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RESEARCH ARTICLE

PREVALENCE AND CHARACTERIZATION OF *CAMPYLOBACTER JEJUNI* IN SMALL-SCALE POULTRY SLAUGHTER HOUSE

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ABSTRACT

The aim of the current work was to evaluate the prevalence, antimicrobial susceptibility and biofilm formation of *Campylobacter jejuni* isolated from different poultry meat retail shop. The samples were collected from different slaughtering level. Chicken sampling at slaughterhouses was performed for Knife, Liver, Chest, Intestine, Machine, Chopping board, weighing balance and washing water (each 7). Totally 41 isolates were identified as *Campylobacter jejuni* and were tested for susceptibility to 10 antimicrobial agents by the disk diffusion method. The highest probability of antimicrobial resistance occurrence of *C. jejuni* was noticed for betalactam group antibiotics tested, such as ampicillin (83%) and second most in a Cephalosporin group of Cephataxime (71%). A more frequent profile of multidrug resistance was noticed for isolates from Intestine (56%). In addition, 20 of biofilm positive isolates were observed. These results reinforce the need of efficient strategy implementation to control and reduce *Campylobacter* in chickens at slaughter levels, and the necessity to reduce the use of antimicrobials in the poultry sector.

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INTRODUCTION

Campylobacteriosis is an infectious disease caused by bacteria of the genus *Campylobacter*. It is also often known as campylobacter enteritis or gastroenteritis. *Campylobacter* is part of the normal flora living in the intestines of healthy chickens and other animals. *Campylobacter spp* are easily spread from contaminated chicken to non contaminated one through contact with infected feces and water source. When an infected bird is slaughtered, *Campylobacter* organisms can be transferred from the intestines to the meat. Although *Campylobacter* has been isolated from different body parts of the chicken such as cloaca, carcasses, feathers and crops, there is little information about the possible sources of contamination (Paulo *et al.,* 2005). In broiler processing, *Campylobacter* numbers found on poultry carcasses usually vary among operations with a peak at defeathering and evisceration, steps (Akram 2016). This enteric infection is mainly caused by *Campylobacter jejuni* and *Campylobacter coli,* with *C. jejuni* being more frequent (80–90%). Most human infections with *C. jejuni* are occurring by ingestion of contaminated water or food and to a minor extent by direct contact with contaminated animals or animal carcasses.

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C. jejuni are acute and self-limiting and do not require antimicrobial chemotherapy. The most frequently recommended drug are erythromycin or a fluoroquinolone such as ciprofloxacin are recommended when severe infection of campylobacteriosis (Skirrow *et al.,* 2000). For the last four decades, antibiotic resistance isolates of *Campylobacter spp* were observed from food and water sources (Beilei, 2003). A number of factors influences the growth of *C. jejuni* especially biofilm formation is suggested to play a significant role in the survival of *C. jejuni* in the food production and also prevent the antibiotic penetration. A number of studies reported the biofilm producing bacteria from slaughter house samples, but there is little information available suggesting the formation of a specific and consistent biofilm morphology by *C. jejuni* as a species. The aim of the study was to determine the prevalence of *Campylobacter* in slaughter house materials. Additionally, the isolated strains were characterized for antimicrobial resistance and the presence of biofilm formation.

METHODS

Sample collection

Samples were collected from 7 poultry slaughter house from different localities of Namakkal district. Eight different samples were collected randomly from each slaughter house

(knife, liver, chest, intestine, dehairing machine, chopping board, weighing balance and washing water) by means of sterilized cotton swabs and transferred to the test tube containing sterilized peptone water. Thus, a total of 56 samples were collected. The samples were transported to laboratory under refrigerated condition by keeping them inside the ice packs.

Isolation and identification

Each sample swab was streaked onto the plates of blood free Karmali *Campylobacter* selective agar which was supplemented with *Campylobacte*r 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 *Campylobacte*r features were selected and sub cultured and tested for gram-staining, motility, production of oxidase, catalase and biochemical tests. These isolates were stored in brain heart infusion agar (BHA).

Antibiotic resistance test

The disc diffusion method was followed (Bauer *et al.,* 1966) to determine the antibacterial activity. The Petri plate containing 20 ml of Mueller Hinton agar was seeded with 24 hours old fresh culture of bacterial isolates. The antibiotic discs were placed on the surface of the media and incubated at 37^0C for 24 hours. The zone of inhibition was measured by making use of Antibiotic zone scale (Hi - media). The resistance patterns were interpreted as per CDC recommendations.

