Journal of Animal Research: v.6 n.2, p. 171-176. April 2016

DOI: 10.5958/2277-940X.2015.00189.8

Prevalence and antimicrobial resistance pattern of *Campylobacter* species among poultry and poultry handlers of Jammu

Javid Ahmad Lone, S.K. Kotwal, Majueeb U Rehman, Najimaana Wani, M.A. Malik and Maninder Singh*

Division of Veterinary Public Health and Epidemiology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology, R.S. Pura, Jammu, (J&K), INDIA

**Corresponding author: M Singh; Email: manindersingh2k2@gmail.com*

Received: 08 March, 2016 **Accepted:**

ABSTRACT

Campylobacter is one of the emerging zoonotic pathogens with poultry and their products serving as an important source of human infections. The present study was aimed to assess the prevalence of *Campylobacter* species among poultry and poultry handlers of R.S. Pura, Jammu and their antibiogram pattern. A total of 177 samples from poultry (n = 167) and poultry handlers (n = 10) were examined and 39 samples were found positive for *Campylobacter* species (32 *C. jejuni,* 6 *C. coli* and 1 *C. lari*). The prevalence of *Campylobacter* was 40.3, 13.2, 7.7 and 30.0% in poultry faeces, poultry meat, poultry carcass swabs and poultry handlers, respectively. Antimicrobial resistance pattern of *C. jejuni* and *C. coli* isolates was studied against nine antibiotics. Multidrug resistance among the isolates was found against ampicillin, metronidazole and cepholathin while high sensitivity was observed towards gentamicin, ciprofloxacin, furazolidone and tetracycline. The results of the present study indicate high prevalence of *Campylobacter* both in poultry and poultry handlers with varying *in vitro* sensitivity to different antibiotics. The outcome enunciates that appropriate control measures ensuring safety of poultry products and human health need to be devised.

Keywords: Antimicrobial resistance pattern, *Campylobacter*, prevalence, poultry, poultry handlers

Campylobacter species cause serious complications related to acute bacterial enteric disease leading to gastroenteritis in humans worldwide (Mazick *et al.* 2006; Kwan *et al.* 2008). Campylobacteriosis is described as an emerging food-borne disease (Houf and Stephan, 2007). The most important pathogenic strains associated with human infections belong to the group of thermo-tolerant *Campylobacter* spp. among which *C. jejuni* and *C. coli* are the most important. The infection can also result in life-threatening disorders like Guillain-Barre syndrome, reactive arthritis, haemolytic uraemic syndrome, meningitis and abortions indicating the public health significance of the organism (Moore *et al.* 2005; Baker *et al.* 2012). The consumption and handling of poultry and poultry products are the major sources of human infection for campylobacteriosis (Corry and Atabay, 2001). Poultry carcasses frequently serve as vehicle for *Campylobacter* transmission as any damage of intestinal tract integrity during slaughtering and dressing processes can lead to bacterial contamination (Son *et al.* 2007). Contamination can also occur directly or indirectly through air, bird to bird, via equipments and water. Cross contamination of *Campylobacters* from live birds to carcasses, poultry products and animal species is also an important route of transmission (Corry and Atabay, 2001).

Poultry meat is one of the popular foods in Jammu and Kashmir state accounting for 19.21% of the total meat production of the state (Economic Survey, J&K, 2013-14). However, majority of poultry meat processing is through unorganized sectorand the transmission of pathogens to humans through poultry meat may occur. The burden of infections due to *Campylobacters* in poultry and humans dealing with them is unknown in Jammu unlike other regions of the country where the status of this pathogen in poultry (Singh *et al.* 2008; Parkar*et al.* 2013) and humans (Jain *et al.* 2005; Rajendran *et al.* 2012) is well documented. The present study provides a comprehensive report on the prevalence and antibiogram of *Campylobacter* species

in poultry and their handlers of R.S. Pura area of Jammu region.

MATERIALS AND METHODS

Sample collection: The present study was conducted in Ranbir Singh Pura (R.S. Pura) area of Jammu region during the period from November 2010 to May 2011.Four types of samples *viz*., raw poultry meat (n=53), poultry carcass swabs (n=52), poultry faeces (n=62)and stool samples from poultry handlers (n=10) were collected from different retail market shops. Meat samples, carcass swabs and poultry faeces were collected in test tubes containing Cairy Blair transport medium. Samples from poultry handlers were collected by providing them with sterile wide mouthed containers containing the transport medium. After collection, all the samples were labelled, kept in containers held over ice packsand brought to the laboratory.

