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MICROBIOLOGY

Prevalence of *Campylobacter* species in raw meat samples sold in open markets of Kolkata city

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Abstract

Campylobacteriosis is a zoonosis, caused by the infection with *Campylobacter*. Raw meat contaminated by *Campylobacter* from faeces of animals during evisceration and by spillage of intestinal content. In recent times, Campylobacter has emerged as an important food borne pathogen of both human and animals, and regarded as one of the most important zoonotic pathogen worldwide. Chicken and mutton meat have repeatedly been implicated as a source of food borne infections for humans, as both these meat are amongst most consumed meat in India. In this study, four Campylobacter spp. viz *C. jejuni*, *C. coli*, *C. lari* and *C. fetus* were isolated from raw meat samples. This study was therefore conducted to determine the prevalence of *Campylobacter* spp. from raw meat samples in Kolkata, India. A total of 200 raw meat samples, chicken (n = 100) & Mutton (n = 100) were collected randomly from open meat markets in Kolkata, India and were tested for the presence of *Campylobacter*. *Campylobacter* spp. was isolated from 136 of 200 (68%) raw meat samples examined. The highest prevalence of *Campylobacter* spp. was found in Chicken meat (72%), followed by mutton meat (64%). The most prevalence *Campylobacter* species isolated from raw meat samples were *Campylobacter jejuni* (58.82%), followed by *Campylobacter coli* (26.47%), *Campylobacter lari (8.82%) & Campylobacter fetus (5.89%)*. It was concluded that a high proportion of raw meat sold in open market in Kolkata, India, was contaminated by Campylobacter spp. and the consumption of undercooked meat possess a possible health risk for consumers.

Highlights

- *Campylobacteriosis* is of serious concern amongst butchers of Kolkata.
- Chicken meat is more contaminated than mutton meat.
- Open meat markets of Kolkata are reservoir of *Campylobacteriosis*.

Keywords: Campylobacter, chicken, Kolkata, mutton, open market

Meat industry plays an important role in the socio-economic upliftment of the people of West Bengal, especially Kolkata city and other states of India. Nowadays more people engaged in poultry business and no doubt this industry also generating lots of employment amongst the youth. The total meat production in the country was reported as 4.3 million tonnes in the year 2008-09 and the production of meat shown an increasing trend during the period 2008-09 to 2012-13 with an average annual growth rate of 8.2%**.** The annual growth rate for the year 2012-13 was 7.8% with highest annual growth rate of 13.2% was reported in 2011-12 due to inclusion of production from commercial poultry by many States during that period. 45% of the production of meat was contributed by Poultry and Sheep meat contributed about 7%. The annual growth rate of meat production during 2012-13 for West Bengal was 6.1%. West Bengal was the third largest meat producer state in the country which produces 10.9%

of the total production. The average meat production from sheep in the state of West Bengal was 0.027 million tonnes per year, and meat production from poultry in the state of West Bengal was 0.329 million tonnes per year for the year 2012-13 (BAHS 2014). *The genus Campylobacter* contains slender, spirally curved rods about 0.2-0.5 μm thick and 0.5-5 *μm long. They are typically comma shaped but may occur as 'S' or multispiral chains. Campylobacter* species are gram-negative, non-spore forming and motile with single unsheathed polar flagella at one or both the poles, microaerophilic organisms i.e. 5% oxygen concentration being optimal and many pathogenic species are thermophilic, which grows well at 42°C, and Strongly oxidase positive. It is reported that *Campylobacter* species are the most common food borne bacterial cause of human enteric diseases in several countries (Scallan *et al*. 2011), and those common *Campylobacters* causing human diseases are *C. jejuni* and *C. coli (*Vaishnavi *et al.* 2015). It is found to be responsible for food-borne enteric infection among consumers world-wide. The infection may be acquired by consumption of undercooked poultry (Shane, 1992) or red meat. Within the genus *Campylobacter, Campylobacter jejuni* and *Campylobacter coli* are the predominant species isolated from fresh meat and, poultry are the most common species associated with human Campylobacteriosis (Corry and Atabay 2001). Virulence factors in *C. jejuni* and *C. coli* are considered useful tools as it assess the risk of poultry as a source of *Campylobacter* infection (Melo *et al*. 2013). In one study, it was found that Campylobacteriosis associated with unscientific handling of raw poultry, and by eating raw or undercooked contaminated meats (especially poultry and/or by-products), cross-contamination of raw or cooked foods and lack of hygiene (Suzki and Yamamoto 2009). Campylobacter spp. is one of the common contaminant of poultry carcasses in different poultry processing plants (Son *et al.* 2007). It is believed that consumption and handling of infected poultry and poultry products act as major source of human campylobacterial enteritis. Yano *et al.* (2013) monitored *C. jejuni* in four chicken farms during the period 2003 to 2006 to elucidate the mechanisms of transmission. *C. jejuni* is the most important campylobacter species as it causes attacks of diarrhea worldwide. Disease caused by *Campylobacter* usually manifests at diarrhea, and severe abdominal pain. There have been many studies regarding isolation of *Campylobacter* spp. from food samples, especially poultry meat. Wide variation (0-100%) in the prevalence of *Campylobacter* in fresh poultry meat have been reported in different countries (Sallam 2007). Although, much attention has been focused on poultry meat, some studies reported that red meat also remains the most common cause of food borne general outbreaks of infectious intestinal disease (Little *et al.* 2008). Currently, there is limited information on the prevalence of *Campylobacter* in raw meat in India; some productive studies have been carried out in Kolkata, Uttar Pradesh, Maharashtra and North Eastern states of India. The aim of the present study is to determine the prevalence of *Campylobacter* species in raw chicken and mutton meat in Kolkata, India.

