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Isolation, Identification and Antimicrobial Resistance Patterns of *Campylobacter* Species from Broiler Meat Sold at KR Market of Bangladesh Agricultural University Campus, Mymensingh

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ABSTRACT

The study was designed with a view to isolate, identifies and characterizes *Campylobacter* species from broiler meat samples (leg muscle, breast muscle and cloacal skin) which were collected from KR market at Bangladesh Agricultural University, Mymensingh during the period from January 2013 to May 2013. A total of 50 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 31 positive *Campylobacter* isolates 70.97% (n = 22) were *Campylobacter jejuni*, and the rest 29.04% isolates (n = 09) 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 31 *Campylobacter* isolates, 86.36% *Campylobacter jejuni*, and 100% *Campylobacter coli* were detected as multidrug resistant. The findings of the study revealed the presence of multidrug resistant *Campylobacter* species in broiler meat of KR market at Bangladesh Agricultural University, Mymensingh. To the best of our knowledge, this study represents the first time report of *Campylobacter jejuni* and *Campylobacter coli* from broiler meat in Bangladesh.

KEYWORDS: Broiler meat, *Campylobacter jejuni*, *Campylobacter coli*, identification and antimicrobial resistance

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 (Doyle, 1990). The ideal environment for optimal recovery of *Campylobacter* spp. is an atmosphere containing approximately 5% O2, 10% CO2, and 85% N2 (Forbes *et al*., 1998). *Campylobacter* is one of the most important bacterial pathogens and is regarded as the major bacterial cause of human gastroenteritis worldwide (Allos 2001). Food animals, mainly poultry, cattle, sheep and pigs, may act as asymptomatic intestinal carriers of *Campylobacter* and animal food products can become contaminated by this pathogen during slaughter and carcass dressing (Berndtson *et al.*, 1996). Poultry and poultry products are considered the primary source of infection (Coker *et al.*, 2002). It is now accepted that campylobacteriosis is predominantly acquired through the consumption of contaminated foods (Humphrey *et al.*, 2007).

The use of antimicrobial agents in food animals has 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 (Looveren *et al.*, 2001). Although *Campylobacter* with resistance to antimicrobial agents has been reported worldwide (Looveren *et al.*, 2001; Isenbarger *et al.*, 2002), the situation seems to deteriorate more rapidly in developing countries, where there is widespread and uncontrolled use of antibiotics (Hart and Kariuki, 1998). Moreover, *Campylobacter* infections pose a serious public health problem for which many countries have

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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 and antimicrobial resistance of *Campylobacter* species in poultry meat in Bangladedsh where broiler meat is widely consumed. Therefore, the aims of this study were to isolate, identify and analyze antimicrobial resistance patterns of *Campylobacter* species from broiler meat sold at KR market of Bangladesh Agricultural University campus, Mymensingh.

MATERIALS AND METHODS

Study area

The samples (leg muscle, breast muscle and cloacal skin of Broiler) which were collected from KR market at BAU, Mymensingh and transported through ice flasks to the laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh for isolation, identification, biochemical and antibiogram study.

Collection and transportation of samples

A total of 50 samples (Leg muscles, breast muscles, cloacal skins) were collected during the period from January 2013 to May 2013 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 meat homogenates and then 100µlof meat homogenates 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), 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 −80^{o}C for further used.

RESULTS AND DISCUSSION

This study was aimed at isolation, identification and biochemical differentiation of *Campylobacter* spp. from the samples (leg muscle, breast muscle and cloacal skin of broiler washing and rinsed water) which were collected from KR market at Bangladesh Agricultural University, Mymensingh and antibiogram characterization of the isolated *Campylobacter* strains were also accomplished. A total of 50 broiler meat samples [leg muscle (n=18), breast muscle (n=18), cloacal skin (n=14)] were subjected to isolation of *Campylobacter* strains by filtration method. A total of 31 *Campylobacter* like organisms [leg muscle (n=13), breast muscle (n=12), cloacal skin (n=7)] as shown in Table 1 were selected from collected samples for biochemical identification. The results of cultural, morphological and motility characteristics of the isolates of *Campylobacter* spp. are summarized in Table 2. The colony characteristics of *Campylobacter* spp. exhibited grey 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 reported by other researchers (Doyle, 1990).

