

Detection of *Campylobacter* spp. from Poultry and Poultry Products in Dhaka, Bangladesh

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Abstract

Poultry meat is frequently contaminated with *Campylobacter* strains and is thought to be the major source of organisms causing human *Campylobacter gastroenteritis*. This study was carried out to detect *Campylobacter* spp. in raw chilled chicken meat, chicken gut, pre-cooked frozen chicken nuggets and chicken sausages from super markets in different areas of Dhaka city, Bangladesh. Isolation of *Campylobacter* spp. was done using the conventional method in which 10g of each sample (except chicken gut) was homogenized with 90ml of enrichment broth and incubated in microaerophilic condition at 42°C for 48hours. Samples were then streaked onto *Campylobacter* charcoal differential agar base supplemented with CCDA selective supplement and was incubated in microaerophilic condition at 42°C for 48h. For chicken gut, swabs are taken from the inner walls of the gut and directly streaked on CCDA plates. Microscopic examination, gram staining, biochemical tests were done for the presumptive identification of *Campylobacter* spp. The results showed that *Campylobacter* spp. can be found in various parts of raw chicken meat, chicken sausages, and chicken gut while chicken nuggets showed the absence of *Campylobacter* spp. This finding reflects the risk of cross-contamination while handling of poultry in our kitchen during the preparation of food.

Keywords: Food safety; Food hygiene; *Campylobacteriosis*; Poultry; Poultry products

Introduction

Human enteritis in developed and developing countries is mostly caused by *Campylobacter* spp. with *C. jejuni*, the most important pathogenic strains belonging to the thermotolerant group, considered as the leading cause for foodborne and

waterborne gastroenteritis worldwide [1]. *Campylobacter jejuni* is most often implicated as the causative agent of *Campylobacteriosis* and are commonly isolated from chicken and cattle, and chicken is expected to be one of the major sources of infection.

Poultry meat and poultry products consumption and production show an upward trend worldwide for a number of reasons including its increased use in food preparations, strong consumer preference, and a great demand on a rich protein source (20–23%) with reasonable cost [2-4].

Campylobacter occurs as a commensal microorganism in the microflora of the gastrointestinal tract of poultry, thus it is likely to occur on the exterior of the bird as well as from the contamination of the growth environment. The normal body temperature of poultry encourages the gastrointestinal establishment of this organism that supports their high optimal growth temperature [5]. It was reported that *Campylobacter* infections in human are usually associated with the ingestion of improperly handled or undercooked food, mainly poultry products [6].

As *Campylobacter* occurs as natural microbial flora in the gastrointestinal tract of poultry, after slaughtering it is possible that they can spread to poultry carcasses, parts and products. Moreover, *C. jejuni* survives well in modified atmosphere and vacuum packaging and also under refrigerated temperatures [5] making it plausible to occur in packaged chicken products that have gone through poor handling and processing. The prevalence of *Campylobacter* spp. on raw poultry products ranges from 0 to 100%, with an average of 62%.

The aim of this study was to evaluate the prevalence of *Campylobacter* spp. in raw chicken meat (chilled), chicken gut and packaged chicken-based products that have been stored in freezing condition.

Materials and Method

Sample collection

At slaughter: One chicken gut was collected from the kitchen in a sterile plastic bag and submitted to the laboratory as soon as possible.

At super market: Three intact packets of different brands of frozen pre-cooked chicken nuggets and chicken sausages were bought from the supermarket and submitted to the laboratory. Raw chilled minced chicken meat was bought and submitted intact to the laboratory in a plastic bag.

Media preparation

Campylobacter Enrichment Broth: 25g was thoroughly mixed in 1liter water. It was swirled and autoclaved 15 min at 121°C (in screw-capped bottles).