Isolation and Identification: Faecal samples from poultry and poultry handlers were directly inoculated on to the Butzler's selective medium (Chattopadhyay *et al.* 2001). Poultry meat samples were cut into small pieces of 10 gram each and homogenized in 90 ml of Normal Saline Solution (NSS) and 10 ml of the homogenate was transferred to Preston enrichment broth and incubated at 42ºC for 48 hrs under microaerophilic conditions. Meat swabs in Cairy Blair transport media were transferred to Preston enrichment broth and incubated as done for meat samples. Samples from each broth were streaked onto Butzler's selective medium. The plates were kept in candle extinction jar along with nutrient agar plate heavily inoculated with *Escherichia coli* and the jars were incubated at 42ºC, 37ºC and 25ºC for 48 hours (Chattopadhyay *et al.* 2001; Saha and Sanyal, 1989) and examined after 48 hours of incubation. If there was no growth, the plates were incubated for further 24 hours and re-examined. Different *Campylobacter* species were identified by morphological characteristics, Gram's staining, motility, oxidase, catalase, nitrate reduction test and other biochemical reactions performed following the method of Smibert (1978). The presumptive *Campylobacter* isolates were subjected to species identification using Hippurate Hydrolysis test (Hwang and Ederer, 1975), H_2S production in Triple Sugar Iron (TSI) agar, growth at 25ºC, indoxyl acetate hydrolysis and sensitivity to cephalothin (30 µg) and nalidixic acid (30 µg) (Table 1).

Table 1: Biochemical tests used for *Campylobacter* species identification

(Goossens and Butzler, 1992).

Biochemical characteristic	*C. jejuni*	*C. coli*	*C. lari*
Growth at 25°C	-	-	-
H_2S production on TSI agar	-	+	-
Nalidixic acid	S	S	R
Cephalothin	R	R	R
Catalase	+	+	+
Hippurate hydrolysis	+	-	-
Nitrate reduction	+	+	+
Indoxyl acetate hydrolysis	+	+	-
Oxidase	+	+	+

S=sensitive, R=resistant.

Antimicrobial susceptibility testing: The antimicrobial susceptibility of *Campylobacter* isolates was performed by disc diffusion method (Bauer *et al.* 1966). The antibiotic discs used were ampicillin (25µg), gentamicin (10µg), nalidixic acid (30µg), ciprofloxacin (5µg), furazolidone (50µg), tetracycline (30µg), metronidazole (05µg), cephalothin (30µg) and erythromycin (15µg) (Hi-Media Mumbai, India). A loopful of growth from Butzler's selective media was taken and mixed with 0.5 ml normal saline to make a fine suspension. A sterile cotton swab was dipped in the bacterial suspension to be tested. The cotton swab was rubbed gently over Muller-Hinton agar plate in several directions by rotating the plate to obtain uniform distribution of inoculum. After drying the plates, antibiotic discs were placed manually using a sterile fine forceps. The seeded plates were incubated at 37ºC in microaerophilic atmosphere. The results were taken after 24 hoursusing the zone interpretation chart (Hi Media, Mumbai, India).

RESULTS AND DISCUSSION

Prevalence of *Campylobacter* species in poultry and poultry handlers: Out of the total 177 samples screened, which included 167 samples from poultry and 10 from poultry handlers, a total of 39 isolates of *Campylobacter* spp. were obtained with an overall prevalence of 22.03% (Table 2). The prevalence was highest in poultry faeces (40.3%) followed by poultry handlers (30%), raw poultry meat (13.2%) and poultry carcass swabs (7.7%) (Fig.1). *C. jejuni* was the most predominant species.

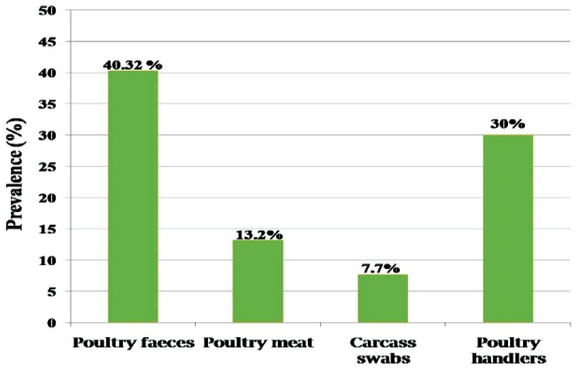

Fig. 1: Prevalence of *Campylobacter* in various sample categories

Poultry faeces: Among all the four types of samples examined, poultry faeces had highest prevalence of *Campylobacter* species with twenty five (40.3%) isolates, out of which twenty one (33.9%) isolates were identified as *C. jejuni*, three (4.8%) as *C. coli* and one isolate (1.6%) as *C. lari* (Table 1). These findings confirm with the previous reports that *C. jejuni* is the predominant *Campylobacter* species isolated from chicken intestinal tract (Sahin *et al.* 2002, Parkar *et al.* 2013).