Materials and Methods

Samples

A total of 200 raw meat samples, chicken (n=100) and Mutton (n=100) were collected from different open markets of Kolkata city. The samples were collected from 5 different zones of Kolkata viz. East, West, North, South and Central. The samples were brought to the laboratory at room temperature and processed within 4 hr.

Isolation of *Campylobacter*

The processing of raw meat samples were done under aseptic conditions. Samples were collected in test tubes containing Normal saline. To ensure proper mixing of meat with Normal saline, vortex machine was used and mixing was carried out for 5-10 minutes. Samples were tested in different dilutions to ensure the accurate result and least possible error. Isolation of *Campylobacter* from meat samples was done by plating the samples onto selective media. Enrichment was very important for those samples where concentrations of microorganisms were very low. Modified charcoal, cefoperazone, desoxycholate agar (mCCDA) was used as selective media. *Campylobacter jejuni* and *C. coli* showed growth on solid media between 24-48 hours at incubation temperature of 42°C. Microaerobic atmospheres of 5-10% oxygen, 5-10% carbon dioxide was given for optimal growth. Anaerobic gas jar evacuations followed by atmosphere replacement with bottled gasses were used. Gas generator kits were available

Table 1: Prevalence of Campylobacter spp. isolated from raw meat samples in Kolkata, India

Meat sample	Number of meat sample	*C. jejuni*	*C. coli*	*C. lari*	*C. fetus*	Campylobacter spp. positive samples
Chicken	100	37 (51.4)	19 (26.4)	9 (12.5)	7 (9.7)	72
Mutton	100	43 (67.2)	17 (26.8)	3 (4.7)	1 (1.6)	64
Total	**200**	**70 (58.8)**	**36 (26.5)**	**12 (8.8)**	**8 (5.9)**	**136**

Table 2: Zone-wise prevalence of Campylobacter spp. in raw chicken meats in Kolkata, India

Campylobacter Spp.	East Kolkata	West Kolkata	North Kolkata	South Kolkata	Central Kolkata	Total
C. jejuni	8 (11.1)	9 (12.5)	7 (9.7)	5 (6.9)	8 (11.1)	37
C. coli	4 (5.5)	1 (1.4)	6 (8.3)	5 (6.9)	3 (4.2)	19
C. lari	2 (2.8)	1 (1.4)	2 (2.8)	2 (2.8)	2 (2.8)	9
C. fetus	2 (2.8)	1 (1.4)	3 (4.2)	1 (1.4)	0	7
Total	**16 (22.2)**	**12 (16.7)**	**17 (23.6)**	**13 (18)**	**13 (18)**	**72**

Table 3: Zone-wise prevalence of Campylobacter spp. in raw mutton meats in Kolkata, India

