academicJournals

Vol. 7(21), pp. 2442-2453, 21 May, 2013
DOI: 10.5897/AJMR2012.2323
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Prevalence, seasonality and antibiotic susceptibility of thermophilic *Campylobacters* in ceca and carcasses of poultry birds in the "live-bird market"

Saba F. D Parkar[1], Dhara Sachdev[2], Nadine deSouza[1], Aniket Kamble[2], Gayatri Suresh[1], Hitendra Munot[3], David Hanagal[4], Yogesh Shouche[3], Balu Kapadnis[1]

[1]Department of Microbiology, University of Pune, Ganeshkhind, Pune 411 007, India.
[2]Institute of Bioinformatics and Biotechnology, University of Pune, Pune 411 007, India.
[3]National Centre for Cell Science (NCCS), University of Pune Campus, Pune, India.
[4]Department of Statistics, University of Pune, Ganeshkhind, Pune 411 007, India.

Accepted 3 May, 2013

Thermophilic *Campylobacter* spp. are the primary cause of human diarrhea. The common source of infection is contaminated poultry. This study aimed to establish the prevalence of *Campylobacter* spp. in poultry obtained from 'pluck shops' and provide a baseline of resistance profiles of *Campylobacter* spp. isolates obtained. A biphasic approach, with qualitative detection as well as quantitative enumeration of *Campylobacter* spp. was used. We examined 240 samples each, of carcasses and cecum of poultry obtained from 'pluck shops. Amongst the cecum samples and carcasses, 59.5 and 57%, respectively were positive for *Campylobacter* spp. The average *Campylobacter* spp. concentration was 2.69 (S.D 0.419) $\log_{10}$ CFU/mL and 4.55 (S.D 0.607) $\log_{10}$ CFU/g for carcass rinsate and cecum, respectively. Of the 225 *Campylobacter* isolates studied, 76.9% were identified as *Campylobacter jejuni* and 23.1% as *Campylobacter coli*. Susceptibilities for 112 strains of *C. jejuni* and 31 strains of *C. coli* were determined for 12 antibiotics by the agar diffusion technique. According to the minimal inhibitory concentration, a marked resistance to gentamycin and chloramphenicol was also demonstrated. According to the antibiotic resistance profiles, the isolates appeared to differ from each other.

Key words: *Campylobacter*, seasonality, antibiotic resistance, India, wet-market, pluck-shop.

INTRODUCTION

Campylobacter jejuni and *Campylobacter coli* are commonly associated with poultry as well as poultry products because they are commensal of the avian gut. *Campylobacter* spp. is the causative agent responsible for human Campylobacteriosis (Humphrey et al., 2007). The central aspect of *Campylobacter* infection in humans is an acute inflammatory gastroenteritis. The disease begins with a battery of symptoms, which may or may not be mutually exclusive. Abdominal cramps, fever, rigors, dizziness, headache, convulsions, delirium, nausea and even myalgia may be among the prodrome preceding copious, runny, watery and bile stained diarrhea. Poultry meat is the principal risk factor associated with *Campylobacter* infections in man (Kapperud et al., 1992; Hudson et al., 1999; Allos, 2001). The different serious sequels such as Guillain-Barré´ syndrome (GBS) or Reiter syndrome are characterized by polyneuritis of the peripheral nerves and may be seen in 1 of 1000 patients infected with *C. jejuni*. These sequelae may be associated with paralysis and severe neurologic deficits (Nachamkin et al., 1998; Kuroki

*Corresponding author. E-mail: bpkapadnis@yahoo.com.

et al., 1993). These bacteria are associated with comercially produced chickens from free range farms as well as mass-produced broilers and laying hens (Rodenburg et al., 2004; Miraglia et al., 2007).

Campylobacter sp*.* contamination commences at the farm level (Byrd et al., 1998). Colonization levels from log 5 to log 9 CFU/g of cecal contents have been reported in broilers (Berndtson et al., 1996). Horizontal transfer of *Campylobacter* ensures the spread of the organism within the whole flock in a few days (Gerdemann, 1996). It has been proposed that human infections follow increased *Campylobacter* in animals (Baker et al., 2012).

In India, the broiler poultry is processed and vended as a fresh product to consumers from a particular location defined as a “pluck shop” (Rodrigo et al., 2005).

The poultry industry thrives solely as a ‘live-bird’ market comprising the pluck shops. Traditional poultry facilities at the wholesale or retail level are manual with negligible sanitary practices taken on the flooring or by the personnel. Only scarce 5% of all poultry meat in India is processed mechanically in industries under hygienic conditions using recommended equipment whereas most poultry meat enters the food chain *via* the ‘live-bird’ market through ‘pluck-shops’ (Reardon and Gulati, 2008). This may be because consumers prefer fresh meat. The fact that mechanical slaughtering process incorporating equipments may reduce the level of contamination by 100 to 1000 times (Rosenquist et al., 2006), is usually overlooked due to cultural disposition to assume that hand slaughtered birds are the fresher and healthier ones.

An infected carcass indicates an infected bird. This is because unlike most other bacteria implicated in food poisoning, *Campylobacter* are fastidious and cannot multiply outside their animal host. Hence, any contamination on the carcass is from within the birds itself. The *Campylobacter* levels on the carcass represent an important source of consumer exposure and potential risk for infection (Stern and Robach, 2003). Thus, the quantification of the *Campylobacter* contamination levels in the birds available through ‘live-market’ is predominantly imperative. However, Indian reports on human Campylobacteriosis or even incidence in poultry are sparse. The above fact has underlying global consequences in light of the increasing numbers of travelers entering and poultry exports exiting the Indian subcontinent.

Campylobacter colonization in poultry has been reported to follow a seasonal pattern, peaking in the warmer months (Jacobs-Reitsma et al., 1995; Boysen et al., 2011; Nylen et al., 2002; Hudson et al., 1999). To date, there have been very few studies investigating the prevalence and seasonality of *Campylobacter* spp. in the Indian poultry industry. In the present study, we have quantified the *Campylobacter* contamination levels on carcasses in the ‘live-market ’and identified the various thermophilic *Campylobacter* found. Additionally, we examined the antibiotic susceptibility of these isolates. To our knowledge, this is the first comprehensive report exploring the seasonality of *Campylobacter* in poultry from tropical environments.

MATERIALS AND METHODS

Determination of sample size

The sample size was calculated using the formula proposed by Thrusfield, for a large (theoretically ‘infinite’) population on assuming simple random sampling with annual expected prevalence (p) of 50%, desired confidence level (Z) of 95%, corresponding to a Zα value of 1.96 (Thrusfield, 1995) and using the formula:

$$n_{\infty} = (Z\alpha)^2 \, p \, (1-p)/L^2$$

The population size is infinite hence; the sample size is 384 with accuracy (L) of 5% and sample size of 196 with accuracy (L) of 10%. Considering practicality of the sampling and the time taken for processing, 240 (L= 6.3%) carcasses were sampled over the course of one year (May 2008 to April 2009) from vendors located at four regions within Pune *viz.* Aundh, Camp, Chinchwad and Hadapsar.

Qualitative detection of *Campylobacter* spp.

