Research Paper

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Prevalence of Campylobacter Jejuni in Poultry Farm and Slaughter House Materials and Study on their Antibiotic Resistance and Virulence Factor

Microbiology

KEYWORDS : C. jejuni, poultry, slaughter house, virB, cadF,antibiotic resistance

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ABSTRACT

Campylobacter is the major cause of gastroenteritis in human. The main risk factor of infection is consumption of contaminated or cross-contaminated poultry meat and other related products. This study aims to determine the prevalence of Campylobacter jejuni from poultry farm and slaughter house materials and its resistance to various antibiotics along with the identification of virulence factors. In this study 19 (59.38%) samples of poultry farm materials were contaminated with Campylobacter jejuni especially fecus, dead bird's liver and intestine had highest prevalence. In case of slaughter house, 22 (68.75%) samples were contaminated with Campylobacter jejuni. The highest incidence was observed in intestine and liver. Screening for virulence genes in the isolated Campylobacter showed that most of the isolates had the virB and cadF genes. In conclusion, the results of this study indicate that C. jejuni is very frequent in poultry farm and slaughter house materials in Namakkal town, suggesting possible risks of infection to people through consumption of contaminated poultry products or by direct contact with infected poultry.

Introduction

Foodborne infections are occurred by food or beverages that contain harmful bacteria, parasites, viruses or chemicals. Common symptoms of foodborne illnesses include vomiting, diarrhea, abdominal pain, fever and chills. The onset of symptoms may occur within minutes to weeks. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors. Contaminated raw meat is one of the main sources of food-borne illnesses. The risk of the transmission of zoonotic infections is also associated with contaminated meat (Nafisa *et al*., 2010). More than 250 different foodborne illnesses are caused by various pathogens or by toxins (Linscott, 2011). Among them, *Campylobacter* is one of the most common causes of food borne infection in human worldwide. Several incidences of Campylobacteriosis have been reported from almost all parts of India (Bandekar *et al*., 2005; Yogesh, 2014). Chickens are major reservoirs and they are frequently colonized by pathogenic *Campylobacter sp.* like *C. jejuni* and *C. coli*.

Campylobacter sp. is a common contaminant of poultry carcasses in poultry processing plants (Jorgensen *et al*., 2002; Son *et al*., 2007). During slaughtering, the damage of intestinal tract integrity can lead to direct contamination. Contamination can also occur directly through air, bird to bird, via equipment and water (Jones *et al*., 1991; Corry & Atabay, 2001). The consumption and handling of poultry and poultry products are major sources of human *Campylobacterial enteritis* (Corry & Atabay, 2001). The prevalence of pathogens in poultry and poultry products is well documented, but their presence in poultry farm has not been extensively investigated. Therefore our present study focused to check the prevalence of *C. jejuni* from poultry farm and slaughter house materials.

Methods

Collection of samples

Samples were collected from 4 poultry farm and 4 poultry slaughter houses in different localities of Namakkal district. Eight different samples were taken randomly from each poultry farm (cage, anus region of hen, feces, feed, water, egg shell, dead bird's intestine and dead bird's liver) and slaughter house (knife, liver, chest, intestine, dehairing machine, chopping board, weighing balance and washing water) by means of sterilised cotton swabs within the test tube containing sterilized peptone water. Thus, a total of 64 samples from poultry farms and poultry slaughter houses were sampled. The collected samples were transported to the laboratory under refrigerated condition by keeping them inside the ice packs.

Each sample swab was streaked onto the plates of blood free-Karmali *Campylobacter* selective agar which was supplemented with *Campylobacter* selective supplement containing Hemin 1.6 mg/5ml, vancomycin, cefoperazon and cycloheximide and incubated at 42°C for 48 h. From the positive agar plates, colonies showing typical *Campylobacter* features were selected and subcultured and thereafter, were tested for gram-staining, motility, production of oxidase and catalase and biochemical tests. These isolates were stored in brain heart infusion agar (BHA).

Antibiotic susceptibility test

Fresh bacterial colonies directly taken from agar slants were inoculated in sterile LB broth. Antimicrobial susceptibility testing for 91 *Campylobacter jejuni* isolated strains was performed by the Kirby-Bauer disc diffusion method in Muller-Hinton agar. The plates were incubated at 42ºC for 48 hours in a microaerophilic atmosphere. Antibiotic discs such as Erythromycin (15mcg), Chloramphenicol (30mcg), Amoxicillin (10mcg), Nalidixic acid (30mcg), Cephataxime (30mcg), Ciprofloxacin (5mcg), Tetracycline (30mcg), Ampicillin (10mcg), Streptomycin (10mcg) and Gentamycin (10mcg) were used. The inhibition zone diameter obtained for 10 antibiotics were compared with standard chart. Multi drug resistance colonies were chosen by comparing the zone diameter with standard chart.

Polymerase chain reaction

All the primers were designed according to Suvamoy Datta (2003) protocol with some modification. Each PCR reaction mixture (20 μl) contained 10µl of 2X master mix (Promega, USA), 0.5 μl of (0.5 pM) each primers, 1 μl of template and 7.0 μl of molecular grade water.