Campylobacter Spp.	East Kolkata	West Kolkata	North Kolkata	South Kolkata	Central Kolkata	Total
C. jejuni	9 (14)	17 (26.5)	8 (12.5)	6 (9.4)	3 (4.7)	43
C. coli	3 (4.7)	4 (6.2)	3 (4.7)	4 (6.2)	3 (4.7)	17
C. lari	0	2 (3.1)	0	1 (1.6)	0	3
C. fetus	0	0	1 (1.6)	0	0	1
Total	**12 (18.7)**	**23 (35.9)**	**12 (18.7)**	**11 (17.2)**	**6 (9.4)**	**64**

from commercial sources. Media was incubated at 42°C to minimize growth of contaminants and to select for optimal growth of *C. jejuni*/*C. coli*. The locally available fungistatic agents were also added to media in order to prevent growth of yeasts and mould at 37°C. *Campylobacter jejuni* and *C. coli* showed growth on solid media between 24-48 hours at 42°C.

Confirmation

Confirmations of the campylobacter organism were done with the findings mentioned below:

(i) *Identification on solid medium:* On charcoal-based media mCCDA, the characteristic colonies were grayish, flat and moistened, showing spreading tendency, and had a metal sheen.

(ii) *Microscopic examination of morphology and motility:* Material from a suspect colony was suspended in saline and evaluated by a phase-contrast microscope, which showed characteristic spiral or curved slender rods with a corkscrew-like motility.

(iii) *Oxidase Test:* Commercial oxidase test kit was used for confirmation; violet or deep blue colour appeared within 10 seconds which indicate oxidase positive and confirmed the organism.

Results and Discussion

The prevalence of Campylobacter spp. isolated from examined raw meat samples summarized in Table 1. Out of 200 meat samples examined, 136 (68%) were found to be contaminated with Campylobacter. Campylobacter spp. was detected in 72% of raw chicken meat & 64% of mutton meat samples. The most prevalent species recovered from samples was *Campylobacter jejuni* with 58.8% of the isolates confirmed. The finding of the present study is comparable with the work of Rajendran *et al*.

(2012) where the prevalence of *Campylobacter jejuni* in chicken sample was 64%. Rahimi *et al*. (2010) also reported high prevalence of *C. jejuni* in chicken meat (61.7%) but in another such study of Geetha, M. (2013), the prevalence of campylobacter jejuni in chicken meat sample was as low as 25.45%. The remaining 26.5%, 8.8% and 5.9% of isolates were identified as *C. coli, C. lari & C. fetus* respectively (Table 1). Campylobacter spp. were isolated from all the five zones of Kolkata under study namely East, West, North, South, Central Kolkata. The organisms isolated from these zones were explained in Table 2, 3. In the present study, 64% of mutton meat samples were found to be *Campylobacter* Positive. Little *et al.* (2008) isolated campylobacter from 12.6% of mutton meat samples, while Hussain *et al.* (2007) isolated 5.1%, which is very low as compared to current study. The higher prevalence in the present study might be due to cross contamination during manual skinning, evisceration and processing in the butcher shops.

Different studies have been carried out in different countries to detect the prevalence of campylobacter in raw chicken and mutton meat samples. In India, the prevalence of campylobacter spp. is variable from one to another state. Pallavi and Kumar (2014) reported 17.33% prevalence of *Campylobacter* species from poultry meat in and around Bareilly area of Uttar Pradesh. In another study, Singh *et al*. (2009) reported 12.7% prevalence of *Campylobacter* from poultry meat and carcass collected from local poultry farms and retail markets of Bareilly. Studies on isolation of *Campylobacter* from poultry meat have been carried out by Chowdhury et al., (1984) using conventional methods, where prevalence of isolates were 62.5% and Varma *et al*. (2000) have reported *C. jejuni* from 40% meat surface samples of poultry. In one Study by Vaishnavi *et al.* (2015), where 44.9% *Campylobacter isolation was done* from intestinal tract of poultry in Chandigarh of India using modern technology. Some Indian studies documented the burden of infections due to *Campylobacters* in poultry Parkar *et al. (*2013) and humans dealing with them Rajendran *et al. (*2012). The isolation rate in chevon samples were found to be 6% in U.P. by Rajkumar *et al*. (2010), which is less compared to current study. Since long *Campylobacter* known as a part of the normal flora in the intestine of most animals including poultry due to their high body temperature which provides an optimum growth to pathogens, leading to more chances of faecal contamination of meat (Noormohamed and Fakhr 2014). The fecal materials contaminate the raw meat, as in most of the roadside meat shops there is no separate infrastructure for slaughter and washing. About 10% cases of human Campylobacteriosis were detected from stool samples in one such study by Salim *et al*. (2014). In another study it is found that the prevalence of Campylobacteriosis is more in males than in females and Campylobacteriosis occurs much more frequently in summer than in winter (Schielke *et al.* 2014), might be because of higher professional exposure of males with meat animals.