The qualitative detection of *Campylobacter* was carried out to understand its prevalence and find the percentage of samples positive carrying *Campylobacter*. Presence or absence testing was performed on 240 carcass and cecum samples each according to modifications of ISO 10272-1:2006(E). Briefly, the method was as follows: 25 g of carcass meat sample was placed into enrichment broth (1:10), homogenized and then incubated at 42 ± 1°C for 48 h under the microaerobic conditions (5% O_2, 10% CO_2, 85% N_2). Sterile Preston broth (HiMedia, India, M899) supplemented with selective supplement (HiMedia, India FD-042) containing polymyxin B (5 IU/mL), rifampicin (10 µg/mL), trimethoprim 10 (µg/mL) and cycloheximide (100 µg/mL) along with 10% horse blood (Haffkine Biopharmaceutical Ltd, Pune) was used as enrichment broth. Then one loopful of enrichment broth was streaked onto mCCDA agar plates (ISO, 10272-1; Stoyanchev et al., 2007; Habib et al., 2011). For qualitative analysis, about 0.5 g of caecal contents were directly plated on mCCDA plates (Hansson et al., 2010)

Both sets of mCCDA plates were then incubated at 42 ± 1°C for 24 h under microaerobic conditions using McIntosh and Filde's anaerobic jar (Hi-Media, IndiaAnaerobic System Mark VI, LE013). Plates were inspected to detect the presence of colonies presumed because of their characteristics of *Campylobacter.*

Quantitative determination to enumerate *Campylobacter* counts

For quantification of *Campylobacter*, modification of the ISO 10272-2:2006(E) was used. Briefly, 48 whole carcasses were rinsed in a large plastic bag containing 500 mL of sterile buffered peptone water to obtain carcass-associated microflora. Decimal dilutions in buffered peptone water were prepared from 1 mL of rinsate. One hundred microlitres of each dilution was spread plated in duplicate onto mCCDA (HiMedia, India, FD-042). Then forty-eight ceca were obtained, 12 from each region. Cecal contents were weighed and diluted in buffered peptone water. Then a 10-fold serial dilution in peptone water was prepared. One milliliter from each dilution was then plated on mCCDA plates (Hansson et al., 2010).

The plates were incubated at 42 ± 1°C for 48 h in a microaerobic atmosphere (85% N_2, 10% CO_2, 5% O_2) (ISO, 2006b, 10272-1). The number of *Campylobacter* was expressed as log CFU/mL car-

cass rinsate and log CFU/g for carcass and cecum, respectively.

Microbiology of presumptive isolates

The plates were examined for round, translucent, raised, convex colonies with entire edge, and a tendency to spread indicating motile nature. The suspected colonies were contradistinguished by the presence of slender, spiral, curved and Gram-negative rods with typical corkscrew, darting motility under hanging drop examination. Presumptive colonies were oxidase positive and unable to grow under aerobic conditions, when incubated on fresh mCCDA plates at 37°C. Presumptive colonies, based on colony morphology, were restreaked on Muller-Hinton-based blood agar plates (Hi-Media India , M-173) supplemented with 10% (v/v) horse blood and incubated microaerobically at 42°C for 24 h. Isolated colonies were then restreaked for purity on mCCDA and incubated microaerobically at 42°C overnight. Biochemical tests, which consisted of hippurate hydrolysis, catalase test, indoxyl acetate hydrolysis and H_2S test, were performed on colonies isolated from the blood agar plates. The Hi*Campylobacter*™ Latex Test Kit (Hi-Media, India), a rapid latex agglutination test was used for confirmation of the isolates as thermophilic *Campylobacter*s.

Molecular typing

The presumptive *Campylobacter* were authenticated by the presence of a 450 bp amplicon obtained using the primers designed to specifically amplify coding regions from the flagellin gene; Pg50 5'-ATGGGATTTCGTATTAAC-3' and Pg3 5'-GAACTTTGAACCGATTTG-3, (Oyofo et al., 1992). Tubes were subjected to 25 cycles of 94°C for 1 min, 37°C for 1 min and 72°C for 1 min, followed by a 5 min extension at 72°C. Also for ten representative isolates, the 16S rRNA gene was amplified using the primers F-27 5'-AGAGTTTGATCMGGCTCAG-3' and R-1525 5'AAGGAGGTGWTCCARCC-3' (Lane, 1991).

For 16S rRNA, tubes were subjected to 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, followed by a 7-min extension at 72°C. The sequencing reaction for either PCR reaction was performed in 25 µl volumes containing 1 µl DNA; 20 mM Tris-HCl (pH 8.3); 50 mM KCl; 2.5 mM $MgCl_2$; 200 µM each dNTP; 0.4 µM of each primer; and 0.625 units of *Taq* DNA polymerase.

Sequencing was performed using degenerate primers 907R (Sigma). Sequence data were obtained using a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). The sequence analysis software used was ChromasPro v1.34. Identity of the isolates was confirmed by BLAST on NCBI.

Antibiotic susceptibility

The minimum inhibitory concentration was determined for 143 selected isolates (112 *C. jejuni*, 31 *C. coli*) of *Campylobacter* which showed resistance towards at least one antibiotic. The agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) (previously National Committee for Clinical Laboratory Standards, NCCLS), subcommittee on Veterinary Antimicrobial Susceptibility Testing (NCCLS, 1999) was used as a standard. The tested antimicrobial agents (HiMedia, India) were as follows: ampicillin, azithromycin, chloramphenicol, ciprofloxacin, doxycycline, gentamycin, nalidixic acid, norfloxacin and tetracycline. There is a dearth of internationally validated criteria for breakpoints of susceptible or resistant isolates for *Campylobacter*. Conse-quently, where breakpoints from CLSI were not available, esta-blished breakpoints were used (Luber et al. 2003a; Yabe et al., 2010; Gu et al., 2009; Luangtongkum et al., 2007).

Suspensions of isolates were prepared and were adjusted to a turbidity equivalent to a 0.5 McFarland standard, which is equivalent to 1.5×10^8 CFU/mL. This was diluted to 1:100 leading to a concentration of 1.5×10^6 CFU/mL. The final inoculum on the agar was approximately 3×10^3 CFU/spot. These suspensions were inoculated onto Mueller-Hinton agar (HiMedia, India) containing the twofold dilution series of antibiotics and supplemented with 5% defibrinated horse blood (Luangtongkum et al., 2007). Plates were incubated at 42°C for 48 h, in a microaerophilic atmosphere. Growth was assessed after incubation and the MIC value was determined to be dilution of the antibiotic, which inhibits the growth of the isolate under study. Antimicrobial susceptibility was determined for concentrations ranging from 0.125 to 256 µg/mL. To ensure reproducibility, MIC determinations were repeated at least thrice.

Statistical analysis

Campylobacter counts were converted to a logarithmic scale to approximate the results to normal distribution. *Campylobacter* detection was recorded in binary variables in terms of *Campylobacter* presence or absence. Enumeration results were recorded as CFU/mL of rinse liquid for carcasses and CFU/g of cecal contents. *Campylobacter* mean counts (carcass and ceca) were compared between months and between seasons. $P \leq 0.05$ was considered statistically significant. Statistical analysis of the data was carried out using Minitab (Version 14).

