Characterization of isolated thermophilic campylobacters and associated risk factors in poultry farms of Uttarakhand, India

Geetika Garhia
GBPUAT
Maansi Mani (maansi2000@rediffmail.com)
GBPUAT https://orcid.org/0000-0002-2466-4085
Deepak Kumar
GBPUAT
Ajay Kumar Upadhyay
GBPUAT

Research

Keywords: thermophilic campylobacters, farms, virulence, antibiotic susceptibility, risk factors

Posted Date: June 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-32058/v1

Abstract

Background

Campylobacters are the common commensals of poultry responsible for several cases of gastroenteritis in humans. The illness, if severe can result into complications causing a nervous disorder named Guillian Barre syndrome. Owing to its serious health implications, the study aimed to screen eight organized poultry farms and their environment (water, litter, manure, and feed) of Uttarakhand state, India for the presence of thermophilic *Campylobacter* species and their virulence and antibiotic resistance profile. It also undertook identification of risk factors associated with the occurrence of campylobacters in each farm using a questionnaire survey comprising eleven potential risk factors (other animals on farm, reuse of litters, use of foot bath, in house or branded feed, chlorination of water, distance of manure heap, housing system, flock size, floor type, shoe use by farm personnels, moist or dry litter and number of broiler floor).

Results

Of eight, six farms showed varying occurrence of *C.jejuni* and *C.coli* with an overall prevalence of 12.29%. Not a single isolate of *C.lari* and *C.upsaliensis* was recorded. Poultry faecal, water and litter samples observed 18.2%, 6% and 1.9% presence, respectively. Feed and manure samples did not appear positive. In 48 revived *Campylobacter* isolates, 100% presence of *cad*F and *fla*A virulence genes were detected followed by *cdt*B (97.9%), *cgt*B (22.9%) and *cia*B (12.5%), respectively. Ten isolates 23.80%) were multidrug resistant (MDR) exhibiting resistance to at least 3 or more antimicrobial classes. The most common MDR patterns were AMP CX CIP TE (n = 2) and AMP CX CIP (n = 2). Feeding of branded feed was found to have significant association with *Campylobacter* presence in the examined broiler flocks (p-value 0.0047).

Conclusions

The study highlights the occurrence of food pathogens, *Campylobacter jejuni* and *C.coli* in the poultry farms and their environment of the state. The organisms possessed significant virulence genes capable of developing critical human illness. Overall, the presence of MDR thermophilic campylobacters appears to be a severe public threat.

Background

The incidences of food-borne illnesses are observed in developing as well as developed nations. These illnesses are mainly caused by pathogenic bacteria present in food (1, 2). As per Center for Disease Control and Prevention (CDC), campylobacters stand as 4th major cause of food-borne illness (9%), 3rd major cause of hospitalization (15%) and 5th main cause of human deaths (6%) due to food-borne

infections annually in the United States (3). India, a developing country, lacks a decent data on foodborne diseases as many cases go unreported. The Integrated Disease Surveillance Programme (IDSP) network, launched in India in 2004, highlights that food-borne outbreaks together with acute diarrhoeal diseases constitute nearly half of all reported outbreaks based on data collected from 2011-15(4). Among the well known food-borne pathogens, thermophilic campylobacters namely *Campylobacter jejuni (C.jejuni*) and *Campylobacter coli (C.coli)* contribute approximately 95% of human infection (3). Two others, *C. lari* and *C. upsaliensis* also account for many diarrhoeal cases in humans (5, 6). These microorganisms constitute the normal gastrointestinal microflora in many animals,.especially birds (7). Poultry birds can be infected with the bacteria at a very high level without showing any visible clinical symptoms. Campylobacters, *C. jejuni* and *C. coli* are well adapted to birds because of their ability to grow at 41–42 (the approximate body temperature of a bird). These organisms have been frequently isolated from the caecal microflora (8). Intestinal content is thus one of the primary suspected source of meat contamination during slaughter. Hence, managing *Campylobacter* spp. in the poultry reservoir is a crucial step in prevention and control of food-borne campylobacteriosis in humans. Other possible sources like contaminated drinking water, consumption of unpasteurized milk and ready to eat food products, faecal run-off of birds and domestic animals contaminating surface water and direct contact with animals are significant in transmitting illness to humans (9).

Campylobacter illness in humans occur worldwide with estimated 500 million infections annually (10). Although it is a self limiting disease, the emergence of antimicrobial resistance in campylobacters has become a concern for food safety. Development of antimicrobial resistant (AMR) campylobacters has been linked to the indiscriminate use of antimicrobials in food animal production system (poultry and swine) for disease prevention and growth promotion (11, 12). Sub-therapeutic use of antimicrobials in food production systems is believed to create selection pressure and force microorganisms to develop resistance in order to survive (13). A rapid increase in the proportion of *Campylobacter* strains resistant to antimicrobial agents, particularly fluoroquinolones and macrolides, has been reported in many countries (14, 15, 16, 17). Nevertheless, there still exists paucity of data on the presence of antimicrobial resistant campylobacters and various risk factors responsible for the prevalence of these organisms in poultry production systems in India. Very few researchers have reported campylobacters in poultry (18, 19, 20, 21) thus, more future research awaits in this direction.

Uttarakhand, an Indian state with high tourist footfall of around 34.36 million with foreign tourist visits over 0.13 million in 2017(22) finds limited data on campylobacter presence in farms. To fill this knowledge gap, the present study was designed to estimate the occurrence of thermophilic campylobacters,virulence,antibiotic resistance and risk factor associated with campylobacters in eight commercial poultry farms located in Kumaon region of Uttarakhand.

Results

Prevalence of thermophilic *campylobacter* s

Out of 545 samples comprising 346 poultry faecal and 199 environmental samples viz; feed (n=52), water (n= 50), litter (n=51) and manure (n=46) samples collected from eight poultry farms, 67 samples tested positive for *Campylobacter* yielding a total prevalence of 12.29% (Table 2). *Campylobacter* genus-specific amplicon of 816 bp(16SrRNA) was present in all the positive isolates. Faecal prevalence of *Campylobacter* was 18.2% (63/346) while environmental sources showed a prevalence of 2.01% (4/199), which included 3(6%) isolates from water and 1 isolate (1.96%) from litter sample. None of the feed and manure samples yielded *Campylobacter* spp. Of the 67 isolates obtained, multiplex PCR targeting *lpx*A gene for species differentiation identified 16(23.88%) as *C. jejuni* (331bp*)* and 51(76.11%) as *C. coli(*391bp*).* None of the isolates produced an amplicon size of 233 bp (*C. lari*) and 206 bp (*C. upsaliensis*).

Varying prevalence was observed among the farms studied. Highest prevalence was detected in Bazpur farm (31.4%) followed by Pantnagar farm 2 (25.0%), Pantnagar farm 1 (24.4%), Haldwani farm (16.3%), Bindukhatta farm (7.5%) and Jawaharnagar farm (5.6%). No *Campylobacter* isolate was recovered from Kiccha and Ramnagar farms.

The species distribution of *Campylobacter* across farms revealed highest prevalence of *C. coli* in Bazpur farm (90.9%) followed by Pantnagar farm 1 (81.81%), Pantnagar farm 2 (66.67%), Jawaharnagar (60%), Haldwani farm (37.5%) and Bindukhatta (33.33%). However, the highest prevalence of *C. jejuni* was observed in Bindukhatta farm (66%) followed by Haldwani farm (62.5%), Jawaharnagar farm (40%) Pantnagar farm 2 (33.33%), Pantnagar farm 1 (18.18%) and Bazpur farm (9.09%)(Table 3).

Prevalence of virulence genes

All the 48 revived *Campylobacter* isolates (39 *C. coli* and 9 *C. jejuni)* showed 100% presence of *cad*F and *fla*A virulence genes followed by *cdt*B (97.9%), *cgt*B (22.9%) and *cia*B (12.5%), respectively. None of the *Campylobacter* isolate harboured *wla*N gene. The *cia*B gene was detected only in *C. jejuni* isolates (66.66%, 6/9). None of the *C. coli* isolates harboured *cia*B gene. Gene *cdt*B was detected in all *C. coli* (100%,39/39) and (88.88%, 8/9) *C. jejuni* isolates. Virulence gene *cgt*B was identified in (33.33%, 3/9) *C. jejuni* and (20.51%, 8/39) *C. coli* isolates(Table 4).

