	7(43.75)
Ciprofloxacin	9(56.25)	4(25.00)	4(25.00)
Norfloxacin	3(18.75)	2(12.50)	11(68.75)

Legends:
S = Susceptible
I = Intermediate resistance
R = Resistance

Table 5. Results of antimicrobial resistance pattern of *Campylobacter* spp.

Isolates	Resistance profiles	No. of isolates (%)
Campylobacter jejuni **(n=47)**	a. No resistance demonstrated	-
	b. Resistant to 1 agent (AMP)	6(12.76)
	c. Resistant to 3 agents (AMP-TET-NA)	9(19.14)
	d. Resistant to 3 agents (AMP-TET-ER)	2(4.26)
	e. Resistant to 4 agents (AMP-TET-ER-NOR)	2(4.26)
	f. Resistant to 5 agents (AMP-ER-AZ-NA-NOR)	6(12.76)
	g. Resistant to 5 agents (AMP-TET-ST-NA-CI)	4(8.51)
	h. Resistant to 5 agents (AMP-TET-ER-NA-NOR)	9(19.14)
	i. Resistant to 6 agents (AMP-TET-ER-NA-CI-NOR)	9(19.14)
	Total Resistant isolates	47(100)
Campylobacter coli **(n=16)**	a. No resistance demonstrated	-
	b. Resistant to 2 agent (AMP-NOR)	2(12.50)

	c. Resistant to 4 agents (AMP-TET-AZ-NA)	2(12.50)
	d. Resistant to 4 agents (AMP-TET-GEN-ER)	4(25.00)
	e. Resistant to 4 agent (AMP-ER-CIP-NOR)	4(25.00)
	f. Resistant to 5 agents (AMP-TET-ER-NA-NOR)	4(25.00)
	Total Resistant isolates	16(100)

Table 6. Frequency distribution of multidrug resistant *Campylobacter* isolates from cloacal swabs and poultry litter (when considered resistant to 2 or more drugs)

Name of isolates	No (%)
C. jejuni	41 (87.23)
C. coli	16 (100)

Table 7. Exposure assessment of *Campylobacter* spp. in poultry farms.

Variables		No. of farms	Frequency (%)
Education Background	Primary School Secondary School Tertiary	10	6(60) 4(40) 1(10)
Electricity power at the farm	Yes No	10	8(80) 2(20)
Source of water	Supply water Tube well Natural/River/Pond	10	3(30) 5(50) 2(20)
Total capacity of farm per batch	>5000 <5000	10	6(60) 4(40)
Source of chicks	Direct from hatchery Supplied by middle man	10	7(70) 3(30)
Type of feed used	Pellet Mesh	10	6(60) 4(40)
Practice of Vaccination schedule	Yes No	10	9(90) 1(10)
Farming system	All-in-all-out system Multi-age group	10	4(40) 6(60)

Table 8. Assessment of biosecurity level in the selected poultry farms for the development of strategies in order to reduce *Campylobacter* spp. on poultry farms.

Variables		No of farms	Frequency (%)
All litter and manure removed when poultry house is empty	Sometimes Always	10	3(30) 7(70)
Disinfectant used after cleaning	Rarely Sometimes Always	10	1(10) 3(30) 6(60)
With good fencing around poultry house	Yes No	10	8(80) 2(20)
Footbath at entrance of poultry farm	Yes No	10	9(90) 1(10)
Vehicles go through a disinfectant at farm gate before entry	Yes No	10	8(80) 2(20)
Poultry cages/egg trays cleaned and disinfected before entry into farm	Yes No	10	7(70) 3(30)
Visitors walk through a disinfection foot bath	Yes No	10	8(80) 2(20)
Farm workers change cloths and farm boots upon entry to farm	Yes No	10	6(60) 4(40)

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