Isolation of biofilm producing isolates

The congo red agar medium was prepared by adding 37 g of the BHI powder, 50 g of sucrose and 10 g of agar No 1 in 1 L of distilled water. The mixture was then autoclaved for 15 min at 121°C. Once the agar solution has cooled down to about 50°C, a solution of Congo red (8 g/L) was added and mixed again and then the media were poured into the Petri plates and allowed to solidify. Once the media had settled, the plates were inoculated with the microorganisms and incubated at 37°C for 24 h. The black colour colonies were indicated as positive isolates and red colonies were negative isolates (Freeman *et al.,* 1989).

RESULTS

Isolation of *Campylobacter jejuni*

A total of 56 retail slaughter house samples were analyzedfor the presence and number of *Campylobacter jejuni.*According to sugar fermentation, Hippurate hydrolase and IMVIC test isolates were confirmed as *Campylobacter jejuni*. Out of 56 samples, 73.2% of samples were contaminated with *Campylobacter jejuni*. Among the 8 sources of samples, the highest range of 100% was noticed in both liver and intestine. The next high percentage of 86% was noticed in Chopping board and Washed water; it may be due to the leakage of cellular content of intestines and liver parts from the chopped poultry chicken. The lower percentages of 57.1% were detected in Knife, Chest and weighing balance in the slaughter house followed by 43% was appeared on a machine (Fig. 1). The percentage values are insisting the importance of hygienic conditions of poultry farm and Slaughter house to prevent the infection of *C. jejuni* to human beings.

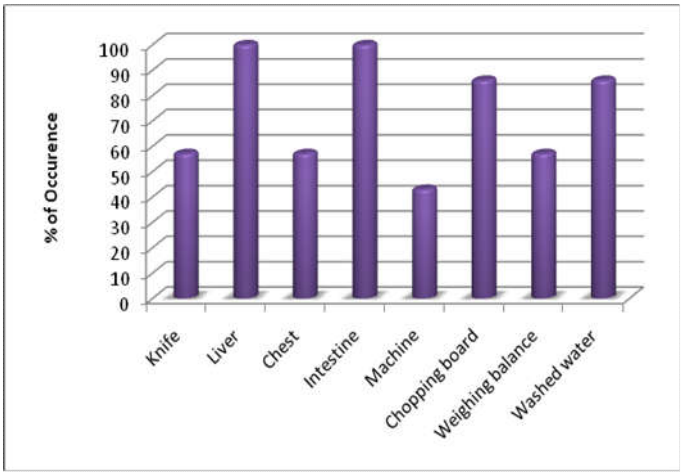

Fig. 1. Occurrence of *C.jejuni* on slaughter house line

Antibiotic resistance test

The antimicrobial resistance of *Campylobacter* was determined in 41 isolates, among them,10% of isolates were resistant to 7 types of antibiotic and 66% of were resistance to 5 types of antibiotic, none of the isolates were sensitive to all antibiotics and 5% of isolates were resistant to 2 types of antibiotics. In this study, more than 4 antibiotic resistances isolate were considered as multidrug resistance isolates. The overall antibiotic resistance pattern, 83% of isolates has resistance against Ampicillin followed by 71% against Cephataxime, 63.4% against Amoxicillin (Fig.2). In this study, lowest resistance was observed against to Tetracycline (7.3%). In the present study, 18 types of resistance patterns were observed, among them, C, S, NA, CTX, G patterns was observed from 17% of isolates and AM, NA, CTX, CIP, AMP pattern was observed from 12.1% of isolates (Table.1). Among the 8 types of sources, highest antibiotic resistance isolates were observed from Intestine (56%) second most were in washed water (52%) and 50% of isolates were observed from knife and weighing balance (Fig.3).

Table 1. Antibiotic resistance patterns of *C.jejuni*

S.No	Resistance patterns	No of isolates	Percentage
1.	C,AM,CTX,CIP,AMP	1	2.4
2.	AM,E,CTX,CIP,AMP	2	4.8
3.	AM,NA,CTX,CIP,AMP	5	12.1
4.	AM,E,CTX	1	2.4
5.	AM	2	4.8
6.	S,NA,CIP,G,AMP	2	4.8
7.	C,AM,CTX,G,AMP	2	4.8
8.	AM.G,AMP	1	2.4
9.	E,CTX,CIP	1	2.4
10.	C,AM,S,CTX,CIP,G,AMP	4	10
11.	C,AM,NA,CIP,AMP	2	4.8
12.	C,S,NA,CTX,G	7	17.0
13.	AM,CTX,CIP,AMP	2	4.8
14.	E,CTX,CIO,AMP	2	4.8
15.	S,NA,AMP	1	2.4
16.	AM,TET,NA,CIP,AMP	3	7.3
17.	C,AM,CTX,G,AMP	1	2.4
18.	C,AM,NA,CTX,AMP	2	4.8