Virulence genes *cad*F and *fla*A were detected in all isolates (100%) recovered from all four farms. Highest frequency of virulence gene *cia*B was detected from Bindukhatta farm (33.3%) followed by Jawaharnagar farm (20%), Bazpur farm(9.09%) and Pantnagar farm 2 (6.66%). Virulence gene *cdt*B was detected from Pantnagar farm 2 (100%), Jawaharnagar farm (100%), Bindukhatta farm (100%) and Bazpur farm (95.4%). Highest frequency of *cgt*B gene was detected from Bazpur farm (36.3%) followed by Jawaharnagar farm (20%), Bindukhatta farm (16.6%) and Pantnagar farm 2 (6.6%). Virulence gene *wla*N was not detected in any of the farms(Table 5).

Phenotypic Antimicrobial Susceptibility

On subjecting 42 revived isolates to disc diffusion test, forty one isolates (n=41, 97.6%) exhibited resistance to at least one antimicrobial on the disc diffusion assay and one isolate (ID.C4) was pan-susceptible. Ten isolates (n=10, 23.80%) were multidrug resistant (MDR) exhibiting resistance to at least 3 or more antimicrobial classes. Three *Campylobacter* isolates were found resistant to four classes of antimicrobials while seven isolates showed phenotypic resistance to three classes of antimicrobials. However, twenty two isolates were found to be resistant to two classes of antimicrobials.

β-lactam antimicrobials (cefoxitin, ceftriaxone and ampicillin) observed higher resistance than other classes studied . Highest frequency of resistance was found against cefoxitin (97.61%) followed by ciprofloxacin (64.28 %), nalidixic acid (33.33 %), ampicillin (28.5%) and ceftriaxone (14.28%). Two isolates (4.76%) were resistant to tetracycline. However, only one isolate showed resistance to clindamycin, sulfafurazole and erythromycin. All isolates (n=42) were susceptible to levofloxacin and gentamicin.

Variable resistance was seen in the two thermophilic campylobacters (*C. jejuni* and *C.coli*). Out of 42 isolates, 41 (97.61%) showed resistance to second generation cephalosporin , cefoxitin. Of these 41, 11 (27.5%) were *C. jejuni* and 30 (73.17%) were *C. coli.* Only 12 (28.5%,12/42) isolates showed resistant to ampicillin, of which, 4 (33.33%) were *C. jejuni* and 8 (66.66%) were *C. coli*. Six isolates (14.28%, 6/42) showed resistance to ceftriaxone of which 4 (66.66%) were *C. jejuni* and 2 (33.33%) were *C. coli*. However, resistance against ciprofloxacin, nalidixic acid and tetracycline was shown by 27 (64.28%,27/42), 14 (33.33%,14/42) and 2 (4.76%,2/42) isolates, respectively. Of which 7 (25.92%), 6 (42.85%) and 2 (100%) were *C. jejuni* and 20 (74.07%), 8 (57.14%) and 0 (0%) *C. coli* respectively(Table 6).

A total of 16 different AMR combinations were detected of which, 8 resistance patterns were MDR represented by 10 isolates. The most common MDR patterns were AMP CX CIP TE (n=2) and AMP CX CIP (n=2). Distribution of antimicrobial resistance patterns across sample types and farm location is detailed in Table7.

Genotypic Characterization of AMR Determinants

Presence of four antibiotic resistance genes (ARGs) namely *bla*OXA-61, *tet*(O), *cme*B and *erm*B conferring resistance to different classes of antibiotics were detected by specific Antibiotic Resistance Genes-PCRs. Out of 41 isolates showing phenotypic resistance, 29 isolates showed presence of at least one resistance genes targeted (*bla*OXA-61, *tet*(O), *cme*B and *erm*B. However, 12 resistant isolates did not harbour any of the four resistance genes. β-lactam resistance gene *bla*OXA-61 was detected in 18 (58.06%) out of 31 isolates showing phenotypic resistance. Resistance gene *cme*B was detected in 19 fluoroquinolone resistant isolates (79.16%) out of 24 tested. One lincosamide (Clindamycin) resistant isolate harboured *cme*B gene. Tetracycline resistant *tet*(O) gene was detected in all isolates showing phenotypic tetracycline resistance (n=2). Macrolide resistance gene *erm*B was absent in a single erythromycin resistant isolate. Most prevalent resistance gene combination was *bla*OXA-61+ *cme*B, which was detected in 11 isolates(Table 8).

Risk factor analysis

Of the 11 parameters studied as risk factors using a questionnaire distributed to farm owners, only one risk parameter i.e., feeding of branded feed was found to have significant association with *Campylobacter* presence in the examined broiler flocks (p-value 0.0047).

Discussion

The present study was designed to determine the prevalence of thermophilic campylobacters in poultry raised at farms and their living environment.

Prevalence of thermophilic campylobacters

The overall *Campylobacter* comprising poultry faeces (n=346) and environmental samples(n=199) was recorded as 12.29% (67/545). Other findings reported from the studies conducted in broiler flocks have also reported almost similar overall prevalence. **Chokboonmongkol *et al.* (23)** reported 11.2% *Campylobacter* spp. prevalence in broiler flocks from Thailand while another study from Ecuador reported 12.4% prevalence of *Campylobacter* in broiler flocks **(24).** In India, limited studies have been done on *Campylobacter* prevalence in poultry. These studies have revealed prevalence ranging from 13.54-21.8% (13.54%,**21;** 15.89%,**19;** 21.8%,**25** 14.28%,**26 and** 20%, **27).** However, a much higher prevalence of *Campylobacter* as high as 72.2% from cloacal swab samples has also been documented from poultry by **Vaz *et al.* (28**). Another study by **Ingresa *et al.* (29)** reported 71.4% prevalence of *Campylobacter* in poultry caecal samples and 69.1% for poultry faecal samples.

Faecal prevalence of *Campylobacter* was 18.2% (63/346).Detection of campylobacters in poultry faeces poses a significant risk for contamination of chicken meat. The organisms frequently colonize the bird's intestine and shed in large numbers through faeces. Faecal shedding of *Campylobacter* spp. is a source of infection to other birds in the flock. Bacteria present in faeces can contaminate feed and water supply of the same flock. Moreover, there is a risk of *Campylobacter* transmission to their flocks by means of frequent human movement.

Only 4 isolates could be recovered from 199 environmental samples with a prevalence of 2.01%, which included 3 isolates from water and 1 isolate from litter sample However, **Vaz *et al.* (28)** recorded much higher 63.8% *Campylobacter* prevalence in litter samples from Brazilian broiler flocks. Similarly, **Lisa *et al.* (30)** reported 64.3%, 64.3% and 45.7% *Campylobacter* prevalence in soil, compost, and processed waste water respectively. Presence of campylobacters in environment is significant as campylobacters are able to form biofilms as a survival mechanism outside the host (31**).** Detection of *Campylobacter* spp. from water samples is important because all the birds in a flock drink water from the same waterer which aid in furthur spread within a flock. Also, capability to form a biofilm poses the risk of its presence in cold water inspite of chemical treatment **(32).**

Feed and manure samples of our study did not reveal any presence of *Campylobacter* spp. However, these sources cannot be neglected as a source of infection. Zero prevalence of *Campylobacter* in manure and feed samples could be due to less number of samples processed.

Interestingly, in this study majority of the isolates were identified as *C. coli* (76.11%) and only 23.88% isolates were *C. jejuni*. (Table4). *C. jejuni* is considered to be the predominant species colonizing poultry **(33,34).** Many studies (**35,36,37)** report the dominance of *C.jejuni* over *C.coli* in poultry. In India, **Chattopadhyay *et al*. (38)** and **Rajendran *et al*. (39)** also showed that *C. jejuni* were more frequent than *C. coli* in poultry faecal samples. However, in accordance to our study, many other authors have reported *C.coli* dominance. **Pergola *et al*. (40)** reported 70.71% prevalence of *C. coli* and 17.14% *C. jejuni* from cloacal swab samples. **Monika (21) and 19)** also reported higher *C.coli* presence of 75% and 67.44%,respectively of the total isolates recovered from poultry faeces of Uttarakhand. Also, **Wieczorek *et al*(41)** in their retrospective study of five-years on prevalence and antimicrobial resistance of *Campylobacter* from poultry carcasses in Poland also found *C.coli* as a dominant species over *C.jejuni* In our opinion, the initial dominance of a species and further spread due to improper control measures can decide the higher presence of a species. Better colonization ability of either of the two species in poultry intestine and persistence in outside environment may decide the dominance.