The prevalence of *Campylobacters* in poultry faeces across other parts of India has been reported to be 22.72% in western Uttar Pradesh (Singh *et al.* 2008) and 32% among broilers in Bareilly region (Malik *et al.* 2014) which is in conformity with the results obtained in our study. However, a comparatively lower prevalence of 15.89% with the predominance of *C. coli* was observed in poultry faeces from Pantnagar, India (Rajagunalan *et al.* 2014) and 17.14% from chicken intestines in Meghalaya and Assam (Rizal *et al.* 2010). These differences may be attributed to the differences in sample size used, varied climatic conditions and survival of the host and the pathogen under different environmental conditions.

Poultry meat and carcass swabs: Among the poultry meat samples tested, higher prevalence of *Campylobacter* spp. was found in raw chicken meat (13.2%) than in chicken carcass swabs (7.7%). The prevalence of *C. jejuni* and *C. coli* was 9.4% and 3.84% in raw chicken meat, and 5.8% and 1.92% in chicken carcass swabs, respectively. Similar results have been observed across other parts of the country. Pallavi and Kumar (2014) reported a prevalence of 17.33% of *Campylobacter* species from poultry meat in and around Bareilly area of Uttar Pradesh. Similarly, Singh *et al.* (2009) reported an overall prevalence of 12.79% in chicken meat and carcass swabs collected from local poultry farms and retail shops of the same area. However, Parkar *et al*. (2013) found 57% (*n*=225) of poultry carcasses positive for *Campylobacter* in Pune area with 76.9% of isolates identified as *C. jejuni* and 23.1% as *C. coli* while Varma *et al*. (2000) have reported *C. jejuni* from 40% meat surface samples of poultry. Although with the present study it could not be deduced that at what point of food chain, *Campylobacters* could have entered in meat, their presence indicates the necessity of adoption of hygienic measures to safeguard public health.

Table 2: Details of *Campylobacter* species isolated

Type of Sample	Samples screened	*Campylobacter* spp.			
		C. jejuni	*C. coli*	*C. lari*	Total (%)
Poultry faeces	62	21	3	1	25(40.32)
Poultry meat	53	5	2	-	7 (13.2)
Poultry carcass swabs	52	3	1	-	4 (7.69)
Poultry handlers	10	3	-	-	3(30)
Total	177	32	6	1	39 (22.03)

Poultry handlers: The analysis of ten poultry handler stool samples revealed three samples (30%) positive for *Campylobacter* species and all the three isolates were *C. jejuni*. Thus *C. jejuni* appeared predominant both in humans and poultry conforming to the earlier reports of Rajendran *et al*. (2012) and Salim *et al*. (2014). Our results are higher as compared to 17.5% isolation of *Campylobacter* spp. among animal handlers from West Bengal (Rashid and Chattopadhyay, 2005), 4.5% among children in South India (Rajendran *et al.* 2012). Similarly, the carriage rate of *C. jejuni* among diarrhoeic and apparently healthy handlers in Kolkata has been reported to be 16.6 and 18.8% respectively (Rathore, 1989). The high prevalence of *Campylobacter* infection among the poultry handlers included in this study could have been due to lack of personal hygiene along with close occupational contact with large number of live poultry birds. Besides,

lack of scientific slaughter facilities and unhygienic conditions of cutting boards prevailing in poultry shops of R.S. Puramay lead to cross-contamination of their foods, thereby increasing their exposure to the pathogen.