Results of percentages (%) of *Campylobacter* spp. were presented in Table 3**.** 22 (70.96%) were detected as *Campylobacter jejuni* and 9 (29.04%) were detected as *Campylobacter coli*. In catalase test, all the isolates (n = 31) produced bubbles those indicated positive for *Campylobacter.* In oxidase test a purple color change was observed in all the isolates (n=31). In hippurate hydrolysis test some of the isolates (n=9) did not develop any purple color that indicated the isolates were *C. coli* and some of the test isolates (n=22) developed purple color that indicated the isolates were *C. jejuni*. In indoxyl acetate test, 1% glycerine and nitrate reduction test all the isolates (n=31) showed positive result. In TSI *C. jejuni* did not produce H_2S but in case of *C. coli* variable results were seen. These results support the findings of Jacobs-Reitsma et al., 1995.

The results of antimicrobial susceptibility pattern of *C. jejuni* and *C. coli* identified by the disk diffusion method are summarized in Tables 4 and 5. In antimicrobial susceptibility testing, Out of 22 *Campylobacter jejuni* isolates, 22 (100%) were resistant to ampicillin, 16 (72.72%) were resistant to tetracycline, 2 (9.09%) were resistant to streptomycin, 13 (59.09%) were resistant to erythromycin, 3 (13.63%) were resistant to azithromycin, 17 (77.27%) were resistant to nalidixic acid, 10 (45.45%) were resistant to ciprofloxacin and 12 (54.54%) were resistant to norfloxacin. On the other hand, Out of 9 *Campylobacter coli* isolates, 9 (100%) were resistant to ampicillin, 6 (66.67%) were resistant to tetracyclin, 2 (22.22%) were resistant to gentamycin, 7 (77.77%) were resistant to erythromycin, 1 (11.11%) were

resistant to azithromycin, 4 (44.44%) were resistant to nalidixic acid, 2 (22.22%) were resistant to ciprofloxacin, 6 (66.67%) were resistant to norfloxacin. These findings are also very close to (Allos, 1998; Allos, 2001; Blaser, 2000; Butzler, 2004).

The results of antimicrobial resistance patterns of *C. jejuni* and *C. coli* are summarized in Table 6. Out of 22 *Campylobacter jejuni* isolates, 1 (4.54%) were resistant to 4 antibiotics. Furthermore, 4 (18.18%) and 1 (4.54%) were resistant to each of 3 antibiotics respectively. Moreover, 3 (13.63%) and 2 (9.09%) were resistant to each of 5 antibiotics. Furthermore, 1 (4.54%) and 2 (9.09%) were resistant to each of 1 antibiotic respectively and 4 (18.18%) and 4 (18.18%) were resistant to each of 6 antibiotics. These findings are also very close to (Kabir *et al.*, 2013; Khachatourians, 1998). On the other hand, Out of 9 *Campylobacter coli* isolates, 1 (11.11%) were resistant to 2 antibiotics, 1 (11.11%), 2(22.22%) and 2 (22.22%) were resistant to each of 4 antibiotics respectively. On the other hand, 19 (86.36%) *Campylobacter jejuni* (n = 22); 9 (100%) *Campylobacter coli* (n = 9) were detected as multidrug resistant isolates as shown in Table 7. These findings are also very close to (Kabir *et al.*, 2013; Khachatourians, 1998). 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 for *C. coli.* Therefore, streptomycin and chloramphenicol might be more effective against *Campylobacter coli.*

Campylobacter species were isolated and characterized successfully from broiler meat sold at KR market of Bangladesh Agricultural University campus 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 broiler meat sold at KR market of Bangladesh Agricultural University campus. 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 broiler meat in Mymensingh.

Origin of sample	No. of sample	No. of *Campylobacter* spp.
Leg muscle	18	13
Breast muscle	18	12
Cloacal skin	14	7
Total	50	31

Table 2. Results of cultural, morphological and motility characteristics of the isolates of *Campylobacter* spp. at a glance.