No *C. lari* and *C. upsaliensis* were detected in this study. However, *C. lari* isolation from poultry is reported by some authors. Very few studies support the presence of *C.lari* in poultry isolates. **Pillai (42)** and **25** isolated 2 and a single isolate of *C.lari* from chicken samples in Bangalore and Bareillly respectively. **Oyarzabal and Hussain (43)** are of the opinion that, with the development of DNA based methods for the identification of isolates; *C. lari* has not been reported for more than 10 years in the United States, which suggests that previous reports may have been misidentifications from the traditional biochemical tests which were used for species confirmation. Further studies on poultry using molecular diagnostic techniques would answer the same. **Acke *et al* (44)** reported that dogs are the main reservoirs for *C. upsaliensis* which could probably be the reason for non-isolation of this organism in our study.

No previous data on *Campylobacter* prevalence in poultry farms is available for selected locations except for Pantnagar and Haldwani. Probably Isolation of *Campylobacter* from the locations except the two (Pantnagar and Haldwani) has not been reported so far. Poultry farms at Pantnagar screened before have reported the prevalence rates of 16 % **(45),** 11.66 % **(46)** and 13.54 % **(21)**. However, a lower prevalence of 6.9 % **(19)** and 5.34 % **(47)** also has been reported from Pantnagar. **Rawat *et al* (20)** reported 4.17 % *Campylobacter* prevalence in faecal samples of broilers collected from an organized farm of Pantnagar.

Prevalence of virulence genes

Total 48 *Campylobacter* isolates including 39 *C. coli* and 9 *C. jejuni* were included for virulence gene detection using PCR. Nineteen isolates (n=19) could not be revived and thus were not included in the virulence gene analysis. The genes associated with bacterial motility (*flaA*) and adhesion to epithelial cells (*ca dF*),were present in all (100%)the isolates.These genes are known to be conserved in *Campylobacter* spp**. (48,49)** and play a key role in the development of *Campylobacter* infection. The *cdt*B

(97.9%) was second most prevalent gene. This gene along with *cdtA* and *cdtC* cytoxin gene has the ability to interfere with the division and differentiation of the intestinal crypt cells, ,thus has an important role in diarrhoea. This combination has been recorded with a prevalence of 96.6–97.6% in positive strains (**50**) which is in accordance with our study. It also suggests that the three genes(*cdtA, cdt*B and *cdtC) should be included together in future studies for assessing toxic property.*

*The cgt*B gene was found in 22.9% of the positive *Campylobacter* spp. isolates. Not much data is available on the presence of this gene in the campylobacters though this gene, as *wlaN*, also codes for a β-1,3-galactosyltransferase enzyme that is required for the production of sialylated lipooligosaccharide responsible for Guillain-Barré syndrome (GBS)(**51)**

Other gene *cia*B exhibited in 12.5% isolates. This gene is important for *Campylobacter* survival in the intestinal tract. The product of the *ciaB* marker, which play a role both in the intestinal invasiveness and in colonization of the epithelial cells **(52),** was identified in campylobacters by other authors also in a lower percentag **(53,54).**The presence of this gene is significant as it helps the organisms to overcome the stress conditions presented by the intestine and cause disease. *Additionally, expression of ciaB* has been observed to reduce under nutritional stress **(55).**

None of the *Campylobacter* isolate harboured *wla*N gene. Many studies conducted on *C. jejuni* and *C.coli* have reported total absence of this gene (**48,56,57)**.However, Kim *et al.***(58)** identified the *wlaN* gene among 100% of 63 human and in 78.6% of 42 animal *C. jejuni* isolated tested in Korea. The product of the *wlaN* gene is also thought to be involved in development of of Guillain–Barre' syndrome after *C. jejuni* infection **(49,58,59).**

In our study, *C.jejuni* (*cad*F(100%), *fla*A(100%), *cia*B(66.66%), *cdt*B(88.88%) and *cgt*B(33.33%)) possessed more number of virulent genes than *C.coli* (*cad*F and *fla*A(100%), *cdt*B(100%) and *cgt*B(20.51%)). Moreover, *ciaB* gene presence (responsible for both epithelial and intestinal mucosal invasion) only in *C.jejuni* isolates may suggest this species dominance over *C.coli* in being more pathogenic **(60)** and a cause for regulars diarrhoeal cases in humans **(7).** The virulent profile of *C. jejuni* **(59, 61)** showed that the greatest potential of this species over the other in causing clinical cases in humans (81.1%) (**62)** is due to the properties of invasion, colonization and toxin production which are essential to elicit its pathogenesis. In contrast, *C. Coli* shows its priority is to ensure the survival through mechanisms **(63).**

Either of the virulence genes except wlaN were found in both faecal and environmental (water(n=3) and litter(n=1)) samples. This indicates potential risk to consumers..

Virulence genes *cad*F and *fla*A were detected in all isolates (100%) recovered from all four farms. **Pant (45)** recorded 100% prevalence of *fla*A and *cad*F genes in the isolates recovered from diverse sources collected from Udham Singh Nagar district. The presence of virulence genes such as *cdt*A and *cdt*B have been reported **(46,64)** who screened the sources from Pantnagar and nearby areas. *Campylobacter* isolates of the same region were also shown to express *wla*N, *iam*, *cia*B and *dna*J virulent genes **(47).**

High frequency of detection of virulence genes *cad*F (100%), *fla*A (100%) and *cdt*B (97.9%) in *Campylobacter* species in farms is a matter of concern. **Casabonne *et al*. (65)** studied the prevalence of seven virulence and toxin genes, i.e. *fla*A, *cad*F, *cia*B, *cdt*B, *cgt*B, *doc*C and *wla*N from the diarrhic patients. He found all the isolates were positive for *flaA, cadF* and *cdtB* genes (100%) and 40.0%, 23.3%, 20.0% and 6.7% were positive for *ciaB, docC, wlaN* and *cgtC*, respectively. **Wieczorek and Osek (66)** showed the presence of *cad*F and *fla*A gene in 100% of the isolates obtained from poultry and human. **Talukder *et al*. (57)** studied pathogenic genes namely *fla*A*, cad*F*, pld*A*, cia*B, *cdt*A*, cdt*B*, cdt*C and *wla*N in 40 *C. jejuni* and 5 *C. coli* strains isolated from diarrheal patients in Bangladesh and found 100% prevalence of *fla*A*, cad*Fand *pld*A genes. The detection rates of *cia*B, *cdtA, cdtB, cdtC* and *wlaN* genes were reported as 95%, 97.5%, 97.5%, 97.5% and 7.5% respectively.

Phenotypic Antimicrobial Susceptibility

Forty two isolates (11 *C. jejuni* and 31 *C. coli*) were revived for the phenotypic antimicrobial susceptibility. *Campylobacter* isolates exhibited highest frequency of resistance to cefoxitin (97.61%) followed by ciprofloxacin (64.28 %), nalidixic acid (33.33 %), ampicillin (28.5%) and ceftriaxone (14.28%) (Fig. 19). Two isolates (4.76%) were resistant to tetracycline. However, only one isolate showed resistance to clindamycin, sulfafurazole and erythromycin. All isolates (n=42) were susceptible to levofloxacin and gentamicin (Table 14).

The antibiotic resistance profile in this study was almost identical to the findings of Rajagunalan **(19)** who observed *C. jejuni* to be 100% sensitive to gentamicin, ampicillin and erythromycin and 100% resistant to cephalothin and co-trimoxazole. Narvaez *et al.* **(67)** reported that 71.4% of *Campylobacter* isolates had sensitivity against nalidixic acid followed by tetracycline (48.1%), ciprofloxacin (5.5%), azithromycin (1.78%) and erythromycin (1.78%). All isolates were susceptible to clindamycin, florfenicol, gentamicin and telithromycin and tetracycline resistance was attributable to the presence of the *tet(*O) gene. Kashoma *et al*. **(68)** reported *Campylobacter* isolates with resistance to ampicillin (63%), ciprofloxacin (9.3%), erythromycin (53.7%), gentamicin (0%), streptomycin (35.2%), and tetracycline (18.5%), azithromycin (42.6%), nalidixic acid (64.8%), chloramphenicol (13%) and tylosin (90.2%) respectively. The variation in the antimicrobial sensitivity pattern of the *Campylobacter* isolates has been reported earlier.