All samples were subjected to 30 cycles of amplification in a DNA thermal cycler (Genei). The cycling was as follows: Denaturation at 94^{0}C for 1 min, Annealing at a 50^{0}C for 1 min, Extension at 72^{0}C for 1 min and the final extension at 72^{0}C for 3 min. After amplification, part of the amplified product was run the agarose gel electrophoresis and then visualized the amplified DNA with UV transilluminator.

Result

Prevalence of *Campylobacter jejuni*

In this study, 64 samples were collected from poultry farm and slaughter house materials for isolation of *Campylobacter jejuni*. Among them 19 (59.38%) samples of poultry farm materials were

contaminated with *Campylobacter jejuni* especially fescus had highest prevalence. In case of slaughter house, 22 (69%) samples were contaminated with *Campylobacter jejuni*. The highest incidence was observed in liver and intestine. The result was tabulated in table 1.

Table 1. Occurrence of *Campylobacter jejuni*

S. No	Poultry farm materials		Slaughter house materials	
	Sampling site	% of occurrence	Sampling site	% of occurrence
1.	Cage	25	Knife	75
2.	Anus	75	Liver	100
3.	Fecus	100	Chest	50
4.	Feed	25	Intestine	100
5.	Water	75	Machine	25
6.	Egg shell	25	Chopping board	75
7.	Dead bird's intestine	75	Weighing balance	50
8.	Dead bird's liver	75	Washed water	75

Table. 2. Antibiotic resistance of *Campylobacter jejuni*

Sample site	Antibiotics Resistance (%)									
	CHL	AMX	ERY	STR	TET	NAL	CTX	CIP	GEN	AMP
Poultry farm	26.3	84.2	10.5	21	0	31.5	84.2	58	42.1	63.1
Slaughter house materials	54.4	95.4	14	27	0	45.4	64	45.4	14	59

Chloramphenicol (CHL), Amoxicillin (AMX), Erythromycin (ERY), Streptomycin (STR), Tetracycline (TET), Nalidixic acid (NAL), Cephataxime (CTX), Ciprofloxacin (CIP), Gentamycin (GEN), Ampicillin (AMP).

From data shown in Table 3 , among 19 isolates recovered from poultry farm, 37% of the isolates were resistant to three antibiotics. Among 22 isolates recovered from slaughter house materials, 63% of the isolates were resistance to five antibiotics.

Table. 3. Antibiotic resistance of *Campylobacter jejuni*

Samples site	No. of Antibiotics resistance	No. of isolates	Total %
Poultry farm	3	7	37
	4	5	26.3
	5	4	21
	6	2	10.5
	7	1	5.2
Slaughter house	1	1	4.5
	3	6	27.2
	4	1	4.5
	5	14	64

Isolated *Camphylobacter jejuni* were utilized for virulence markers determination with use of PCR amplification. The *cadF* gene was seen in 31.5% of isolates obtained from poultry farm samples and 40.91% of isolates obtained from slaughter house materials (Fig.1). The 16% of isolates obtained from poultry farm samples and 59.09% of isolates obtained from slaughter house isolates had *virB* gene (Fig. 1).

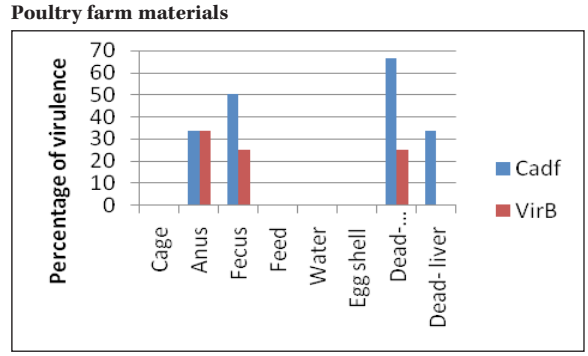
Antimicrobial Resistance Profiling

The isolated *Campylobacter jejuni* strains (19 from poultry farm and 22 from slaughter house materials) were tested for their antimicrobial resistance towards 10 different antibiotics. The table 2 was illustrated that the percentage of antibiotic resistance of poultry farm isolates. Cefotaxime and Amoxicillin were resistance to 84.2% and followed by Ampicillin (63.1%), Ciprofloxacin (58%), Gentamycin (42.1%), Nalidixic acid (31.5%), Chloramphenicol (26.3%), Streptomycin (21%) Erythromycin (10.5%). All isolates were sensitive to tetracycline antibiotic.

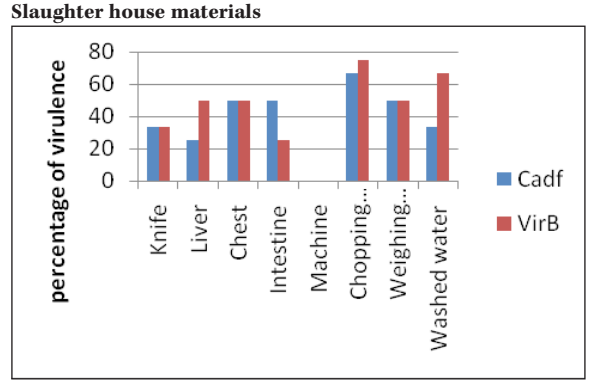
In case of slaughter house isolates, the highest resistance to Amoxicillin (95.4%) and followed by Cephataxime (64%), Ampicillin (59%), Chloramphenicol (54.5%), Ciprofloxacin and Nalidixic acid (45.4%), Streptomycin (27%), Erythromycin (14%), Gentamycin (14%). All isolates were sensitive to tetracycline antibiotic.