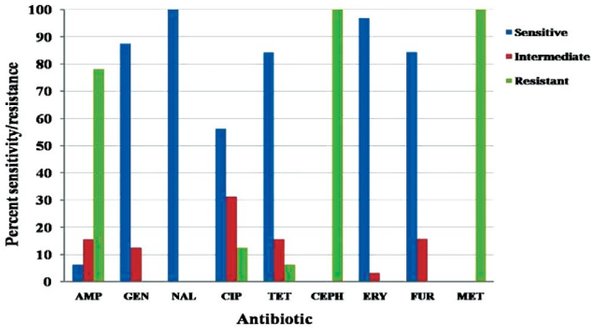

Fig. 2: Antibiogram pattern of *C. jejuni* isolates (n=32); AMP-ampicillin, GEN-gentamicin, NAL-nalidixic acid, CIP-ciprofloxacin, TET-tetracycline, CEPH-cephalothin, ERY-erythromycin, FUR-furazolidone, MET-metronidazole

Antimicrobial susceptibility/resistance pattern of *Campylobacter* isolates: Thirty two *C. jejuni* and 6 *C.coli* isolates obtained from the different samples were analysed for their antibiogram pattern against nine antibiotics. All *C. jejuni* and *C. coli* isolates were sensitive to nalidixic acid (Fig. 2 and 3). Majority of the isolates (96.8% *C. jejuni* and 83.3% *C. coli*) were sensitive to erythromycin. High sensitivity was observed against gentamicin, ciprofloxacin, furazolidone and tetracycline with 87.5, 56.2, 84.4 and 84.3% sensitivity in *C. jejuni* isolates and 66.6, 83.3, 66.6 and 16.6% sensitivity in *C. coli* isolates, respectively. *C. jejuni* isolates were resistant to cephalothin and metronidazole (Figs. 2 and 3). Resistance was also observed against ampicillin (78.1% in *C. jejuni* and 83.3% in *C. coli*). *C. coli* isolates were more resistant than *C. jejuni* which corroborates with the reports of Wilson (2003) and Pezzoti *et al.* (2003). The higher sensitivity of *Campylobacters* to gentamicin, nalidixic acid, erythromycin, furazolidone and other aminoglycosides has earlier been reported (Varma *et al.* 2000; Wilson, 2003).

Campylobacteriosis is among top 5 foodborne zoonotic infections in United States while the data for developing countries such as India is not available. In this regard, the data generation and continuous surveillance of foodborne pathogens becomes significant to evaluate the risk posed by these pathogens through different food categories.

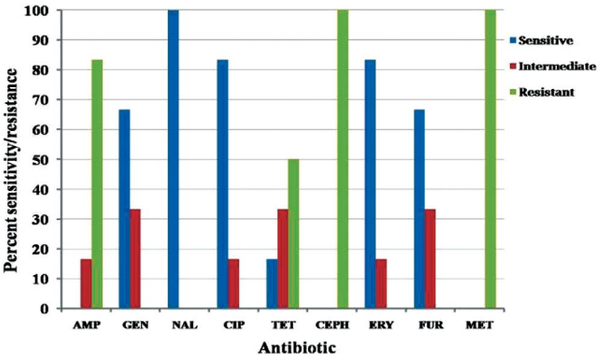

Fig. 3: Antibiogram pattern of *C. coli* isolates (n=6); AMP-ampicillin, GEN-gentamicin, NAL-nalidixic acid, CIP-ciprofloxacin, TET-tetracycline, CEPH-cephalothin, ERY-erythromycin, FUR-furazolidone, MET-metronidazole.

The simultaneous occurrence of *Campylobacter* in poultry and poultry handlers probably indicate the transmission of the bacterium via occupational exposure; however, such interpretations need to be studied thoroughly using molecular techniques. Nevertheless, the high prevalence of *Campylobacter* in poultry and poultry handlers with varying sensitivity to antibiotics indicates the necessity of implementation of appropriate control measures ensuring safety of poultry products and human health.

ACKNOWLEDGEMENTS

The authors are thankful to Dean, FVSc & AH, SKUAST-Jammu for providing financial assistance to carry out this work.

REFERENCES

Baker, M.G., Kvalsvig, A., Zhang, J., Lake, R., Sears, A. and Wilson, N. 2012. Declining Guillain-Barré Syndrome after Campylobacteriosis Control, New Zealand, 1988–2010. *Emerg. Infect. Dis.,***18**(2): 226-33.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am.J. Clin. Pathol.*, **45**(4): 493-96.

Chattopadhyay, U.K., Rashid, M., Sur, S.K. and Pal, D. 2001.The occurrence of campylobacteriosis in domestic animals and their

handlers in and around Calcutta. *J. Med. Microbiol.*, **50**: 933-34.

Corry, J.E. and Atabay, H.I. 2001. Poultry as a source of *Campylobacter* and related organisms. *J. Appl. Microbiol.*,**90**: 96-114.

Economic Survey, J&K, 2013-2014. Directorate of Economics and Statistics, J&K. Government of Jammu and Kashmir.