Ten isolates of 41 (n=10, 23.80%) were multidrug resistant (MDR) exhibiting resistance to at least 3 or more antimicrobial classes. Only one isolate (ID.C4) was pan-susceptible. Higher resistance to β-lactam antimicrobials was detected in our study such as cefoxitin, ceftriaxone and ampicillin. Resistance to ampicillin (28.5%), a “critically important antimicrobial”, crucial in human medicine is alarming, since it limits our options to treat critical human infections. Resistance was also detected against tetracycline (n=2) and clindamycin (n=1); antibiotics classified as “highly important” in human medicine according to WHO. Clinical management of *Campylobacter* infection becomes more difficult because of increasing development of resistance against antibiotics.

Of 16 different AMR combinations, 8 resistance patterns were MDR represented by 10 isolates. The most common MDR patterns were AMP CX CIP TE (n=2) and AMP CX CIP (n=2). Resistance pattern AMP CX CIP TE (n=2) had faecal origin and was identified from two separate locations,viz Haldwani and Pantnagar farm 2. Another MDR pattern AMP CX CIP (n=2) also had faecal origin. However, this pattern was identified from Bazpur and Bindukhatta farms. Distribution of antimicrobial resistance patterns across sample types and farm location is detailed in (Table 17). Most number of AMR patterns were detected from Bazpur farm (n=7), followed by Pantnagar farm 2 (n=6) and Bindukhatta farm (n=5). Four AMR patterns per farm were detected from Haldwani, Pantnagar farm 1 and Jawaharnagar farm. Significant diversity in the AMR patterns was detected across different farms and sample types. This may conclude the presence of genotypic diversity among the isolates circulating across locations and within a single location.

Genotypic Characterization of AMR Determinants

Out of 41 isolates showing phenotypic resistance, 29 isolates showed presence of at least one resistance genes targeted (*bla*OXA-61, *tet*(O), *cme*B and *erm*B). Most prevalent resistance gene combination was *bla*OXA-61+ *cme*B, which was detected in 11 isolates. A variety of antimicrobial resistance genes (ARGs) conferring resistance to various classes of antibiotics detected in this study is a matter of concern because these antibiotics are frequently used in human medicine and also these resistant determinants can be transferred to susceptible bacterial population by horizontal gene transfer (HGT). Nesme and Simonet **(69)** reported that soil is prone to genetic exchange by means of horizontal gene transfer between ecologically distinct lineages present in other ecosystems. Kashoma *et al*. **(68)** reported antimicrobial resistance genes *bla*OXA-61 (52.6%), *cme*B (26.3%), *tet*(O) (26.3%) and *aph*-3-1 (5.3%) in *Campylobacter* isolates.

Risk factor analysis

Out of 11 risk parameters tested, only feeding of branded feed was found to be significantly associated with *Campylobacter* colonization of the examined broiler flocks (p-value 0.0047). In a similar study, Hald *et al*. **(70)** reported that 35% *Campylobacter* positive flocks used purchased wheat. Authors further reported that farmers who purchased wheat from a feedstuff dealer (p value 0.026) had a higher risk of *Campylobacter* infections in their broiler flocks compared to farmers who fed home-grown wheat. Various studies have been conducted to determine potential risk factors for *Campylobacter* infection in poultry farms **(70,71,72,73,74). Cardinale (75)** reported that an elevated risk of *Campylobacter* infection at poultry farms was associated with several factors namely presence of other animals (mainly laying hens, cattle and sheep) in the farm, farm staff not wearing proper work clothing while working in poultry houses, un-cemented poultry-house floors and the use of cartons that transport chicks from the hatchery to the farm as feed plates (rather than specifically designed feed plates). However, thorough cleaning and disinfection of poultry-house surroundings and manure disposal outside the farm were associated with decreased flock risk. In our study, the strength of association of risk factors with the prevalence of

Campylobacter organism could be better identified with more number of samples screened at much larger number of farm locations.

Conclusion

The study highlights the occurrence of food pathogens, *Campylobacter jejuni* and *C.coli* in the poultry farms and their environment of the state. The organisms possessed significant virulence genes capable of developing critical human illness. Moreover, their resistance for frequently used antibiotics and attaining multi drug resistance is a point of concern. Eight different multi drug resistant patterns point towards reinforcing strict regulations against frequent misuse of antibiotics in farms for commercial gains. Majority of isolates possessing *blaOXA*-61+ *cme*B gene combination may increase the peril by further possible horizontal spread in the surrounding microflora. Evaluation of potential risk factors in colonization of campylobacters suggests a thorough examination of feed before use, though this finding needs a more detailed study with more number of samples. To conclude, improved biosecurity in farms is of paramount importance. Also, pre-harvest and post harvest interventions are valuable in reducing the risks linked with consumption /contamination of poultry meat.

Materials And Methods

Study Design and Sample collection

The present study was conducted in the Uttarakhand state of India. Samples were collected from eight poultry farms (n=8) farms located at Haldwani, Pantnagar, Kiccha, Ramnagar, Bazpur, Jawaharnagar and Bindukhatta regions of the state, India from September 2016 to May 2017. A total of 545 samples collected comprised poultry faeces (n=346) and environment samples (n=199). The environmental samples represented water (n=50), poultry feed (52), litter (51) and manure (46). Sterile 100 ml whirlPak bags (Nasco, Fort Atkinson, WI) were used to collect poultry faeces, poultry feed, litter and manure. The water samples were collected in 100ml sterile sample container (Abdos India). The samples were collected aseptically and immediately brought to the laboratory for processing as per previously published protocols **(76, 77, 78).**

Isolation and Molecular Confirmation

Poultry faecal samples were streaked directly onto the modified Charcoal-Cefoperazone-deoxycholate agar (mCCDA, Hi media) plates and incubated at 42°C with 5% CO_2 in a CO_2 incubator for 48 hrs (OIE terrestrial manual 2008). The poultry feed and the environmental samples however were initially enriched in 9 ml Bolton broth (Oxoid, UK) supplemented with 5% sheep blood. Thereafter, a loopful of the enriched broth suspension was streaked onto mCCDA plates and were incubated at same time-temperature combination. The characteristic campylobacter colonies (1-2 mm size, circular, flat to slightly raised, sticky, spreading and shiny grey) were selected from each plate and tested biochemically. All the presumptive *Campylobacter* isolates that were catalase and oxidase positive while urease and TSI

negative were subjected to DNA isolation using heat-shock method. A simplex PCR and a multiplex PCR assay targeting the 16SrRNA**(79)** and lipid gene lpxA **(80)** respectively were used for the *Campylobacter* genus and species identification. The primer sequence and the cyclic conditions used were as per references (**79 , 80** for *Campylobacter* genus and species, respectively). All PCR confirmed *Campylobacter* isolates were stored as 20% glycerol stock at -80°C.

Antibiotic Susceptibility Testing (AST)

The antimicrobial resistance (AMR) profile of *Campylobacter* isolates was determined using standard Kirby-Bauer disc diffusion method. A total of 42 isolates out of 67 isolates could be recovered for antimicrobial sensitivity testing. A panel of eleven antibiotics representing 5 classes of antimicrobials included Ampicillin (AMP,10µg), Gentamicin (GEN,10µg), Erythromycin(E,15 µg) ciprofloxacin(CIP,5µg) , levofloxacin (LE,5µg), nalidixic acid(NA,30µg),ceftriaxone(CTR,30 µg),cefoxitin(CX, 30µg),sulfafurazole(SF,300 µg), tetracycline(TE, 30µg)and clindamycin(CD, 30µg). The isolates were revived on mCCDA plates supplemented with (FD009) supplement. The growth suspension prepared in PBS (0.5 McFarland) was spread on Mueller-Hinton agar (MHA) plates and incubated at 37 °C for 24h. Zone diameter was measured and break points were interpreted based on the recommendations of Clinical and Laboratory Standards Institute standards for disk-diffusion assay **(81).** The isolates showing resistance to three or more classes of antimicrobials were classified as Multidrug Resistant (MDR) **(82).** The isolates with intermediate level of resistance were categorized as susceptible to avoid overestimation of resistance.

Detection of antimicrobial resistance genes (ARGs)

Campylobacter isolates were screened for the presence of five antimicrobial resistance genes coding resistance to the antimicrobials used. PCR was performed to detect the presence of β-lactam resistance coding *bla*OXA-61 gene**(83),** gentamicin resistance coding *aph*A-3-1 gene **(84),** tetracycline resistance coding *tet*(O) gene(**84**), macrolide resistance coding *erm*Bgene **(85))** and a multidrug resistance gene *cme*B coding for fluroquinolone and lincosamide antibiotics **(83).** PCR reaction and cycling conditions were used as described in respective references.