RESULTS

Overview of *Campylobacter* contamination

Table 1 shows prevalence of *Campylobacter* spp. in chicken carcasses and intestine from broilers at pluck shops. All the live processing units were found to be positive for *Campylobacter*. More than half (59.6%) of the cecal samples and 57% of the carcasses tested were positive for *Campylobacter* spp. The prevalence of *Campylobacter* spp. in contaminated carcasses was highest in the Aundh area (65%), and lowest in the Camp region (48.3%). The prevalence of *Campylobacter* contaminated ceca was highest in the Camp region (66.7%) and lowest in Hadapsar (55%). Of the 225 isolates studied, 76.9% (173) were identified as *C. jejuni* and 23.1% (52) *C. coli.*

The isolates were positively identified using the PCR based assays for identification of *Campylobacter* spp. Since the biochemical analysis revealed the presence of only *C. jejuni* and *C. coli* amongst the isolates, a single PCR assay that amplified a part of the flagellin gene was deemed sufficient for the study. The PCR yielded the expected amplicon of product size of 450 bp, with the primers specific for flagellin gene. Further, the PCR assay confirmed that the isolates obtained belonged to *Campylobacter* spp. On amplification of 16SrRNA gene and carrying out BLAST, along with differentiation based on biochemical tests, the isolates were identified as *C. jejuni* or *C. coli*.

The count data for carcass contamination showed that 81.7% of the samples were contaminated with 10^2 to 10^3 CFU/mL, while 16.7% of the samples showed contamina-

Table 1. Prevalence of *Campylobacter* in chicken carcasses and intestine from broilers at pluck shops.

Area[a]	Number (%) of samples positive for *Campylobacter*		Mean microbial count	
	Intestine[c]	Carcass[c]	Cecca[d]	Carcass[d]
Aundh	36 (60.00)	39(65.00)	4.38 ± 0.60[b]	2.92 ± 0.77[b]
Camp	40 (66.66)	29 (48.33)	4.65 ± 0.64[b]	2.69 ± 0.27[b]
Chinchwad	34 (56.66)	37 (61.66)	4.40 ± 0.62[b]	2.62 ± 0.29[b]
Hadapsar	33 (55.00)	32 (53.33)	4.77 ± 0.54[b]	2.61 ± 0.20[b]
Total	143(59.58)	137 (57.08)		

[a]For the study, samples were collected from four 'pluck shops' at each area; [b]Numbers without common superscripts differ significantly ($P \leq 0.05$) within a column; [c]Total number of samples tested was 240, that is, 60 from each region; [d]Total number of samples tested was 48, that is, 12 from each region. Microbial counts for cecca and carcasses are expressed as log cfu/g and log cfu/ml, respectively.

tion greater than 10^3 CFU/mL. The average *Campylobacter* concentration was 2.69 $\log_{10}$ CFU/mL, with a standard deviation of 0.41 $\log_{10}$ CFU/mL. The count data for cecal samples showed that 14.6% of the samples were contaminated with 10^3 to 10^4 CFU/g, while 58.3% of the samples showed contamination between 10^4 and 10^5 CFU/g and 27% greater than 10^5 CFU/g. The average *Campylobacter* concentration was 4.55 $\log_{10}$ CFU/g, with a standard deviation of 0.60 $\log_{10}$ CFU/g.

Seasonality of *Campylobacter*

Qualitative study of prevalence

This entails the percentage of samples positive for *Campylobacter*. Figure 1A and B show monthly occurrence of *Campylobacter* spp. in chicken intestine and carcasses, respectively. In the 12 months, highest and lowest poultry prevalence of *Campylobacter* was seen in May and January, respectively among the carcass samples, whereas the cecal samples showed highest and lowest prevalence in June and December, respectively. Prevalence studies show that highest prevalence in cecum as well as carcasses was noted in monsoon. Lowest prevalence in cecal and carcasses was seen in post-monsoon and winter, respectively.

Quantitative enumeration

This entails the *Campylobacter* load and thus the numbers on the carcass. Figure 2A and B show seasonal occurrence of *Campylobacter* spp. in chicken intestine and carcasses, respectively. Highest and lowest numbers of *Campylobacter* were seen in October and December, respectively among the carcass samples, whereas the cecal samples showed highest and lowest numbers of *Campylobacter* in May and September, respectively. The highest and lowest numbers of *Campylobacter* in poultry ceca were seen in the post-monsoon and monsoon season, whereas the highest and lowest numbers of *Campylobacter* in poultry carcasses were seen in summer and winter, respectively.

Antimicrobial resistance of the *Campylobacter* isolates

The prevalence of antimicrobial resistance patterns in the *C. jejuni and C. coli* isolates are presented in Tables 2 and 3, respectively. Amongst all the *C. jejuni* and *C. coli* isolates, 64.7 and 59.6% respectively were resistant to one or more antibiotics. 35.9 and 25% of *C. jejuni* and *C. coli* isolates respectively showed multidrug resistance to 4 or more antibiotics.

The MIC was determined for only those isolates, which showed resistance to one or more antibiotic. Minimal inhibitory concentrations of 12 antimicrobial agents were determined via agar dilution for 112 isolates of *C. jejuni* and 31 isolates of *C. coli* (Table 4). The MIC_{90}s for *C. jejuni* were 64 µg/mL for ciprofloxacin and nalidixic acid, 32 µg/mL for ampicillin, erythromycin, gentamycin, norfloxacin and tetracycline, 4 µg/mL for streptomycin, 2 µg/mL for chloramphenicol and doxycycline, 0.5 µ g/mL for clindamycin and ≥ 0.0625 µg/mL for azithromycin. The MIC_{90}s for *C. coli* were 64 µg/mL for ciprofloxacin and chloramphenicol, 32 µg/mL for ampicillin, nalidixic acid, norfloxacin and tetracycline, 16 µg/mL for gentamycin, 4 µg/mL for clindamycin, erythromycin and streptomycin and 1 µg/mL for azithromycin.

The proportion of isolates resistant to each antimicrobial agent for *C. coli* was as follows: 48.0% for ampicillin, 5.7% azithromycin, 15.4% for chloramphenicol and ciprofloxacin, 9.6% for clindamycin and doxycycline, 0% for erythromycin, 36.5% for gentamycin, 19.2% for nalidixic acid, 15.4% for norfloxacin, 3.84% for streptomycin and 30.8% for tetracycline. The proportion of isolates resistant to each antimicrobial agent for *C. jejuni* was as follows: 19.6% for ampicillin, 6.3% for azithromycin, 7.5% for chloramphenicol, 28.3% for ciprofloxacin, 10.4% for clindamycin, 8.6% for doxycycline, 26.6% for erythromycin, 43.1% for gentamycin, 25.4% for nalidixic acid, 26.5% for norfloxacin, 6.3% for streptomycin and 23.7% for tetracycline.