Houf, N. and R. Stephan, 2007. Isolation and characterisation of the emerging food borne pathogens *Arcobacter* from human stools. *J. Microbiol. Method.*, **68**: 408-413.

Hwang, M. N. and Ederer, G.M. 1975. Rapid hippurate hydrolysis method for presumptive identification of group B Streptococci. *J. Clin. Microbiol.,* **37**: 956-57.

Jain, D., Sinha, S., Prasad, K.N. and Pandey, C.M. 2005. *Campylobacter* species and drug resistance in a north Indian rural community. *Trans. R. Soc. Trop. Med. Hyg.*, **99**(3): 207-14.

Kwan, P.S., Birtles, A. and Bolton, F.J. 2008. Longitudinal study of molecular epidemiology of *C. Jejuni* in cattle on dairy farms. *Appl. Environ. Microbiol.*,**74**: 3626-36.

Malik, H., Kumar, A., Rajagunalan, S., Kataria, J.L., Anjay and Sachan, S. 2014. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* among broilers in Bareilly region. *Vet. World*, **7**(10): 784-87.

Mazick, A., Ethelberg, S., Nielsen, E.M., Molbak, K. and Lisby, M. 2006. An outbreak of *Campylobacter jejuni* associated with consumption of chicken, Copenhagen, 2005. *Euro Surveill.*, **11**(5): 137-9.

Moore, J.E., Corcoran, D., Dooley, J.S.G., Fanning, S., Lucey, B., Matsuda, M., McDowell, R., O'Riordan, L., O'Rourke, M., Rao, J.R., Rooney, P.J., Sails, A. and Whyte, P. 2005. Campylobacter. *Vet. Res.*, **36**: 351-82.

Pallavi and Kumar, A. 2014. Prevalence and antibiotic resistance pattern of *Campylobacter* species in foods of animal origin. *Vet. World*, **7**(9): 681-84.

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.

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.

Rajagunalan, S., Bisht, Garima., Pant, Sheetal., Singh, S.P., Singh, R. and Dhama, K. 2014. Prevalence and molecular heterogeneity analysis of *Campylobacter jejuni* and *Campylobacter coli* isolated from human, poultry and cattle, in Pantnagar, India. *Vet. Arhiv,* **84**(5): 493-504.

Rashid, M. and Chattopadhyay, U.K. 2005. Incidence of campylobacteriosis in domestic animals, birds and animal handlers. *Indian Vet. J.*, **82**: 1214-15.

Rathore, R.S. 1989. Isolation and characterisation of Campylobacter spp. from some of domestic animals and their handlers. M.V.P.H. Thesis submitted to University of Calcutta.

Rizal, A., Kumar, A. and Vidyarthi, A.S. 2010. Prevalence of Pathogenic Genes in *Campylobacter jejuni* Isolated from Poultry and Human. *Int. J. Food Safety*, **12**: 29-34.

Saha, S.K and Sanyal, S.C. 1989. Better growth of *Campylobacter jejuni* using simple Fortner's principle or candle extinction jar. *Indian J. Med. Res.*, **89**: 24-27.

Sahin, T., Morishita, Y. and Zhang, Q. 2002. Campylobacter colonization in poultry, sources of infection and modes of transmission. *Ani. Health*, **3**: 95-105.

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

Singh, R., Singh, P.P., Rathore, R.S., Dhama, K. and Malik, S.V.S. 2009. Prevalence of C*ampylobacter 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. Dis.*, **30**: 35-38.

Singh, R., Singh, P.P., Rathore, R.S., Dhama, K. and Malik, S.V.S. 2008. Studies on effects of seasonal variation on the prevalence of *Campylobacter jejuni* in the poultry faecal samples collected from western Uttar Pradesh. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, **29**: 1-2.

Smibert, R.M. 1978. The genus *Campylobacter*. *Ann. Rev. Microbiol.*, **32**: 673-709.

Son, I., Englen, M.D., Berrang, M.E., Fedorka, P.J. and Harrison, M.A. 2007. Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *Int. J. Food Microbiol.*, **108**: 401-03.

Varma, K.S., Jagadeesh, N., Mukhopadhyay, H.K. and Dorairajan, N. 2000. Incidence of *Campylobacter jejuni* in poultry and their carcasses. *J. Food Sci. Tech.*, **37**(6): 639-41.

Wilson, I.G. 2003. Antibiotic resistance of *Campylobacter* in raw retail chickens and imported chicken portions. *Epidemiol. Infect.*, **131**: 1181-86.