Procedure for enrichment and isolation

For Chicken nuggets, Chicken Sausages and Minced Chicken Meat: 10 gram of weighed sample was homogenized with 90 ml of *Campylobacter* enrichment broth and incubated for 24 hours at microaerophilic condition at 42°C. After that streaking was done on CCDA (Campylobacter Charcoal Differential Agar) this is selective for *Campylobacter* spp. and incubated at microaerophilic condition at 42°C for 48 hours. Plates were observed. For chicken gut, cotton swabs are taken from inner walls of the colon and directly streaked on CCDA plates.

Reagents used

Reagents used are listed in Appendix I and II.

Observation and Result

Plates were observed after 48 hours of incubation (Figure 1).

Colony characteristics of *Campylobacter* spp. on CCDA plate

For chicken sausages and raw chilled minced meat, heavy to moderate growth was found. The colonies were gray in color, glistening, spreading to round shaped, elevated and the texture was creamy. For chicken nuggets samples, no growth was found.

Microscopic examination of morphology and motility

Material from a suspected colony was suspended in saline and evaluated by light microscope for the characteristic. Spiral or curved slender rods with a corkscrew-like motility were observed though not very clearly. Older cultures showed less motile coccoid forms.

Gram staining

Gram staining was performed from suspected colonies. All were gram negative.

Oxidase test

The oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) was taken on a filter paper and placed in a Petri dish. The filter paper was moistened with distilled water. The colony from the CCDA agar was picked with help of a tooth pick and soaked on the oxidase solution region. The appearance of the blue color within 10 seconds would indicate a positive result.

Catalase test

A small amount of growth (single, discrete colony) from CCDA plate was placed on a clean microscope slide. A few drops of H₂O₂ was added to the smear. It was mixed with a toothpick. A positive result was indicated by the rapid evolution of O₂ as evidenced by bubbling.

Hippurate hydrolysis test

This test is used to determine whether a microorganism, by the action of the enzyme hippurate hydrolase, can hydrolyze sodium hippurate to benzoic acid and glycine. 0.4 ml of sodium hippurate solution was inoculated with heavy inoculums (single colony). It was shaken by vortex. Incubated for 2 hours at 37°C aerobically. 0.2 ml of Ninhydrin solution was added slowly down the side to form an overlay. It cannot be shaken. Tubes reincubated at 37°C and checked for color change after 10 to 20 minutes.

Presence of a deep blue or purple color indicates a positive reaction. The tubes that were colorless showed negative for hippurate hydrolysis test (Table 1).

Discussion

In this study, *Campylobacter* spp. was isolated from poultry and poultry products. Enrichment of chicken nugget samples, chicken sausage samples and chilled raw minced meat samples

Table 1: Biochemical test results of the colony found on CCDA plate

Sample	Growth at 42°C	Oxidase test	Catalase test	Hippurate hydrolysis test
Chicken Nuggets				
Sample 1	-	-	-	-
Sample 2	-	-	-	-
Sample 3	-	-	-	-
Chicken Sausages				
Sample 1	+	+	+	+
Sample 2	+	+	+	+
Sample 3	+	+	+	+
Raw Chilled Minced chicken meat				
	+	+	+	-
Chicken Gut				
	+	+	+	+



Figure 1: *Campylobacter* colonies on CCDA plates

was performed prior to isolation because organisms might be under stress due to refrigeration. In case of the chicken gut, cotton swabs were taken from the inner walls of the gut (as they are microaerophilic, cotton swab stick was pushed inside) and streaked directly on selective media. Results showed that chicken sausages, chilled raw minced meat and chicken gut contained *Campylobacter* spp. *Campylobacter* was absent in chicken nugget samples. All the incubations were done at 42°C as most of the pathogens cannot grow at this temperature and thus the contamination risk is low here.