Gentamycin, erythromycin, ciprofloxacin, norfloxacin and tetracycline resistance was common amongst the

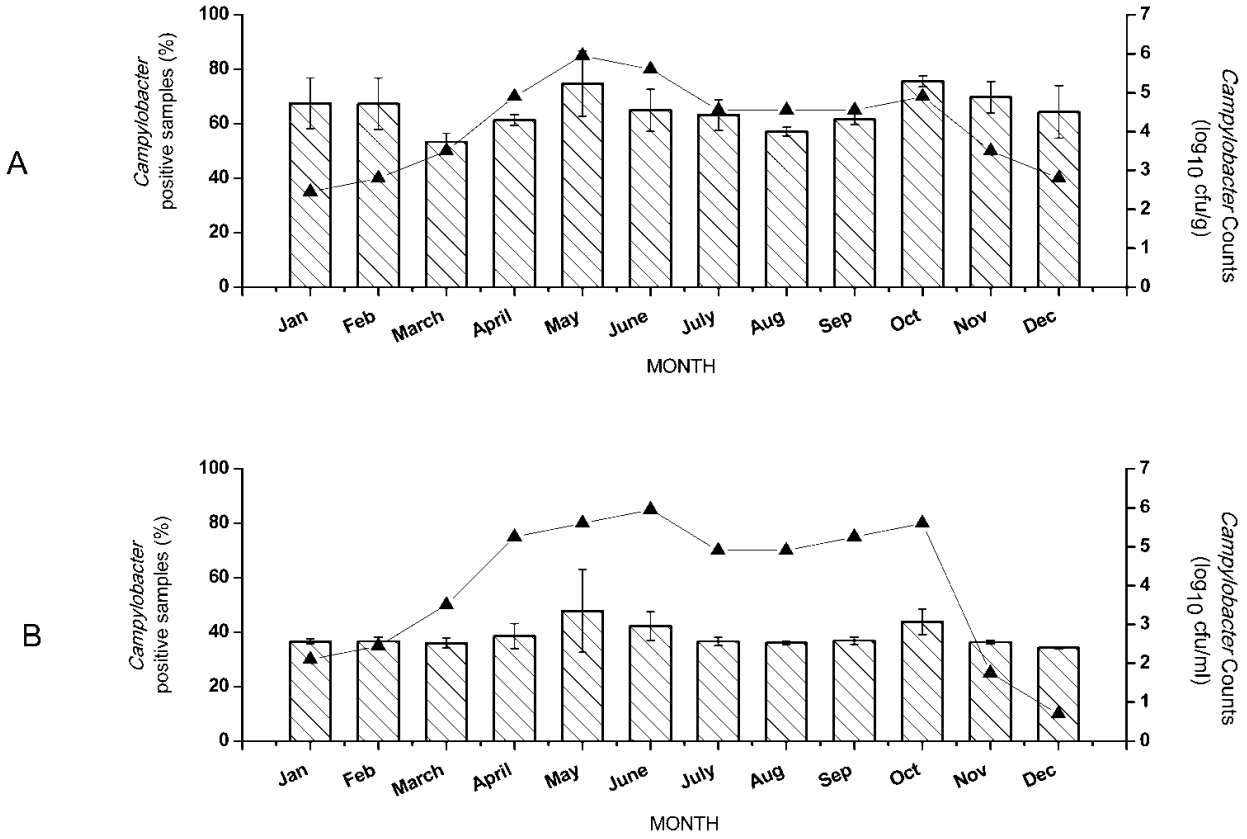

Figure 1. Monthly occurrence of *Campylobacter* in chicken intestine (A) and in carcasses (B) from May 2007 to April 2008. ▲, Percentage of positive samples; lined columns, Microbial counts for cecca and carcasses expressed as log cfu/g and log cfu/ml, respectively.

isolates. The *C. jejuni* isolates had norfloxacin MICs as high as 256 µg/mL, indicating a high-level resistance to fluoroquinolones. Amongst quinolones (ciprofloxacin, norfloxacin and nalidixic acid), tetracyclines (tetracycline and doxycycline), macrolides (erythromycin and azithromycin) and clindamycin, the overall resistance rates were statistically similar between *C. jejuni* and *C. coli*. The MIC values however in *C. jejuni* were higher than in *C. coli*. As compared to other antimicrobial agents, the resistance rates to ampicilin were significantly different in *C. jejuni* and *C. coli* with the *C. coli* isolates showing higher prevalence of ampicillin resistance (48%). The overall prevalence of azithromycin resistance was low in *C. jejuni* (6.3%) and *C. coli* (5.7%). Another notable observation of this study was erythromycin resistance, which was moderate among the *C. jejuni* isolates (26%), whereas not a single case of erythromycin resistance was seen amongst the *C. coli* isolates.

DISCUSSION

Prevalence of *Campylobacter* in retail poultry meat from "pluck-shops"

In countries where frozen or chilled poultry is predominantly consumed, rates of isolation of thermophilic *Campylobacter*s from carcasses were: 49.5% in Spain (Domínguez et al., 2002), 35.2% in Bulgaria (Stoyanchev et al., 2007), 44% Germany (Näther et al., 2009) and 52.5% in the US (Son et al., 2007). The rates of isolation of *Campylobacter* contamination are higher in countries with traditional "pluck shop" based wet markets: 63% in Iran (Taremi et al., 2006), 68.3% in Korea (Han et al., 2007) and 83.9% in Trinidad. Studies in Malaysia have shown the overall rate of contamination for *Campylobacter* in modern processing plants and in traditional wet markets were 61.1 and 85.6%, respectively (Rejab et al., 2012). The prevalence of *Campylobacter*-positive carcasses, found in this study, was observed to be comparable to prevalence reports for chicken carcasses from other countries were poultry wet-markets is common (Rodrigo et al., 2005). Previous studies in India also concur with our findings with 39.3% of the tested poultry positive for *Campylobacter* in Calcutta (Chattopadhyay et al., 2001), 64% in Vellore (Rajendran et al., 2012) and 17.14% in the Meghalaya- Assam region (Rizal et al., 2010). Our findings on the prevalence of *Campylobacter*, based on cecal samples, corresponds well with those reported by Stern and Robach (2003) with an average population of 4.6 $\log_{10}$CFU/g in 1995 and an average population of 5.17 $\log_{10}$CFU/g in 2001 (Stern and Robach, 2003). These