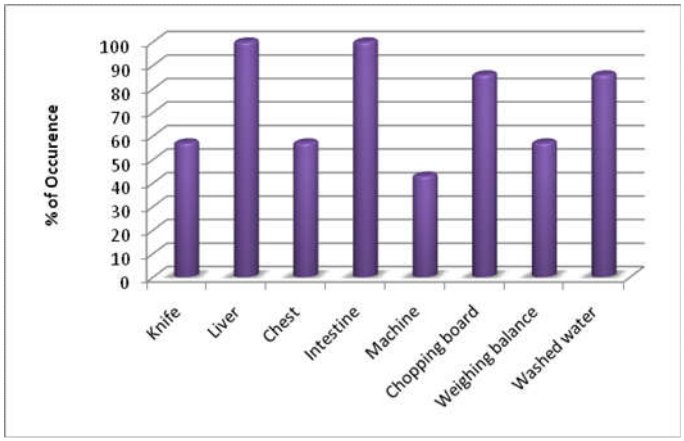

Fig. 2. Antibiotic resistance of *C. jejuni* isolates

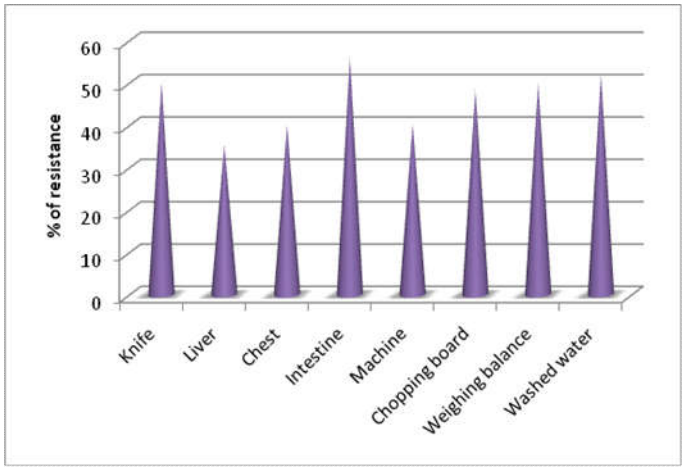

Fig. 3. Percentage of antibiotic resistance on slaughter house line

Isolation of biofilm producing isolates

Among 41 isolates of *C. jejuni* assessed for biofilm formation, only 20 of them developed biofilm within a 24 hrs period at 37°C. Results in Table 2 showed that *Campylobacter jejuni* isolated from chopping board and washed water (67%) had the highest prevalence, followed by isolates from the knife, chest, liver and weigh balance (50%).In present investigation, most of the biofilm producers were observed from multidrug resistance isolates than non multidrug resistant isolates.

Table.2 Prevalence of biofilm producing *C.jejuni* from slaughter house line

Slaughter house	
Samples name	% of occurrence
Knife	50
Liver	50
Chest	50
Intestine	28.5
Machine	33.3
Chopping board	67
Weighing balance	50
Washed water	67

DISCUSSION

Campylobacter spp. is recognized as a main cause of human enteritis outbreaks in both developed and developing countries. Birds and animals are the primary sources contributing to human infection. Mostly, *Campylobacter* infection was transferred to human beings through handling of infected poultry samples, especially at evisceration and plucking phases (Bojan *et al.,* 2017). In the current study it was noticed that the frequency of *Campylobacter* in samples collected at the flock-based slaughter houses. The entry point to the slaughterhouse of flocks positive for *Campylobacter* is the main reason for the pathogen dissemination all over the line's plant (Reich *et al.,* 2008; Hue *et al.,* 2010). In this study, the highest prevalence was observed in liver and intestine of poultry samples. In India, Chetana *et al.,* (2015) were observed the *Campylobacter jejuni* from poultry intestine samples. In this study, *C. jejuni* were found from not only the carcasses of poultry samples, it also is within slaughter line, namely as, defeathering instruments, weighing balance, chopping boards and knives. Among the 8 types of samples, the highest incidence was observed from chopping board and washed water followed by other sources. The cross contamination of these pathogens from one step to another on the slaughter line (scalding, defeathering, evisceration, carcass chilling, deboning), along with the hazard analysis critical control point plan implemented in the plant, is important (Hue *et al.,* 2010).