Detection of virulence genes

All *Campylobacter* isolates were screened for the presence of various virulence genes by PCR. Virulence genes screened were *Cad*F**(86),***fla*A and *Cia*B **(56)** , *cdtB*(**87**), *wlaN* **(88)** and *cgtB* **(89)** . PCR reaction and cycling conditions were used as described earlier in respective references.

Risk factor analysis

A questionnaire was prepared to study various risk factors associated with the *Campylobacter* prevalence in the poultry farms. All farm owners were requested to respond to the questionnaire (Table 1). However, no records were taken if an owner showed unwillingness to answer the questionnaire.

Statistical analysis

Univariate analysis was used to analyze differences in the proportion of *Campylobacter* in various poultry farms. The statistical significance level was defined as a two-tailed $p \leq 0.05$. All data analysis was carried out using Statistix7 software (Tallahassee, Florida, US).

Declarations

Ethics approval

The study was performed under the project on Zoonotic diseases.The project involves routine commection of probable samples for isolation and identification of food pathogens. The samples were analyzed using referred analytical methods. Therefore no informed consent was obtained.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its additional information files. Strains are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests.

Funding

The funds for the research work was provided by the Indian Council of Agricultural Research, New Delhi, India to carry out the research work under a project on “Outreach Programme on Zoonotic Diseases (F.No. 14(1)2009-ASR-IV).

Author’s contribution

GG collected the samples and analyzed the samples. M designed the study. DK performed analysis of the data and AKU provided help as and when required and edited the manuscript. All authors have read and approved the final manuscript.

Acknowledgements

The authors acknowledge the funds provided by the Indian Council of Agricultural Research, New Delhi, India to carry out the research work under a project on “Outreach Programme on Zoonotic Diseases (F.No. 14(1)2009-ASR-IV).

Authors information

All authors contributed equally to this work

Afiliations

Department of Veterinary Public Health and Epidemiology, College of Veterinary and Animal Sciences,GBPUAT,Pantnagar.Uttarakhand-263145,India.

References

1. Hugas M, Tsigarida E, Robinson T, Calistri P. The EFSA scientific panel on biological hazards first man date: may 2003–may 2006. In sight into food borne zoonoses. Trends Food Sci Technol. 2009;20:188–93.
2. EFSA.EU summary. report on trends and sources of zoonoses, Zoonotic agents and food-borne outbreaks in 2013. EFSAJ. 2015;13:3991.
3. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy S, Jeffery L, Jones JL. Griffin PM. Foodborne illness acquired in the United States -major pathogens. Emerg Infect Dis. 2011;17:7–15.
4. NCDC..Food-borne Diseases and Food Safety in India. 2017 – National Centre for Disease Control, New Delhi,India.
5. Volokhov D, Chizhikov V, Konstantin Chumakov K, Rasooly A. Microarray-Based Identification of Thermophilic Campylobacter jejuni, C. coli, C. lari, and C. Upsaliensis. Journal Of Clinical Microbiology. 2003;41(9):4071–80.
6. Vondrakova L, Pazlarova J, Demnerova K, Detection, identification and quantification of *Campylobacter jejuni*, *coli* and *lari* in food matrices all at once using multiplex qPCR. *Gut Pathog.*2014. *6*, 12.
7. Epps SVR, Harvey RB, Hume ME, Phillips TD, Anderson RC, Nisbet DJ. Foodborne *Campylobacter*: infections, metabolism, pathogenesis and reservoirs. Int J Environ Res Public Health. 2013;10:6292–304.
8. Agunos A, Waddell L, Leger D, Taboada E. A systematic review characterizing on-farm sources of *Campylobacter* spp. for broiler chickens. PLoS One. 2014;9:1–20.
9. Vaishnavi C, Singh M, Thakur JS, Thapa BR. Low Prevalence of campylobacteriosis in the Northern Region of India. Advances in Microbiology. 2015;5(03):155–65.
10. World Health Organization (WHO). The global view of campylobacteriosis, 2013. Vol. 57. Report of expert consultation, World Health Organization.
11. Lekshmi M, Ammini P, Kumar S, Varela MF. The Food Production Environment and the Development of Antimicrobial Resistance in Human Pathogens of Animal Origin. Microorganisms. 2017;5(1):11.
12. Osaili TM, Alaboudi AR. Antimicrobial Resistance of *Campylobacter* sp. Foodborne Pathogens and Antibiotic Resistance. 2017. pp: 417–429.

13. Chang Q, Wang W, Regev-Yochay G, Lipsitch M, Hanage WP. Antibiotics in agriculture and the risk to human health: how worried should we be? Evol Appl. 2015;8(3):240–7.
14. Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms & trends in human isolates. Emerg Infect Dis. 2001;7:24–34.
15. Isenbarger DW, Hoge CW, Srijan A. Comparative antibiotic resistance of diarrheal pathogens from Vietnam and Thailand, 1996–1999. Emerg Infect Dis. 2002;8(2):175–80.
16. Putnam SD, Frenck RW, Riddle MS. Antimicrobial susceptibility trends in *Campylobacter jejuni* and *Campylobacter coli* isolated from a rural Egyptian pediatric population with diarrhea. *Diagn. Microbiol. Infect. Dis*. 2003.47(4): 601–608.
17. Tjaniadi P, Lesmana M, Subekti D. Antimicrobial resistance of bacterial pathogens associated with diarrheal patients in Indonesia. Am J Trop Med Hyg. 2003;68(6):666–70.
18. Jain D, Sinha S, Prasad KN, Pandey CM. *Campylobacter* species and drug resistance in a north Indian rural community. Trans R Soc Trop Med Hyg. 2005;99(3):207–14.
19. Rajagunalan S, Isolation, PCR based identification and fla typing of thermophilic campylobacters. M.V.Sc. Thesis submitted to G.B.P.U.A & T, 2010. Pantnagar.
20. Rawat N, Maansi, Kumar D, Upadhyay AK. Virulence typing and antibiotic susceptibility profiling of thermophilic Campylobacters isolated from poultry, animal, and human species. Veterinary World. 2018;11(12):1698–705.
21. Monika J, Isolation. Epidemiology, Molecular Characterization and Antibiogram of *Campylobacter* from meat. M.V.Sc. Thesis submitted to G.B.P.U.A & T, 2014.Pantnagar.
22. IBEF. 2018. www.ibef.org.Uttarakhand.
23. Chokboonmongkol C, Patchanee P, Golz G, Zessin KH, Alter T. Prevalence, quantitative load, and antimicrobial resistance of Campylobacter spp. from broiler ceca and broiler skin samples in Thailand. Poult Sci. 2013;92(2):462–7.
24. Vinueza-Burgos C, Wautier M, Martiny D, Cisneros M, Damme IV, Zutter LD. Prevalence, antimicrobial resistance and genetic diversity of *Campylobacter coli* and *Campylobacter jejuni* in Ecuadorian broilers at slaughter age. Poultry Science. 2017;96(7):2366–74.
25. Sharma AK. (2004). Detection of zoonotic *Campylobacter* species in animals, human beings and foods. M.V.Sc thesis submitted to Indian Veterinary Research Institute.
26. Singh HD. 2006. Isolation and PCR-based detection of *Campylobacter jejuni* and *Campylobacter* fetus in man, animals and foods of animal origin. M.V.Sc thesis submitted to Indian Veterinary Research Institute.
27. Khurana RK, Kumar A. Examination of fresh meat and eggs for *Campylobacter jejuni* and *Campylobacter coli*. Indian J Comp Microbiol Immunol Infect Dis. 1996;17:41–4.
28. Vaz CSL, Voss-Rech D, Pozza JS, Coldebella A, Silva VS. Isolation of *Campylobacter* from Brazilian broiler flocks using different culturing procedures. Poult Sci. 2014;93(11):2887–92.