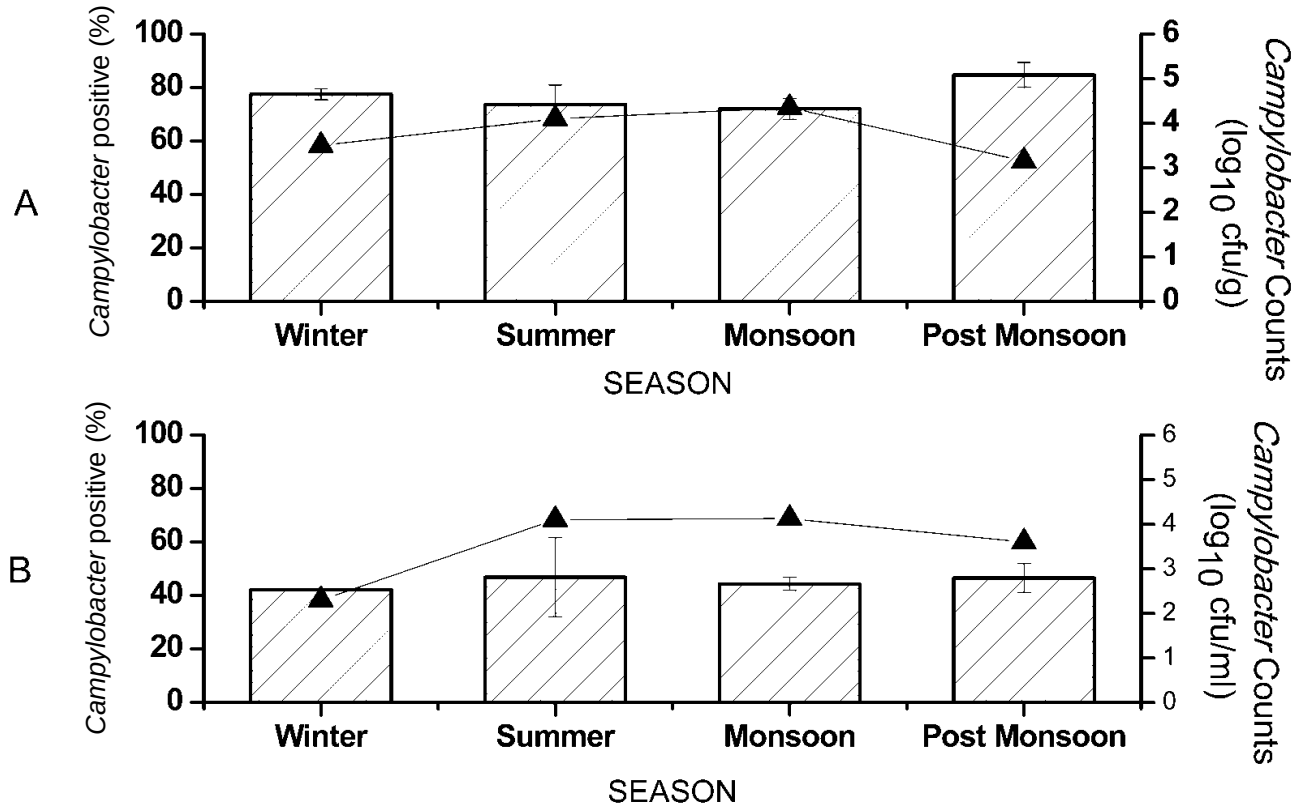

Figure 2. Seasonal occurrence of *Campylobacte*r in chicken intestine (A) and in carcasses (B). ▲, Percentage of positive samples; lined columns, Microbial counts for cecca and carcasses expressed as log cfu/g and log cfu/ml, respectively.

findings are also in agreement with values reported by Berrang et al. (2000) and Hansson et al., (2010).

We postulated that since the numbers of this organism in the cecal contents correspond with those observed in other countries, the higher numbers of contaminated carcasses as well as higher *Campylobacter* loads can possibly be due to lack of industrial processing. Industrial poultry processing comprises of killing, scalding, defeathering and evisceration of birds mechanically.

Each step is followed by washing with chlorinated water. Finally, the processing is culminated with a rapid cooling on carcass chillers. The number of *Campylobacter* sps. microorganisms sequentially reduce at each step, followed by a final wash with chlorinated water and processing aids that almost finishes the contamination, leading to a low count on the end product (Keener et al., 2004). The mechanical slaughtering reduces *Campylobacter* contamination by a 100 to 1000 fold (Rosenquist et al., 2006).

These reasons may explain the high level of contamination found in poultry carcasses from India. Manual slaughtering and evisceration by hand leading to rupture of intestinal viscera may lead to fecal contamination of carcasses. In addition, the lack of washing steps with water or processing aids may be responsible for the increased numbers of *Campylobacter*s observed in Indian poultry.

Seasonality

We are not confirming a definite seasonality in the *Campylobacter* numbers or its prevalence in birds. However, noticeable peaks are seen during the warmer months. Consequently, we support this concept of seasonality due to the presence of dual peaks, one in the month of May and the second in October. Both peaks occur during warmer months. Earlier studies on seasonality of *Campylobacter* sp. by Singh and colleagues (2008) showed that the highest prevalence of *C. jejuni* in faecal samples was reported during rainy season. This is in agreement with our results. However, among the different months, the highest prevalence was found in September, whereas our study shows highest prevalence was seen in October. These findings can be explained by the regional differences in temperature. In addition, higher isolation rates of *Campylobacter* spp. during the summer and monsoon months in children has been reported in Calcutta (Bhadra et al., 1992).

The seasonal structure seen in India is very different from that of European countries with an absence of the spring and autumn seasons and presence of monsoon and the post monsoon seasons. Even so, higher recovery rates were reported during the warmer months of the year in the U.S (Willis and Murray, 1997) as well as India (Singh et al., 2008). Seasonality in *Campylobacter*

Table 2. Drug resistance patterns in *Campylobacter* isolates from raw chicken in India, during the period of May 2008 to April 2009.

Number of classes of antibiotics present	Resistance pattern	Number of isolates of *C. jejuni*	Number of isolates of *C. coli*
1	A[b]	**2**	**2**
	G	2	-
	T[b]	**2**	**2**
	Q[a]	6	-
	C	-	1
2	**A, G**	**5**	**2**
	A, Q	**3**	**1**
	A, T	1	-
	C, T	1	-
	G, M	1	-
	G, Q	8	-
	M, Q	2	-
	M, T	1	-
	Q, T	**2**	**1**
3	A, C, G	-	1
	A, C, Q	-	1
	A, G, P	-	1
	A, G, Q[b]	**2**	**4**
	A, G, T	4	-
	A, Q, T	-	2
	C, G, Q	2	-
	C, Q, T	1	1
	G, M, Q[a]	12	-
	G, M, T	2	-
	G, P, Q	2	-
	G, Q, T	2	-
	M, P, T	2	-
	M, Q, T	3	-

Q, Fluoroquinolones (nalidixic acid, ciprofloxacin and norfloxacin); M, macrolides (erythromycin and azithromycin); C, clindamycin; P, phenicols (chloramphenicol); T, tetracyclines (tetracyclin and doxycycline); A, ampicillin; G, gentamicin and streptomycin. [a]indicates prevalent pattern for *C. jejuni*; [b]indicates prevalent pattern for *C. coli*; letters in bold indicates overlapping patterns in *C. jejuni* and *C. coli*

colonization of poultry has been studied (Berndtson et al., 1996). Seasonal peak in humans coincides with a peak in poultry isolates (Meldrum et al., 2005). The *Campylobacter* population in the intestines of cattle, lamb and poultry differ considerably with changing seasons (Wallace et al., 1997; Stanley et al., 1998a, b). Hence it is deducible that carcass contamination potentially varies with the season (Jones, 2001). Despite these pressing arguments, seasonality is not often studied with reference to *Campylobacter* spp.

Studies on packed meats are not conducive to understanding seasonal variations because of the longer shelf life of these products. Frozen or chilled poultry is stored by companies and released as per demand and may be further stored by retailers before the actual sale. Furthermore, time lapses occur during poultry processing, transportation and retail storage. Studies from “pluck-shops” make an interesting model because birds are sold immediately after culling.

Antibiotic resistance

The resistance rates of *Campylobacter* to each antimicrobial agent differ considerably in several countries (Payot et al., 2004; Luberet al., 2003b; Aquino et al., 2002; Gupta et al., 2004). In our data set, the susceptibility of *Campylobacter* isolates has been evaluated by using the minimal inhibitory concentration method, therefore

Table 3. Multi drug resistance patterns in *Campylobacter* isolates from raw chicken in India, during the period of May, 2008 to April 2009.