Presence of *Campylobacter* spp. in pre-cooked frozen poultry products (chicken sausages) indicates improper handling. Due to the fact that *Campylobacter* survives well in modified atmosphere and vacuum packaging and also under refrigerated temperatures, there are possibilities that it could occur in packaged chicken products that have gone through poor handling and processing. Consumption of insufficiently cooked frozen poultry products might be the cause of acquiring foodborne infections. Chicken gut leaving the slaughterhouses contained *Campylobacter* spp. This signifies the risk of cross-contamination while handling of poultry in our kitchen. As the intestinal tract of chicken, especially the cecum and colon can harbor a large number of *Campylobacter* spp. as commensal microflora, during processing the intestinal tract may leak or rupture and the contents are transferred to the skin of the carcass. Thus it is likely to found *Campylobacter* spp. in meat (poultry carcass) if contamination occurs. However, the supermarkets provide facilities for raw chicken storage and displaying in chilled or frozen condition. This is proof that efforts are being taken to control the quality and safety of the raw chicken sold in those premises. But it is often that if one part of the chicken has been contaminated, there are chances that the organism will spread to other parts as well. Thus, it is not surprising to find that even the inner parts of the chicken were also contaminated, most likely due to cross-contamination during processing.

The chilled meat samples were taken from various supermarkets where chicken meat was stored and displayed in chilled condition. Supermarkets, usually offer chicken meat under conditions that appear more hygienic than those in wet markets with better control of the storage temperatures. Even though foodstuffs in supermarkets appear more hygienic, the preparation before packing could be unhygienic and the packing facilities itself could be in poor conditions.

Chicken nuggets showed the absence of *Campylobacter* spp. Failure of isolating the organism could be either due to a high concentration of salt and other ingredients contained in the product or the nuggets have gone through proper handling process. *Campylobacter* can usually survive up to 2.0% of salt. So in this case, it was possible that the samples might have been contaminated by *C. jejuni*, but later was killed by the use of salt and spices. Another reason might be they had undergone minimal cooking process prior to freezing. This may have reduced the risk of occurrence of *Campylobacter*.

Microscopic examinations and biochemical tests were done to presumptively identify the *Campylobacter* spp. Hippurate hydrolysis test was done to differentiate between *C. jejuni* and other species. As the samples showed positive result for hippurate hydrolysis test other than raw chilled meat, it can be inferred that *C. jejuni* might be present in sausages and chicken gut. Other species such as *C. coli*, *C. lari*, *C. fetus*, *C. upsaliensis* might be present in raw chilled meat as these do not hydrolyze hippurate. Again it should be kept in mind that *Campylobacter* spp. may occasionally be misclassified when species identification is based on a single phenotypic trait such as the hydrolysis of hippurate. Molecular analysis should be performed to confirm at species level identification. In a study, it was reported that *C. jejuni* was found in various parts of fresh and chilled raw chicken but the absence of *C. jejuni* was reported for the analysis done on marinated raw chicken meat and frozen chicken-based products [7].

Conclusion

Consumption of insufficiently cooked frozen poultry products poses the risk of acquiring foodborne infection caused by *Campylobacter* spp. Supermarkets provide healthier environment than wet market to store chilled raw chicken but the packaging itself may be the source of contamination. Improper handling of poultry in the kitchen may cause cross-contamination in the kitchen as *Campylobacter* reside as the normal microbiota of gastrointestinal tract of poultry.

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Appendix I

Reagents

1	Gram staining	Crystal violet Gram's iodine Decolorizing agent Safranin
2	Oxidase test	1% solution of N,N,N,N-Tetramethyl-p-phenylenediamine Dihydrochloride
3	Catalase test	3% H ₂ O ₂
4	Hippurate hydrolysis test	Ferric chloride Concentrated hydrochloric acid

Appendix II

Media: Campylobacter Enrichment Broth

Components

Item	g/L
Peptic Digest of Animal Tissue	10.00
Beef Extract	10.00
Sodium Chloride	5.00

Final pH (at 25 °C) 7.5 ± 0.2

Media: CCDA (Campylobacter Charcoal Differential Agar)

Composition

Ingredients	g/L
Meat extract	10.00
Peptone	10.00
Casein enzymic hydrolysate	3.00
Sodium chloride	5.00
Sodium deoxycholate	1.00
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Charcoal, bacteriological	4.00
Agar	12.00

Final pH (at 25°C) 7.4 ± 0.2