Most of the meat shops in India, especially in Kolkata city are unorganized. The meat sellers or butchers lack the knowledge of proper meat handling and slaughter of meat animals. Majority of the people purchase and consume the chicken and mutton meat from such open markets. The butchers and meat traders are less aware of the hazards from meat, i.e. meat borne diseases and lack the knowledge of zoonotic potentials. The wholesomeness of the water used for washing the carcasses after slaughter and dressing is still doubtful. There are very few open meat shops where proper drainage system exist, which acts as the commonest source of different hazards in Kolkata city. Proper hygienic conditions while processing Chicken and mutton meat can reduce the load of *Campylobacter* on the meat surfaces.

Conclusion

The prevalence of Campylobacter spp. in Chicken and mutton meat in Kolkata city, India was found to be high (68%). One of the major causes of this high prevalence in meat was cross contamination of meat with handler's faecal materials, as during slaughter process in open meat market, it is found that, after defecation they don't wash their hands with any soap, and don't even use plenty of plain water to wash hands, which might contaminate the meat samples. A gross survey regarding the health of meat handlers reveals majority of meat handlers had recent history of chronic diarrhea. This point can't be ruled out in contrast of the higher prevalence of campylobacter in meat samples in Kolkata, India. Therefore, Campylobacter contamination of carcasses during slaughter and processing

constitutes a risk for consumers which can only be prevented or minimized by proper health education to all concern persons.

References

Chowdhury, S., Balakrish Nair, G., Pal, S.C. 1984. Occurrence of Campylobacter jejuni in country chicken in Calcutta. *Indian J. Med. Res* **79**: 171-173.

Corry, J.E., Atabay, H.I. 2001. Poultry as a source of Campylobacter and related organisms. *Journal of Applied Microbiology* **90**: 96S-114S. http://dx.doi.org/10.1046/j.1365-2672.2001.01358.x

Geetha, M. 2013. Molecular Identification of Campylobacter Jejuni in Chicken Meat by Polymerase Chain Reaction. *Indian Vet. Journal* **90**(12): 87

Hussain, I., Mahmood, M.S., Akhtar, M., Khan, A. 2007. Prevalence of Campylobacter species in meat, milk and other food commodities in Pakistan. *Food Microbiology* **24**: 219-222. http://dx.doi.org/10.1016/j.fm.2006.06.001

Little, C.L., Richardson, J.F., Owen, R.J., de Pinna, E., Threlfall, E.J. 2008. Campylobacter and Salmonella in raw red meats in the United Kingdom: Prevalence, characterization and antimicrobial resistance pattern, 2003-2005. *Food Microbiol* **25**: 538-543. http://dx.doi.org/10.1016/j.fm.2008.01.001

Melo, R.T., Nalevaiko, P.C., Mendonça, E.P., Borges, L.W., Fonseca, B.B., Beletti, M.E. and Rossi, D.A. 2013. Campylobacter jejuni strains isolated from chicken meat harbor several virulence factors and represent a potential risk to humans. *Food Control* **33**: 227-231. http://dx.doi.org/10.1016/j.foodcont.2013.02.032

Noormohamed, A. and Fakhr, M.K. 2014. Molecular typing of Campylobacter jejuni and Campylobacter coli Isolated from Various Retail Meats by MLST and PFGE. *Foods* **3**: 82-93. http://dx.doi.org/10.3390/foods3010082

Pallavi and Kumar, A. 2014. Prevalence and antibiotic resistance pattern of Campylobacter species in foods of animal origin. *Vet. World* **7**(9): 681-84. http://dx.doi.org/10.14202/vetworld.2014.681-684

Parkar, S.F.D., Sachdev, D., deSouza, N., Kamble, A., Suresh, G., Munot, H., David Hanagal, D., Shouche, Y and Kapadnis, B. 2013. Prevalence, seasonality and antibiotic susceptibility of thermophilic Campylobacters in ceca and carcasses of poultry birds in the "live-bird market". *Afr. J. Microbiol. Res* **7**(21): 2442-53.