Number and classes of antibiotics present	Resistance pattern	Number of isolates *C. jejuni*	Number of isolates *C. coli*
4	A, C, G, P	1	-
	A, G, M, Q	**1**	**1**
	A, G, P, Q	1	-
	A, G, Q, T[b]	**1**	**7**
	A, M, Q, T	1	-
	C, G, M, Q	5	-
	C, G, Q, T	1	-
	C, M, Q, T	1	-
	G, M, Q, T[a]	15	-
	G, P, Q, T	-	1
5	A, C, G, M, P	1	-
	A, C, G, M, Q	1	-
	A, C, G, M, T	1	-
	A, G, M, P, Q	1	-
	A, G, M, Q, T[ab]	**7**	**2**
	A, G, P, Q, T	-	1
	C, G, M, P, Q	1	-
	C, G, M, Q, T	1	-
	G, M, P, Q, T	3	-
			-
6	A, C, G, M, P, Q	1	-

Q, Fluoroquinolones (nalidixic acid, ciprofloxacin and norfloxacin); M, macrolides (erythromycin and azithromycin); C, clindamycin; P, phenicols (chloramphenicol); T, tetracyclines (tetracyclin and doxycycline); A, ampicillin; G, gentamicin and streptomycin. [a]Indicates prevalent pattern for *C. jejuni*; [b]indicates prevalent pattern for *C. coli*; letters in bold indicates overlapping patterns in *C. jejuni* and *C. coli* isolate.

enabling the determination of precise concentration at which the microorganism failed to grow. *Campylobacter* enteritis are usually treated using fluoroquinolones and macrolides (Allos, 2001). On the other hand, recent studies have reported the appearance of fluoroquinolones resistant *Campylobacter* spp. among poultry flocks (Niwa et al., 2001), necessitating the survey of prevalence of *Campylobacter* spp. in poultry and their antimicrobial resistances. Earlier reports from India show 30.6% of strains were multidrug resistant (Jain et al., 2005). This is in agreement with our result where 35.83 and 25% of *C. jejuni* and *C. coli* isolates respectively showed multidrug resistance to four or more antibiotics.

Previous studies have shown that antibiotic resistance of *Campylobacter* species was ampicillin 81.6%, ciprofloxacin 71.4%, tetracycline 26.5%, gentamycin 10.2% and erythromycin 6.1% (Jain et al., 2005).

C. coli strains are reported to have tendency to acquire resistance to macrolides, specifically erythromycin. In contrast, *C. jejuni* isolates remain sensitive to erythromycin. *C. coli* has frequently been found to be resistant to erythromycin and other macrolides (Nayak et al., 2005; Kim et al., 2006). This propensity was not observed is our data set. Gentamycin resistance has been reported to be absent in studies from Europe and U.S (Hariharan et al., 2009). However, data from Asian countries suggests otherwise. The findings in our study are in accordance with the data obtained from other Asian countries (Chen et al., 2010). Earlier studies from this region have indicated that the frequency of antibiotic resistance is high in *Campylobacters* (Baserisalehi et al., 2005). In 2011, a new policy on containment of antimicrobial resistance

Table 4. Distributions of MICs of 12 antimicrobial agents for 112 *C. jejuni* isolates and 31 *C. coli* isolates from "pluck-shop" broiler chicken.

Antibiotic	Number of isolates with MIC (µg/ml) of													MIC50	MIC90	Number of resistant isolates
	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256			
Ampicillin										I						
C. jejuni	0	0	0	0	0	6	6	0	6	4	0	0	0	8	32	34
C. coli	0	0	0	0	0	0	6	0	0	5	0	0	0	32	32	25
Azithromycin						I										
C. jejuni	96	0	0	0	5	11	0	0	0	0	0	0	0	0.0625	0.0625	11
C. coli	18	0	0	0	10	0	3	0	0	0	0	0	0	0.0625	1	3
Chloramphenicol										I						
C. jejuni	0	0	0	0	36	63	0	0	0	12	1	0	0	2	2	13
C. coli	10	0	0	0	8	5	0	0	0	3	5	0	0	1	64	8
Ciprofloxacin							I									
C. jejuni	0	0	0	0	34	29	0	1	1	6	41	0	0	2	64	49
C. coli	0	0	0	2	16	5	0	0	0	2	6	0	0	1	64	8
Clindamycin							I									
C. jejuni	0	0	19	67	4	4	6	7	2	3	0	0	0	0.5	0.5	18
C. coli	0	0	3	11	12	0	5	0	0	0	0	0	0	1	4	5
Doxycycline									I							
C. jejuni	4	0	62	0	7	10	2	12	10	5	0	0	0	0.25	2	15
C. coli	0	0	0	25	0	0	0	3	0	0	3	0	0	0.5	8	3
Erythromycin								I								
C.jejuni	0	0	0	0	39	18	0	2	11	42	0	0	0	2	32	55
C.coli	0	0	0	0	21	0	10	0	0	0	0	0	0	2	4	0
Gentamycin									I							
C. jejuni	0	0	0	14	3	9	0	10	38	38	0	0	0	16	32	76
C. coli	0	0	0	0	12	0	0	0	19	0	0	0	0	16	16	19
Nalidixic Acid										I						
C. jejuni	0	0	0	0	0	16	9	4	39	4	37	3	0	32	64	44

Table 4. Contd.

C. coli	0	0	0	0	11	6	4	0	0	10	0	0	0	2	32	10
Norfloxacin									I							
C. jejuni	0	0	0	0	0	23	40	3	2	14	2	1	27	4	32	46
C. coli	0	0	0	0	14	3	6	0	4	4	0	0	0	2	32	8
Streptomycin											I					
C. jejuni	0	0	0	0	0	18	72	0	0	11	11	0	0	4	4	11
C. coli	0	0	0	0	2	7	20	0	0	0	0	0	2	4	4	2
Tetracycline									I							
C. jejuni	0	0	0	0	13	14	37	7	9	32	0	0	0	4	32	41
C. coli	0	0	0	0	9	8	0	0	7	7	0	0	0	2	32	14

Ivertical lines indicate breakpoints for resistance.

was enacted in India. It will be interesting to note any change of antimicrobial resistance patterns in future surveys of *Campylobacter* isolates in this scenario.

There is a paucity of information on the levels of *Campylobacter* and its prevalence in poultry carcasses from “pluck-shops” or wet poultry markets. Data emerging from western countries is usually from chicken carcasses picked at the end of stringent mechanical poultry processing. However, this is not always the case in many Asian countries. Assuring good manufacturing along with food safety practices is crucial. The manual and laborious method of poultry processing used in India needs to be streamlined and regulated. The cultural confines, which endorse the consumption of freshly culled birds, may or may not change. Hence, a more hygiene oriented, education based approach needs to be taken and poultry processors or “pluck-shop” owners need to be educated on a more informed protocol of processing. Minimum hygiene standards must be set and adherence to the same must be regulated.

This research shows that higher mean concentrations in the cecum have been seen in warmer months. Earlier research postulates that low intestinal *Campylobacter* concentration leads to low fecal contamination of the carcasses during the slaughter steps and conversely with high concentrations (Rosenquist et al., 2006). It has been proposed that the probability of meat contamination increases when the prevalence of *Campylobacters* is high within the flock and higher numbers of *Campylobacters* are present in the intestines (Nauta et al., 2009). Due consideration must be given to these factors and a real time surveillance system must be brought into practice.