29. Ingresa CS, González BS, Jiménez TE, Marco JF, Catalá P, Vega S, Marin C. Comparison of different sampling types across the rearing period in broiler flocks for isolation of *Campylobacter* spp. Poultry Science. 2015;94(4):766–71.
30. Lisa MT, Walid QA, Kristen EG, Steven CR, Philip C, Divya J, Mark B, Mussie YH. Prevalence and concentration of Salmonella and *Campylobacter* in the processing environment of small-scale pastured broiler farms. Poult Sci. 2013;92(11):3060–6.
31. Hanning R, Jarquin M, Slavik. *Campylobacter jejuni* as a secondary colonizer of poultry biofilms. J Appl. Micrbiol. 2008.105(4).1199–1208.
32. 4;9:2092
10.3389/fmicb.2018.02092
PMID: 30233556; PMCID: PMC6131577.
Zhang X, Tang M, Zhou Q, Zhang J, Yang X, Gao Y. Prevalence and Characteristics of *Campylobacter* Throughout the Slaughter Process of Different Broiler Batches. Front Microbiol. 2018 Sep 4;9:2092. doi: 10.3389/fmicb.2018.02092. PMID: 30233556; PMCID: PMC6131577.
33. Oosterom J, Notermans S, Karman H, Engels GB. Origin and prevalence of *Campylobacter jejuni* in poultry processing. J Food Prot. 1983;46:339–44.
34. Berndtson E, Danielsson-Tham M, Engvall A. *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. International Journal of Food Microbiology. 1996;32(1–2):35–47.
35. Lawes JR, Vidal A, Clifton-Hadley FA, Sayers R, Rodgers J, Snow L, Evans SJ, Powell LF. Investigation of prevalence and risk factors for *Campylobacter* in broiler flocks at slaughter: results from a UK survey.Epidemiol Infect. 2012. 140: 1725–1737.
36. Perdoncini G, Sierra-Arguello YM, Lima LM, Trindade MM, Gomes MJ, Santos LR, Nascimento VP. Occurrence of *Campylobacter jejuni* and *C. coli* on broiler carcasses after chilling in southern Brazil. Pesquisa Veterinária Brasileira. 2015;35(4):349–52.
37. Vidal AB, Colles FM, Rodgers JD, Mccarthy ND, Davies RH, Maiden MC, Clifton-Hadley FA. Genetic Diversity of *Campylobacter* jejuni and *Campylobacter* coli Isolates from Conventional Broiler Flocks and the Impacts of Sampling Strategy and Laboratory Method. Appl Environ Microbiol. 2016;82(8):2347–55.
38. Chattopadhyay UK, Rashid M, Sur SK, Pal D. The occurrence of Campylobacteriosis in domestic animals and their handlers in and around Calcutta. J Med Microbiol. 2001;50:933–4.
39. Rajendran P, Babji S, George AT, Rajan DP, Kang G, Ajjampur SS. Detection and species identification of *Campylobacter* in stool samples of children and animals from Vellore, south India. Indian J Med Microbiol. 2012;30:85–8.
40. Pergola S, Franciosini M, Comitini F, Ciani M, Luca SD, Bellucci S, Proietti PC. Genetic diversity and antimicrobial resistance profiles of *Campylobacter coli* and *Campylobacter jejuni* isolated from broiler chicken in farms and at time of slaughter in central Italy. J Appl Microbiol. 2017;122(5):1348–56.

41. Wieczorek, K, Bocian, L. Osek, J..Prevalence and antimicrobial resistance of *Campylobacter* isolated from carcasses of chickens slaughtered in Poland – a retrospective study. Food Control. 2020, Volume 112, 107159.
42. Pillai RM. Studies on *Campylobacter jejuni* infection in chicken. 1986 Ph. D. Thesis submitted to University of Agri. Sc., Bangalore.
43. Oyarzabal OA, Hussain SK. Microbial Analytical Methodology for Processed Poultry Products. Handbook of Poultry Science Technology. 2009;2:527–44.
44. Acke E, McGill K, Golden O, Jones BR, Fanning S, Whyte P. Prevalence of thermophilic *Campylobacter* species in household cats and dogs in Ireland. Vet Rec. 2009;164:44–7.
45. Pant K, Isolation, Identification & Molecular Characterization of *Campylobacter jejuni* and *C. coli*. 2011. M.V.Sc. Thesis submitted to G.B.P.U.A & T, Pantnagar.
46. Kumar P. Isolation and molecular characterization of *C. jejuni* and *C. coli* from human and poultry caeca as well as meat. 2013. M.V.Sc. Thesis submitted to G.B.P.U.A & T, Pantnagar.
47. Pandey R. Biochemical and Molecular Characterization of Thermophilic campylobacters Isolated from Man and Farm Animals. 2015. M.V.Sc. Thesis submitted to G.B.P.U.A & T, Pantnagar.
48. Koolman L, Whyte P, Burgess C, Bolton D. Distribution of virulence-associated genes in a selection of *Campylobacter* isolates. Foodborne Pathog Dis. 2015;12(5):424–32.
49. Lapierre L., Gatica MA, Riquelme V, Vergara C, Yañez JM., San Martin B., Sáenz L, Vidal M, Martínez MC, Araya P, Flores R, Duery O, Vidal R. Characterization of antimicrobial susceptibility and its association with virulence genes related to adherence, invasion, and cytotoxicity in *Campylobacter jejuni* and *Campylobacter coli* isolates from animals, meat, and humans. Microbial Drug Resist. 2016.22, 432–444. 10.1089/mdr.2015.0055.
50. Wieczorek K, Wołkowicz T, Osek J. Antimicrobial Resistance and Virulence-Associated Traits of Campylobacter jejuni Isolated From Poultry Food Chain and Humans With Diarrhea. Front. Microbiol. 2018. 9:1508. doi: 10.3389/fmicb.2018.01508.
51. Guirado P, Paytubi S, Miró, Iglesias TY, Navarro F, Cerdà CM, Attolini CS, Balsalobre C, Madrid C. Differential Distribution of the wlaN and cgtB Genes, Associated with Guillain-Barré Syndrome, in Campylobacter jejuni Isolates from Humans, Broiler Chickens, and Wild Birds. Microorganisms. 2020;8:325. doi:10.3390/microorganisms8030325.
52. Poly F, Guerry P. Pathogenesis of Campylobacter. Curr Opin Gastroenterol. 2008 Jan;24(1):27–31.
53. Ziprin RL, Young CR, Byrd JA, Stanker LH, Hume ME, Gray SA, Kim BJ, Konkel ME. Role of Campylobacter jejuni potential virulence genes in cecal colonization. Avian Dis. 2001;45(3):549–57.
54. Hanning I, Biswas D, Herrera P, Roesler M, Ricke SC. Prevalence and characterization of Campylobacter jejuni isolated from pasture flock poultry. J Food Sci. 2010.75(7):M496-502.
55. Ma Y, Hanning I, Slavik M. Stress-induced adaptive tolerance response and virulence gene expression in Campylobacter jejuni. J Food Safety. 2009;29:126–43.

56. Datta S, Niwa H, Itoh K. Prevalence of 11 pathogenic genes of *Campylobacter* jejuni by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. *Journal of Medical Micrbiology.2003* 52(4): 345–348.
57. Talukder KA, Aslam M, Islam Z, Azmi IJ, Dutta DK, Hossain S, Nur-E-Kamal A, Nair GB, Cravioto A, Sack DA, Endtz HP. Prevalence of Virulence Genes and Cytolethal Distending Toxin Production in *C. jejuni* Isolates from Diarrheal Patients in Bangladesh. J Clin Microbiol. 2008;46(4):1485–8.
58. Kim JS, Lee MY, Kim S.J, Jeon SE. Cha I, Hong S, Chung GT, Huh MJ Kang YH, Yoo CK, Kim J. High-level ciprofloxacin-resistant *Campylobacter jejuni* isolates circulating in humans and animals in Incheon, Republic of Korea. Zoonozes Public Hea.lth..2016 63, 545–554.
59. Thakur S, Zhao S, McDermontt P, Harbottle H, Abbott J, English L, Gebreyes WA, White DG.. Antimicrobial resistance, virulence, and genotypic profile comparison of *Campylobacter jejuni* and *Campylobacter coli* isolated from humans and retail meats. Foodborne Pathog.2010. Dis. 7, 835–844. 10.1089/fpd.2009.0487.
60. Liu KC, Jinneman KC, Neal-McKinney J, Wu WH, Rice DH. Simultaneous identification of *Campylobacter jejuni, Campylobacter coli*, and *Campylobacter lari* with smart cycler-based multiplex quantitative polymerase chain reaction. Foodb Pathog Dis. 2017;14:371–8.
61. Melo RT, Mendonça EP, Valadares J, Edson C, Monteiro Guilherme P, Peres, Phelipe ABM, Rossi DA. Campylobacter jejuni and Campylobacter coli originated from chicken carcasses modulate their transcriptome to translate virulence genes in human cells. Pesquisa Veterinária Brasileira. 2019;39(8):592–9.
62. EFSA. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. EFSA J. 2014;12(2):1–312.
63. Stintzi A, Whitworth, L. Investigation of the *Campylobacter jejuni* cold-shock response by global transcript profiling. Genome Lett. 2003. 2:18–27.
64. Shaheen H. 2011. Molecular characterization of *C. jejuni* and *C. coli* isolated from humans, animals and poultry. M.V.Sc. Thesis submitted to G.B.P.U.A & T, Pantnagar.
65. Casabonne C, Gonzalez A, Aquili V, Subils T, Balague C. Prevalence of Seven Virulence Genes of *Campylobacter jejuni* Isolated from Patients with Diarrhea in Rosario, Argentina. *Int. J. Infect.* 2016. 3(4).
66. Wieczorek K, Osek J. Identification of virulence genes in *C. jejuni* and *C. coli* by PCR. Bull. Vet Inst Pulawy. 2008;52:211–6.
67. Narvaez B, Taboada CEN, Mutschall SK, Aslam M. Epidemiology of antimicrobial resistant *Campylobacter* spp. isolated from retail meats in Canada. Int J Food Microbiol. 2017;253:43–7.
68. Kashoma IP, Kassem II, John J, Kessy BM, Gebreyes W, Kazwala RR, Rajashekara G. Prevalence and Antimicrobial Resistance of *Campylobacter* Isolated from Dressed Beef Carcasses and Raw Milk in Tanzania. Microbial Drug Resistance. 2016;22(1):40–52.
69. Nesme J, Simonet P. The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. Environ Microbiol. 2014;17:13–30.