Sources of isolates	Colony morphology	Staining characteristics	Motility
S 1 to S 50 except SAMPLE (1,2,3,7,11,14,15,19, 21,23,26,27,31,32,33,40,42,47,49)	Grey color colony	Gram (-ve) curved shaped bacteria	+ ve

Table 3. Results of percentages (%) of *Campylobacter* spp. available in broiler meat samples.

Name of isolates (n=31)	% of the isolates recovered from broiler meat
Campylobacter jejuni **(n=22)**	70.96
Campylobacter coli **(n=9)**	29.04

Table 4. Antimicrobial susceptibility pattern of *Campylobacter jejuni* identified by the disk diffusion method.

Antimicrobial agents	Number (%) of *Campylobacter* isolates		
	S (%)	I (%)	R (%)
Ampicillin	0(0)	0(0)	22(100)
Tetracycline	4(18.18)	2(9.09)	16(72.72)
Chloramphenicol	16(72.72)	6(27.27)	0(0)
Streptomycin	14(63.63)	6(27.27)	2(9.09)
Gentamicin	18(81.81)	4(18.18)	0(0)
Erythromycin	7(31.81)	2(9.09)	13(59.59)

Azithromycin	14(63.63)	5(22.72)	3(13.63)
Nalidixic acid	2(9.09)	3(13.63)	17(77.27)
Ciprofloxacin	7(31.81)	5(22.72)	10(45.45)
Norfloxacin	8(36.36)	2(9.09)	12(54.54)

Legends:

S = Susceptible
I = Intermediate
R = Resistance

Table 5. Antimicrobial susceptibility pattern of *Campylobacter coli* identified by the disk diffusion method.

Isolates	Resistance profiles	No. of isolates (%)
***Campylobacter jejuni* (n=22)**	a. No resistance demonstrated	-
	b. Resistant to 1 agent (AMP)	3(13.63)
	c. Resistant to 3 agents (AMP-TET-NA-CI)	4(18.18)
	d. Resistant to 3 agents (AMP-TET-ER)	1(4.45)
	e. Resistant to 4 agents (AMP-TET-ER-NOR)	1(4.45)
	f. Resistant to 5 agents (AMP-ER-AZ-NA-NOR)	3(13.63)
	g. Resistant to 5 agents (AMP-TET-ST-NA-CI)	2(9.09)
	h. Resistant to 5 agents (AMP-TET-ER-NA-NOR)	4(18.18)
	i. Resistant to 6 agents (AMP-TET-ER-NA-CI-NOR)	4(18.18)
	Total Resistant isolates	22(100)
***Campylobacter coli* (n=09)**	a. No resistance demonstrated	-
	b. Resistant to 2 agent (AMP-NOR)	1(11.11)
	c. Resistant to 4 agents (AMP-TET-AZ-NA)	1(11.11)
	d. Resistant to 4 agents (AMP-TET-GEN-ER)	2(22.22)
	e. Resistant to 4 agent (AMP-ER-CIP-NOR)	2(22.22)
	f. Resistant to 5 agents (AMP-TET-ER-NA-NOR)	3(33.33)
	Total Resistant isolates	09(100)

Legends:

S = Susceptible
I = Intermediate
R = Resistance

Table 6. Results of antimicrobial resistance pattern of *Campylobacter* spp.

Antimicrobial agents	Number (%) of *Campylobacter* isolates		
	S (%)	I (%)	R (%)
Ampicillin	0(0)	0(0)	9(100)
Tetracycline	2(22.22)	1(11.11)	6(66.67)
Chloramphenicol	6(66.67)	3(33.33)	0(0)
Streptomycin	6(66.67)	3(33.33)	0(0)
Gentamicin	4(44.44)	3(33.33)	2(22.22)
Erythromycin	2(22.22)	0(0)	7(77.77)
Azithromycin	5(55.55)	3(33.33)	1(11.11)
Nalidixic acid	3(33.33)	2(22.22)	4(44.44)
Ciprofloxacin	5(55.55)	2(22.22)	2(22.22)
Norfloxacin	2(22.22)	1(11.11)	6(66.67)

Table 7. Frequency distribution of multidrug resistant *Campylobacter* isolates from broiler meat (when considered resistant to 2 or more drugs).

Name of isolates	No (%)
C. jejuni	19 (86.36)
C. coli	9 (100)

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