Presence of bacteria on surfaces of cutting boards can lead to transmission of bacterium to uncontaminated meat products. This chopping board is inanimate objects which don't have basic nutrients for survival of microbes. However, microbe's survival was due to the continuous use, remaining of wastes or small pieces of flesh during the process, improper handling of equipment, improper cleaning and failing to disinfect the equipment which is used during the slaughtering process. Several reports are available for occurrence of *C. jejuni* from inanimate objects (Gough and Dodd 1998; Newell and Wagenaar, 2000; Tang *et al.,* 2011). In this study, apart from chopping board, other inanimate products of the knife and weighing balance also consist of *C. jejuni* isolates. This incidence was agreed with previous studies of Luber *et al.,* (2006). They were observed the *C. jejuni* from knife samples and suggested that the isolates were contaminated through the cutting of infected carcasses. Cross-contamination is a well known route for transmission of bacteria from contaminated sources of the finished products (Kusumaningrum *et al.,* 2004). Washing of cutting boards with hot water and soap oil or detergents after use is an efficient method to remove the bacteria fromthe surface of cutting boards, knife and weighing balance, etc., unfortunately, most of the slaughter house cleaners are not aware of the health risks associated with bacterial infection. Improper cleaning procedures might concomitantly displace bacteria from contaminated equipments to another area within the processing environment. (Taormina & Beuchat, 2002).

Carcasses are commonly washed with chlorinated water to remove contamination, such as blood, tissue fragments, and fecal contamination, as part of the regular processing procedure. During the period of sample collection, most of the slaughter house handlers were not properly washed the carcasses. In the current study, 86% of occurrence was observed from washing water. Several studies addressed the prevalence of *C. jejuni* from poultry washed water (Hoon *et al.,* 2002; Bashor *et al.,* 2004; Thomas *et al.,* 2006; Hinton *et al.,* 2007). Antimicrobials are used in food of poultry and animal farms as a therapeutic agents and growth promoters. The use of

effective drugs has been essential to guarante the high indices of productivity reached in the last decades, propitiating a reduction of mortality and morbidity and the maintenance of poultry chicken well–being. However, the indiscriminate use of antimicrobials can lead to cause the resistant to bacteria for particular antibiotics in food and poultry chicken, then its subsequently be transmitted to humans and finally causes a serious public health problem (McEwen and Fedorka–Cray, 2002). The highest probability of antimicrobial resistance occurrence of *C. jejuni* was noticed for betalactam group antibiotics tested, such as ampicillin (83%) and Amoxicillin (63.4%) and second most in Cephalosporin group of Cephataxime (71%). Also, a high probability of Ciprofloxacin resistance occurrence was revealed for the *Campylobacter jejuni* tested (61%).The Resistance rate to ampicillin was lower than those findings of Hungaro *et al.,* 2015; Ozkan 2017. Generally, *Campylobacter spp.* are immanently resistant to β-lactam antibiotics, due to production of β-lactamases enzymes, which was reducing the binding of β-lactams to the target (penicillin-binding proteins [PBPs]), or failure of the drugs to penetrate the outer membrane porins (Usha *et al.,* 2010). Tetracyclines were listed as an alternative treatment for campylobacter gastroenteritis in the past. It was used as subtherapeutically feed additives for livestock and poultry. In our study, the resistance to tetracycline was 7.3 %. This resistance percentage was low compared to previous studies of Marija *et al.,* 2005. In this study, quinolones group of Nalidixic acid also resistance to 54% of isolates. Mostly these drugs are utilized for the treatment of human gastrointestinal infection, so the increased resistance of such isolates poses a public health problem (Wieczorek *et al.,* 2012).

The persistence of *C. jejuni* in the slaughter environment is possibly due to their capacity for resistance even under oxidative stress or low temperatures and improved adaptation to biofilm formation (Gundogdu *et al.,* 2011; Sulaeman *et al.,* 2012). Therefore, all isolates were subjected for determination of biofilm formation. Among the 8 types of sources, predominant biofilm producers were observed from chopping board and washed water (Yavuz *et al.,* 2011). A number of studies conducted under the experimental conditions have reported that *C. jejuni* can be present in biofilms found in animal production watering systems and may play a role in the colonization of these animals. Several authors raise the question as to whether *C. jejuni* forms biofilms as a survival mechanism in the environment or if it just attaches to surfaces. In this study, substantially biofilm producers were observed from most the isolates, even inanimate objects (chopping board, knife). The extracellular matrix is an essential component of bacterial biofilms, and usually accounts for more than 90% of the dry mass of a biofilm (Flemming and Wingender, 2010). It allows cells to remain hydrated and metabolically active by trapping nutrients and liquid near the bacterial cells. It also prevents the penetration of antibiotics (Mulcahy *et al.,* 2008; Billings *et al.,* 2013). This current study of *C. jejuni* biofilm formation under laboratory condition assigns to our understanding of their survival in the environment. The conclusion from the present study with poultry slaughter line at the retail level, it can be seen that the percentage of *Campylobacter*-positive samples were higher, however sample were collected from different stages of slaughter line. Furthermore, most of the isolates were resistant to fluoroquinolones and quinolones antibiotics. These antibiotics are considered as the drugs of choice for the treatment of human gastroenteritis infections. These finding suggest that the consumption of uncooked meat or food cross-contaminated with *Campylobacter* may pose a serious threat to consumer health.

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