Since *Campylobacter* sp. is considered a major pathogen associated with food borne disease worldwide, this study revealed the prevalence of antimicrobial resistance in *Campylobacter* sp. from poultry, which is the most consumed animal food in India. Surveillance systems must be brought into place to monitor the use of antibiotics in poultry. The results of this study suggest that timely scrutiny of the presence of *Campylobacter* sp. needs to be conducted and reported in poultry to reduce their numbers thereby preventing cases of

human Campylobacteriosis.

REFERENCES

Allos B (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. Clin. Infect. Dis. 32:1201–1206.

Aquino MHC, Filgueiras ALL, Ferreira MCS, Oliveira SS, Bastos MC, Tibana A (2002). Antimicrobial resistance and plasmid profiles of *Campylobacter jejuni* and *Campylobacter coli* from human and animal sources. Lett. Appl. Microbiol. 34:149–153.

Baker MG, Kvalsvig A, Zhang J, Lake R, Sears A, Wilson N (2012). Declining Guillain-Barré syndrome after campylobacteriosis control, New Zealand, 1988-2010. Emerg. Infect. Dis. 18:226–233.

Baserisalehi M, Al-Mahdi AY, Kapadnis BP (2005). Antimicrobial susceptibility of thermophilic *Campylobacter* spp. isolated from environmental samples. Ind. J. Med. Microbiol. 23:48–51.

Berndtson E, Danielsson-Tham ML, Engvall A (1996). *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. Int. J. Food Microbiol. 32:35–47.

Berrang ME, Buhr RJ, Cason JA (2000). *Campylobacter* recovery from external and internal organs of commercial broiler carcass prior to scalding. Poult. Sci. 79:286–290.

Bhadra RK, Dutta P, Bhattacharya SK, Dutta SK, Pal SC, Nair GB (1992). *Campylobacter* species as a cause of diarrhoea in children in Calcutta. J. Infect. 24:55–62.

Boysen L, Vigre H, Rosenquist H (2011). Seasonal influence on the prevalence of thermotolerant *Campylobacter* in retail broiler meat in Denmark. Food Microbiol. 28:1028–1032.

Byrd J, Corrier D, Hume M, Bailey R, Stanker L, Hargis B (1998). Incidence of *Campylobacter* in crops of preharvest market-age broiler chickens. Poult. Sci. 77:1303–1305.

Chattopadhyay UK, Rashid M, Sur SK, Pal D (2001). The occurrence of Campylobacteriosis in domestic animals and their handlers in and around Calcutta. J. Med. Microbiol. 50:933–934.

Chen X, Naren GW, Wu CM, Wang Y, Dai L, Xia LN, Luo PJ, Zhang Q, Shen JZ (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. Vet. Microbiol. 144:133–139.

Domínguez C, Gómez I, Zumalacárregui J (2002). Prevalence of Salmonella and *Campylobacter* in retail chicken meat in Spain. Int. J. Food Microbiol. 72:165–168.

Gerdemann MM (1996). *Campylobacter jejuni* strains : the importance of food hygiene for the production of poultry. Fleischwirtschaft 76:58–60.

Gu W, Siletzky RM, Wright S, Islam M, Kathariou Sophia (2009). Antimicrobial susceptibility profiles and strain type diversity of *Campylobacter jejuni* isolates from turkeys in eastern North Carolina. Appl. Environ. Microbiol. 75:474–482.

Gupta A, Nelson JM, Barrett TJ (2004). Antimicrobial Resistance among *Campylobacter* Strains , United States,1997-2001. Emerg. Infect. Dis.10:1102–1109.

Habib I, Uyttendaele M, De Zutter L (2011). Evaluation of ISO 10272:2006 standard versus alternative enrichment and plating combinations for enumeration and detection of *Campylobacter* in chicken meat. Food Microbiol. 28:1117–1123.

Han K, Jang SS, Choo E, Heu S, Ryu S (2007). Prevalence, genetic diversity, and antibiotic resistance patterns of *Campylobacter jejuni* from retail raw chickens in Korea. Int. J. Food Microbiol. 114:50–59.

Hansson I, Pudas N, Harbom B, Engvall EO (2010). Within-flock variations of *Campylobacter* loads in caeca and on carcasses from broilers. Int. J. Food Microbiol. 141:51–55.

Hariharan H, Sharma S, Chikweto A, Matthew V, DeAllie C (2009). Antimicrobial drug resistance as determined by the E-test in *Campylobacter jejuni*, *C. coli*, and *C. lari* isolates from the ceca of broiler and layer chickens in Grenada. Comp. Immun. Microbiol. Infect. Dis. 32:21–28.

Hudson JA, Nicol C, Wright J, Whyte R, Hasell SK (1999). Seasonal variation of *Campylobacter* types from human cases, veterinary cases, raw chicken, milk and water. J. Appl. Microbiol. 87:115–124.

Humphrey T, O'Brien S, Madsen M (2007). *Campylobacter*s as zoonotic pathogens: a food production perspective. Int. J. Food Microbiol. 117:237–257.

ISO 10272-1:2006 And ISO/TS 10272-2:2006. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Campylobacter* spp. Part 1: Detection method; Part 2: Colony count technique. International Organisation for Standardisation (ISO), ISO Central Secretariat, 1 rue deVarembé, Case Postale 56, CH – 1211, Geneva 20, Switzerland.

Jacobs-Reitsma WF, van de Giessen a W, Bolder NM, Mulder RW (1995). Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. Epidemiol. Infect. 114:413–421

Jain D, Sinha S, Prasad KN, Pandey CM (2005). *Campylobacter* species and drug resistance in a north Indian rural community. Trans. R. Soc.Trop. Med. Hyg. 99:207–214.

Jones K (2001). The Campylobacter conundrum. Trend. Microbiol. 9:365-366.

Kapperud G, Skjerve E, Bean NH, Ostroff SM, Lassen J (1992). Risk factors for sporadic *Campylobacter* infections: results of a case-control study in southeastern Norway. J. Clin. Microbiol. 30:3117–3121.

Keener KM, Bashor MP, Curtis PA, Sheldon BW, Kathariou S (2004). *Campylobacter* and Poultry Processing. Compr. Rev. Food. Sci. F. 3:105–116.

Kim J, Carver DK, Kathariou S (2006). Natural transformation-mediated transfer of erythromycin resistance in *Campylobacter coli* strains from turkeys and swine. Appl. Environ. Microbiol. 72:1316–1321.

Kuroki S, Saida T, Nukina M, Haruta T, Yoshioka M, Kobayashi Y, Nakanishi H (1993). *Campylobacter jejuni* strains from patients with Guillain-Barré syndrome belong mostly to Penner serogroup 19 and contain beta-N-acetylglucosamine residues., Ann. Neurol. 33:243–247.

Lane DJ (1991). 16S/23S rRNA sequencing. In Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics, John Wiley and Sons, New York, pp. 115–175.

Luangtongkum T, Morishita TY, El-Tayeb AB, Ison AJ, Zhang Q (2007). Comparison of antimicrobial susceptibility testing of *Campylobacter* spp. by the agar dilution and the agar disk diffusion methods. J. Clin. Microbiol. 45:590–594.