70. Hald B, Wedderkopp A, Madsen M. Thermophilic *Campylobacter* spp. in Danish broiler production: A cross-sectional survey and a retrospective analysis of risk factors for occurrence in broiler flocks. Avian Pathol. 2000;29(2):123–31.
71. Lyngstad TM, Jonsson ME, Hofshagen M, Heier BT. Risk Factors Associated with the Presence of *Campylobacter* Species in Norwegian Broiler Flocks. Poult Sci. 2008;87(10):1987–94.
72. Chowdhury S, Sandberg M, Themudo GE, Ersbøll AK. Risk factors for *Campylobacter* infection in Danish broiler chickens. PoultSci. 2012;91(10):2701–9.
73. Sandberg M, Sørensen LL, Steenberg B, Chowdhury S, Ersbøll AK, Alban L. Risk factors for *Campylobacter* colonization in Danish broiler flocks, 2010 to 2011. Poult Sci. 2015;94(3):447–53.
74. Hog,BB., Sommer HM, Larsen, LS, Sørensen, AIV, David,B, Hofshagen H, Rosenquist H.Farm specific risk factors for *Campylobacter* colonisation in Danish and Norwegian broilers. Preventive Veterinary Medicine. 2016. Vol.130. 137–145.
75. Cardinale E, Tall F, Guèye EF, Cisse M, Salvat G. Risk factors for *Campylobacter* spp. infection in Senegalese broiler-chicken flocks. Prev Vet Med. 2004. 64(1):15–25.
76. Thakur S, Brake J, Keelara S, Zou M, Susick E. Farm and environmental distribution of *Campylobacter* and *Salmonella* in broiler flocks. Research in Veterinary Science. 2013;94:33–42.
77. Corry JEL, Atabay HI, Forsythe SJ, Mansfield LP. Culture media for the isolation of campylobacters, helicobacter and arcobacters. *In*: Handbook of Culture Media for Food Microbiology, Second Edition, Corry J.E.L., Curtis G.D.W. & Baird R.M. eds. Elsevier, Amsterdam, The Netherlands. 2003. pp: 271–315.
78. OIE Terrestrial Manual. *C. jejuni* and *C. coli*. 2008pp: 1185–1191.
79. Linton D, Owen RJ, Stanley J. Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. Res Microbiol. 1996;147:707–18.
80. Klena JD, Parker CT, Knib BK, Ibbitt JC, Devane PML, Horn ST, Miller WG, Konkel ME. Differentiation of *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter upsaliensis* by a multiplex PCR developed from the nucleotide sequence of the lipid A gene *lpxA*. J Clin Microbiol. 2004;42:5549–57.
81. CLSI. CLSI Subcommittee on Antimicrobial Susceptibility Testing CLSI AST News Update. 2016. Volume 1, Issue 2.
82. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268–81. doi: 10.1111/j.1469-0691.2011.03570.x. Epub 2011 Jul 27.
83. Obeng AS, Rickard H, Sexton M, Pang Y, Peng H, Barton M. Antimicrobial susceptibilities and resistance genes in *Campylobacter* strains isolated from poultry and pigs in Australia. J Appl Microbiol. 2012;113:294–307.

84. Gibreel A, Tracz DM, Nonaka L, Ngo TM, Connell SR, Taylor DE. Incidence of antibiotic resistance in Campylobacter jejuni isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet(O)-mediated tetracycline resistance. Antimicrob Agents Chemother. 2004;48:3442–50.
85. Zhou J, Zhang M, Yang W, Fang Y, Wang G, Hou F. A seventeen-year observation of the antimicrobial susceptibility of clinical *Campylobacter* jejuni and the molecular mechanisms of erythromycin-resistant isolates in Beijing, China. Int J Infect Dis. 2016;42:28–33.
86. Konkel ME, Gray SA, Kim BJ, Garvis ST, Yoon J. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cad*F virulence gene and its products. *J. Clin. Microbiol*. 1999.37: 510–517.
87. Pickett CL, Pesci EC, Cottle DL, Russell G, Erdem AN, Zeytin H. Prevalence of cytolethal distending toxin production in *Campylobacter* jejuni and relatedness of *Campylobacter* spp. *cdt*B genes Infection Immunity. 1996;64:2070–8.
88. Muller J, Schulze F, Muller W, Hanel I. PCR detection of virulence associated genes in *Campylobacter jejuni* strains with differential ability to invade Caco-2 cells and to colonize the chick gut. Vet Microbiol. 2006;113(1–2):123–9.
89. Linton D, Gilbert M, Hitchen PG, Dell A, Morris HR, Wakarchuk WW. Phase variation of a beta-1,3 galactosyltransferase involved in generation of the ganglioside GM1-like lipo-oligosaccharide of *Campylobacter jejuni*. Mol Microbiol. 2000;37(3):501–14.

Tables

Table 1 : **Questionnaire for risk factor analysis for *Campylobacter* prevalence in poultry farms.**

Sl. No.	Questions	Response
1.	Other animals on farm	Yes/No
1.	How many times litter is reused?	Once/twice/more
1.	Use of foot bath at entrance site	Yes/No
1.	Feed	In house/Branded
1.	Chlorination for drinking water	Yes/No
1.	Distance of manure heap from farm	<200m/>200m
1.	Housing System	Free moving/Cage system
1.	Flock size	As informed
1.	Floor type	Cemented/Earthen
1.	Use of shoes by farm personnel	Yes/No
1.	Litter	Moist/Dry
1.	No. of broiler flocks	As informed

Table 2: Prevalence of *Campylobacter* from different source

Sample	Total samples	Isolates found	Prevalence (%)
Poultry faeces	346	63	18.2%
Feed	52	0	0.0%
Litter	51	1	1.96%
Water	50	3	6.0%
Manure	46	0	0.0%
Total	**545**	**67**	**12.29%**

Table 3: *Campylobacter* species distribution at different poultry farms

Farms	Location	Total samples	Isolates recovered (%)[a]	*C. jejuni*	*C. coli*
Haldwani farm	Haldwani	49	8 (16.3)	5 (62.5%)	3(37.5%)
Pantnagar farm 1	Pantnagar	45	11 (24.4)	2 (18.18%)	9 (81.81%)
Pantnagar farm 2	Pantnagar	60	15 (25.0)	1 (33.33%)	14 (66.67%)
Bazpur farm	Bazpur	70	22 (31.4)	2 (9.09%)	20 (90.9%)
Ramnagar farm	Ramnagar	71	0 (0)	0 (0%)	(0%)
Kiccha farm	Kiccha	80	0 (0)	0 (0%)	0 (0%)
Jawaharnagar farm	Jawaharnagar	90	5 (5.6)	2 (40%)	3 (60%)
Bindukhatta farm	Bindukhatta	80	6 (7.5)	4 (66%)	2 (33.33%)
Total	**-**	**545**	**67 (12.29)**	**16 (23.88 %)**	**51 (76.11 %)**