Fig .1. Virulence factors of *Campylobacter jejuni*

Poultry farm materials



Slaughter house materials



Discussion

All isolates of *Campylobacter* was identified by morphological and biochemical tests, viz.: oxidase, catalase and indoxyl acetate hydrolysis tests and H_2S production in triple sugar iron slant, 41 samples were found positive. Further, all the isolates were subjected to hippurate hydrolysis test to identify *C. jejuni* (hippurate hydrolysis positive).

In this study, 59.38% of the poultry farm samples and 68.75% of the slaughter house samples were positive for *Campylobacter jejuni*. Previous studies in India have shown that 60.46 % of the poultry samples positive for *Campylobacter* in Gujarat (Tayde and Brahmbhatt 2014), 64% in Vellore (Rajendran *et al*., 2012), 13% in Maharashtra (Bandekar, 2005) and 17.14% in the Meghalaya- Assam region (Rizal *et al*., 2010).

The contamination rate of poultry farm was due to unhealthy birds present in farms. The microbes transmitted through oral route of unhealthy bird to the other. The unhealthy birds contaminate the feeds, water and other inanimate objects in and

around the farms through their feces. The prevalence and risk factors associated with *Campylobacter* infections in broiler flocks were reported to be 76% in southern Iran, 90% in Great Britain (Erans and Sayer, 2000), 42% in Northern Ireland (McDowell *et al*., 2008), 35% in Quebec, Canada (Arsenault *et al*., 2007), 47% in India (Baserisalehi *et al*., 2007).

High prevalence occurred in feces (100%), anus region (75%), dead bird's intestine (75%) and liver (75%). This is because of *Campylobacter sp.* are the predominant microbes, which colonize in the intestine of birds. In dead birds, the microbes were present in higher level and lead to cross contamination.

The contamination rate of poultry slaughter house samples in this study was 68.75% which was higher than poultry farm. This may be due to improper handling during processing of poultry meat. Colonization of *Campylobacter jejuni* in gastrointestinal tracts is the most significant contributing factors in the contamination of poultry meat (Grant *et al*., 1980). The organisms are transferred onto the meat during mechanized processing of the birds (Genigeorgis *et al*., 1986). *Campylobacter sp.* are frequently found in the intestinal tract of poultry where colonization leads to contamination of carcasses during processing, especially at the de-feathering, evisceration and chilling stages (Franchin *et al*., 2007).

Campylobacter jeuni contamination occurred in different poultry slaughterhouse sources such as knife, chopping board, weighing balance, washing water, de-feathering machine and liver, chest and intestinal region of slaughtered hen. These sources are inanimate objects which don't have basic nutrients for survival of microbes. This was due to the continuous use, remaining of wastes or small pieces of flesh during process, improper handling of equipment, improper cleaning and failing to disinfect the equipment which is used during the slaughtering process.

Antibiotic resistance in *Campylobacter* is emerging globally and has already been described by several authors as a problem of public health importance (Mena *et al*., 2008; Moore *et al*., 2006).Poultry farm isolates showed higher resistance to Cefotaxime and Amoxicillin (84.2%). Poultry slaughter house isolates showed highest resistance to Amoxicillin (95.4%), followed by Cefotaxime (64%), Ciprofloxacin(45.4%) and Nalidixic acid (45.4%). All the isolates were sensitive to Tetracyclin (100%). Ge *et al*., (2003) found that turkey isolates showed significantly higher rates of resistance to ciprofloxacin, erythromycin, nalidixic acid and doxycycline than chicken isolates.

Multidrug resistance of *C. jejuni* isolates were examined for the presence of virulence factor. In this study, two important virulence genes were screened by PCR method. The cadF gene was present in 31.5% of poultry farm isolates and 40.91% of slaughter house isolates. The virB gene was present in 15.79% of poultry farm isolates and 59.09% of slaughter house isolates. The products of these genes are responsible for the expression of adherence and colonization (Nuijten *et al*., 2000, Ziprin *et al*., 2001). The findings are in agreement with earlier observations regarding the presence of *cad*F and virB genes in *C. jejuni* species isolated from human as well as chicken (Rozynek *et al*., 2005).

The occurrence of *C. jejuni* in chicken intestinal samples indicates the public health hazard due to this emerging food borne organism in the region. The contamination of carcasses may take place from intestinal contents during slaughtering and/or post slaughtering processes. The isolates showed a wide variation for the presence of pathogenic factors; however, presence of important virulence factors revealed the pathogenic potential of the isolates. So it is mandatory to have for proper preventive measures to control the infection through food production and consumption.

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