Luber P, Bartelt E, Genschow E (2003a). Comparison of broth microdilution, E test, and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. J. Clin. Microbiol. 41:1062–1068.

Luber P, Wagner J, Hahn H, Bartelt E (2003b). Antimicrobial Resistance in *Campylobacter jejuni* and *Campylobacter coli* Strains Isolated in 1991 and 2001-2002 from Poultry and Humans in Berlin, Germany. Antimicrob. Ag. Chemother. 47:3825–3830.

Meldrum RJ, Griffiths JK, Smith RMM, Evans MR (2005). The seasonality of human *Campylobacter* infection and *Campylobacter* isolates from fresh, retail chicken in Wales. Epidemiol. Infect. 133:49–52.

Miraglia D, Ranucci D, Branciari R, Cioffi a, Mammoli R, Cenci Goga BT, Avellini P (2007). Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in chicken hybrids with different growth rates, reared according to conventional and "free-range" production methods. Vet. Res. Comm. 1:381–384.

Nachamkin I, Allos BANM, Ho T (1998). *Campylobacter* Species and Guillain-Barre ´ Syndrome. Clin. Microbiol. Rev. 11:555–567

Näther G, Alter T, Martin a, Ellerbroek L (2009). Analysis of risk factors for *Campylobacter* species infection in broiler flocks. Poult. Sci. 88:1299–1305.

Nauta MJ, van der Wal FJ, Putirulan FF, Post J, van de Kassteele J, Bolder NM (2009). Evaluation of the "testing and scheduling" strategy for control of *Campylobacter* in broiler meat in The Netherlands. Int. J. Food Microbiol. 134:216–222.

Nayak R, Stewart TM, Nawaz MS (2005). PCR identification of *Campylobacter coli* and *Campylobacter jejuni* by partial sequencing of virulence genes. Mol. Cell. Probes, 19:187–193.

Niwa H, Chuma T, Okamoto K, Itoh K (2001). Rapid detection of mutations associated with resistance to erythromycin in *Campylobacter jejuni/coli* by PCR and line probe assay. Int. J. Antimicrob. Ag. 18:359–364

Nylen G, Dunstan F, Palmer SR, Andersson Y, Bager F, Cowden J, Feierl G, Galloway Y, Kapperud G, Megraud F, Molbak K, Petersen

LR, Ruutu P (2002). The seasonal distribution of *Campylobacter* infection in nine European countries and New Zealand. Epidemiol. Infect. 128:383–390.
Oyofo BA, Thornton SA, Burr DH, Trust TJ, Pavlovskis OR, Guerryl P (1992). Specific Detection of *Campylobacter jejuni* and *Campylobacter coli* by Using Polymerase Chain Reaction. J. Clin. Microbiol. 30:2613–2619.
Payot S, Dridi S, Laroche M, Federighi M, Magras C (2004). Prevalence and antimicrobial resistance of *Campylobacter coli* isolated from fattening pigs in France. Vet. Microbiol. 101:91–99.
Rajendran P, Babji S, George AT, Rajan DP, Kang G, Ajjampur SS (2012). Detection and species identification of *Campylobacter* in stool samples of children and animals from Vellore, south India. Ind. J. Med. Microbiol. 30:85–88.
Reardon T, Gulati A (2008). The Rise of Supermarkets and Their Development Implications. International Experience Relevant for India. IFPRI Discussion Paper 00752, IFPRI, New Delhi Office.
Rejab SBM, Zessin KH, Fries R, Patanchee P (2012). Comparison of *Campylobacter* Contamination Levels on Chicken Carcasses between Modern and Traditional Types of Slaughtering Facilities in Malaysia. J. Vet. Med. Sci. 74:121–124.
Rizal A, Kumar A, Vidyarthi AS (2010). Prevalence of Pathogenic Genes in *Campylobacter jejuni* Isolated from Poultry and Human. Int. J. Food Safety, 12:29–34.
Rodenburg TB, Van Der Hulst-Van Arkel MC, Kwakkel RP (2004). *Campylobacter* and Salmonella infections on organic broiler farms. NJAS-Wagen J. Life. Sci. 52:101–108.
Rodrigo S, Adesiyun A, Asgarali Z, Swanston W (2005). Prevalence of *Campylobacter* spp. on chickens from selected retail processors in Trinidad. Food Microbiol. 22:125–131.
Rosenquist H, Sommer HM, Nielsen NL, Christensen BB (2006). The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. Int. J. Food Microbiol. 108:226–232.
Singh R, Singh P, Rathore R, Dhama K, Malik SV (2008). Studies on effect of seasonal variations on the prevalence of *Campylobacter jejuni* in poultry faecal samples collected from Western Uttar Pradesh, India. J. Comp. Microbiol. Immunol. Infect. Dis. 29:45–48.
Son I, Englen MD, Berrang ME, Fedorka-Cray PJ, Harrison MA (2007a). Antimicrobial resistance of Arcobacter and *Campylobacter* from broiler carcasses. Int. J. Antimicrob. Ag. 29:451–455.
Son I, Englen MD, Berrang ME, Fedorka-Cray PJ, Harrison MA (2007b). Prevalence of Arcobacter and *Campylobacter* on broiler carcasses during processing. Int. J. Food Microbiol. 113:16–22.
Stanley K, Wallace J, Currie J, Diggle P, Jones K (1998a). The seasonal variation of thermophilic *Campylobacter*s in beef cattle, dairy cattle and calves. J. Appl. Microbiol. 85:472–480.
Stanley KN, Wallace JS, Currie JE, Diggle PJ, Jones K (1998b). Seasonal variation of thermophilic *Campylobacter*s in lambs at slaughter. J. Appl. Microbiol. 84:1111–1116.
Stern NJ, Robach MC (2003). Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. J. Food Protect. 66:1557–1563.
Stoyanchev T, Vashin I, Ring C, Atanassova V (2007). Prevalence of *Campylobacter* spp. in poultry and poultry products for sale on the Bulgarian retail market. Antonie. Leeuwenhoek, 92:285–258.
Taremi M, Mehdi Soltan Dallal M, Gachkar L, MoezArdalan S, Zolfagharian K, Reza Zali M (2006). Prevalence and antimicrobial resistance of *Campylobacter* isolated from retail raw chicken and beef meat, Tehran, Iran. Int. J. Food Microbiol. 108:401–403.
Thrusfield M (1995). Surveys. In: Thrusfield M (ed) Veterinary Epidemiology , Blackwell Scientific Ltd., Oxford, U.K., pp. 178–198.
Wallace JS, Stanley KN, Currie JE, Diggle PJ, Jones K (1997). Seasonality of thermophilic *Campylobacter* populations in chickens. J. Appl. Microbiol., 82:219–224
Willis W, Murray C (1997). *Campylobacter jejuni* seasonal recovery observations of retail market broilers. Poult. Sci. 76:314–317.
Yabe S, Higuchi W, Takano T, Razvina O, Iwao Y, Isobe H, Yamamoto T (2010). In vitro susceptibility to antimicrobial agents and ultrastructural characteristics related to swimming motility and drug action in *Campylobacter jejuni* and *C. coli*. J. Infect. Chemother. 16:174–185.