Rahimi, E., Kazemeini, H.R., Safaei, S., Allahbakhshi, K., Momeni, M. and Riahi, M. 2010. *African Journal of Microbiology Research* **4**(15): 1620.

Rajendran, P., Babji, S., George, A.T., Rajan, D.P., Kang, G. and Ajjampur, S.S. 2012. Detection and species identification of Campylobacter in stool samples of children and animals from Vellore, South India. *Indian J. Med. Microbiol* **30**: 85-88. http://dx.doi.org/10.4103/0255-0857.93049

Rajkumar, R.S., Yadav, A.S., Rathore, R.S., Mohan, H.V. and Singh, R.P. 2010. Prevalence of Campylobacter jejuni and Campylobacter coli from unorganized and organized small scale poultry dressing units of Northern India. *J. Vet. Public. Health* **8**: 1-5.

Salim, S.M., Mandal, J. and Parija, S.C. 2014. Isolation of Campylobacter from human stool samples. *Indian J. Med. Microbiol* **32**: 35-38.

Sallam, K.I. 2007. Prevalence of Campylobacter in chicken and chicken by-products retailed in Sapporo areas, Hokkaido, Japan. *Food Control* **18**: 1113-1120. http://dx.doi.org/10.4103/0255-0857.124294

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A. and Roy, S.L. 2011. Food borne illness acquired in the United States major pathogens. Emerg. *Infect. Diseases* **17**: 7-15. http://dx.doi.org/10.3201/eid1701.p11101

Schielke, A., Rosner, B.M. and Stark, K. 2014. Epidemiology of Campylobacteriosis in Germany—Insights from 10 Years of Surveillance. *BMC Infectious Diseases* **14**: 30. http://dx.doi.org/10.1186/1471-2334-14-30

Shane, S.M. 1992. The significance of Campylobacter jejuni infection in poultry: a review. *Avian Pathol* **21**(2): 189-213. http://dx.doi.org/10.1080/03079459208418836

Singh, R., Singh, P.P., Rathore, R.S., Dhama, K. and Malik, S.V.S. 2009. Prevalence of Campylobacter jejuni and Campylobacter coli in chicken meat and carcasses collected from local poultry farms and retail shops of Bareilly, Uttar Pradesh, India. *Indian. J. Comp. Microbiol. Immunol. Infect. Diseases* **30**: 90-93.

Son, I., Englen, M. D., Berrang, M. E., Fedorka-Cray, P. J., Harrison, M.A. 2007. Prevalence of Acrobacter and Campylobacter on broiler carcasses during processing. *International Journal of Food Microbiology* **113**(1): 16-22. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.06.033

Suzuki, H., Yamamoto, S. 2009. Campylobacter contamination in retail poultry meats and by-products in Japan: A literature survey. *Food Control* **20**(6): 531-537. http://dx.doi.org/10.1016/j.foodcont.2008.08.016

Vaishnavi, C., Singh, M. and Kapoor, P. 2015. Isolation of Campylobacters from intestinal tract of Poultry in Northern Region of India. *Advances in Microbiology* **5**: 797-806. http://dx.doi.org/10.4236/aim.2015.512084

Vaishnavi, C., Singh, M., Thakur, J.S. and Thapa, B.R. 2015. Low Prevalence of campylobacteriosis in the Northern Region of India. *Advance in Microbiology* **5**: 155-165. http://dx.doi.org/10.4236/aim.2015.53015

Varma, S.K., Jagadeesh, N., Mukhopadhyay, H.K., Dorairajan, N. 2000. Incidence of Campylobacter jejuni in poultry and Their Carcasses. *J. Food Sci. Technol* **37**: 639-641.

Yano, S., Kira, T., Morishita, Y., Ishihara, K., Asai, T., Iwata, T., Akiba, M. and Murase, T. 2013. Colonization of Chicken Flocks by Campylobacter jejuni in Multiple Farms in Japan. *Poultry Science* **92**: 375-381. http://dx.doi.org/10.3382/ps.2012-02710