[a]Figure in parentheses indicates prevalence

Table 4: Species-wise distribution of various virulence genes

Species	*cadF*	*flaA*	*ciaB*	*cdtB*	*wlaN*	*cgtB*
C. jejuni(9)	9 (100)	9 (100)	6 (66.66)	8 (88.88)	0 (0)	3 (33.33)
C. coli(39)	39 (100)	39 (100)	0 (0)	39 (100)	0 (0)	8 (20.51)
Total	**48(100)**	**48(100)**	**6(12.5)**	**47(97.9)**	**0(0)**	**11(22.9)**

Table 5: Distribution of virulence genes in *Campylobacter* isolates (n=48) at different farms

Farm (no. of isolates)	Number of positive isolates (%)					
	cadF	*flaA*	*ciaB*	*cdtB*	*wlaN*	*cgtB*
Pantnagar farm 2 (15)	15(100)	15(100)	1(6.66)	15(100)	0(0)	1(6.6)
Bazpur farm (22)	22(100)	22(100)	2(9.09)	21(95.4)	0(0)	8(36.3)
Jawaharnagar farm (5)	5(100)	5(100)	1(20)	5(100)	0(0)	1(20)
Bindukhatta farm (6)	6(100)	6(100)	2(33.3)	6(100)	0(0)	1(16.6)
Total (48)	**48(100)**	**48(100)**	**6(12.5)**	**47(97.9)**	**0(0)**	**11(22.9)**

Table 6:

Distribution of resistant *Campylobacter* isolates in *C. jejuni* and *C. coli*

Antibiotics	Resistant isolates	*C. jejuni* (%)	*C. coli* (%)
Ampicillin (AMP)	12/42	4(33.33)	8(66.66)
Clindamycin (CD)	1/42	0(0)	1(100)
Ceftriaxone (CTR)	6/42	4(66.66)	2(33.33)
Cefoxitin (CX)	41/42	11(27.5)	30(73.17)
Levofloxacin (LE)	0/42	0(0)	0(0)
Ciprofloxacin (CIP)	27/42	7(25.92),	20(74.07)
Nalidixic acid (NA)	14/42	6(42.85)	8(57.14)
Erythromycin (E)	1/42	1(100)	0(0)
Tetracycline (TE)	2/42	2(100)	0(0)
Gentamicin (G)	0/42	0(0)	0(0)
Sulphafurazole (SF)	1/42	0(0)	1(100)

Table 7: Distribution of antimicrobial resistance patterns as per type of sample and farm location

Resistance Pattern (N)[a]	Samples			Farm/Location[b]					
	Poultry faeces	Water	Litter	H	P1	P2	BA	JW	BI
CX (6)	6	0	0	0	3	2	0	1	0
CX NA (2)	2	0	0	0	0	2	0	0	0
AMP CX (3)	3	0	0	1	0	0	0	1	1
CX CIP (11)	9	2	0	0	2	1	6	1	1
AMP SF NA (1)	1	0	0	0	0	1	0	0	0
CX CIP CD (1)	1	0	0	0	0	0	1	0	0
CX CTR NA (1)	1	0	0	0	1	0	0	0	0
CX CIP CTR (1)	1	0	0	0	0	0	0	0	1
AMP CX CIP (2)	2	0	0	0	0	0	1	0	1
CX CIP NA (6)	5	0	1	1	1	2	1	1	0
CX CIP CTR NA (1)	1	0	0	0	0	0	0	0	1
AMP CX CTR NA (1)	0	1	0	0	0	0	1	0	0
AMP CX CIP TE (2)	2	0	0	1	0	1	0	0	0
AMP CX CIP NA(1)	1	0	0	0	0	0	1	0	0
AMP CX E CIP CTR (1)	1	0	0	1	0	0	0	0	0
AMP CX CIP CTR NA (1)	1	0	0	0	0	0	1	0	0

[a]Resistance pattern (Number of isolates)

[b]H (Haldwani farm), P1(Pantnagar farm 1), P2 (Pantnagar farm 2), BA (Bazpur farm), JW (Jawaharnagar farm), BI (Bindukhatta farm).

Table 8: Antimicrobial resistance (AMR) as per phenotype and genotype of *Campylobacter* isolates

D	Resistance Pattern	Species	No. of antibiotics	No. of classes	Resistance genotype	Farm	Source
C1	CX	*C. coli*	1	1	-	Pantnagar 1	Poultry faeces
C2	CX CIP	*C. coli*	2	2	*cme*B	Bazpur	Poultry faeces
C3	AMP CX CIP TE	*C. jejuni*	4	4	*tet*(O)	Haldwani	Poultry faeces
C5	AMP CX E CIP CTR	*C. coli*	5	4	*bla*OXA-61, *cme*B	Haldwani	Poultry faeces
C6	CX CIP NA	*C. jejuni*	3	2	-	Haldwani	Poultry faeces
C7	AMP CX CIP TE	*C. coli*	4	4	*cme*B, *tet*(O)	Pantnagar 2	Poultry faeces
C8	CX CIP CTR NA	*C. jejuni*	4	2	*bla*OXA-61, *cme*B	Bindukhatta	Poultry faeces
C9	CX CIP	*C. coli*	2	2	-	Pantnagar 2	Poultry faeces
10	AMP CX CIP CTR NA	*C. coli*	5	3	*cme*B	Bazpur	Poultry faeces
11	CX CIP NA	*C. coli*	3	2	*cme*B	Pantnagar 1	Poultry faeces
12	CX CIP	*C. coli*	2	2	-	Pantnagar 1	Water
13	AMP CX	*C. coli*	2	2	-	Jawaharnagar	Poultry faeces
14	CX CIP NA	*C. coli*	3	2	-	Pantnagar 2	Poultry faeces
15	CX CIP NA	*C. coli*	3	2	*cme*B	Pantnagar 2	Poultry faeces
16	CX CIP NA	*C. coli*	3	2	*cme*B	Bazpur	Litter
17	CX CIP	*C. coli*	2	2	*bla*OXA-61, *cme* B	Bazpur	Poultry faeces
18	CX CIP	*C. coli*	2	2	*bla*OXA-61, *cme* B	Bazpur	Poultry faeces
19	CXCIP	*C. coli*	2	2	-	Pantnagar 1	Poultry faeces
20	CX CIP	*C. coli*	2	2	*cme* B	Bazpur	Water
21	AMP SF NA	*C. coli*	3	3	*bla*OXA-61	Pantnagar 2	Poultry faeces
22	CX NA	*C. coli*	2	2	*bla*OXA-61, *cme* B	Pantnagar 2	Poultry faeces
23	AMP CX CIP	*C. coli*	3	3	*bla*OXA-61, *cme* B	Bazpur	Poultry faeces

24	CX	*C. coli*	1	1	*bla*OXA-61	Pantnagar 1	Poultry faeces
25	AMP CX CIP	*C. coli*	3	3	*bla*OXA-61	Bindukhatta	Poultry faeces
26	CX CIP CD	*C. coli*	3	3	*bla*OXA-61, *cme*B	Bazpur	Poultry faeces
27	CX CTR NA	*C. jejuni*	3	2	*bla*OXA-61	Pantnagar 1	Poultry faeces
28	CX	*C. jejuni*	1	1	-	Pantnagar 1	Poultry faeces
29	CX CIP	*C. jejuni*	2	2	-	Bazpur	Poultry faeces
30	CX CIP NA	*C. jejuni*	3	2	*bla*OXA-61, *cme* B	Jawaharnagar	Poultry faeces
31	CX	*C. coli*	1	1	-	Pantnagar 2	Poultry faeces
32	CX CIP	*C. coli*	2	2	*bla*OXA-61, *cme*B	Bazpur	Poultry faeces
33	CX	*C. jejuni*	1	1	-	Pantnagar 2	Poultry faeces
34	AMP CX CTR NA	*C. jejuni*	4	3	*cme*B	Bazpur	Water
35	AMP CX CIP NA	*C. coli*	4	3	*bla*OXA-61, *cme*B	Bazpur	Poultry faeces
36	CX NA	*C. coli*	2	2	*cme*B	Pantnagar 2	Poultry faeces
37	CX CIP	*C. coli*	2	2	*bla*OXA-61, *cme* B	Jawaharnagar	Poultry faeces
38	CX	*C. coli*	1	1	*bla*OXA-61	Jawaharnagar	Poultry faeces
39	CX CIP CTR	*C. jejuni*	3	2	-	Bindukhatta	Poultry faeces
40	CX CIP	*C. coli*	2	2	*bla*OXA-61	Bindukhatta	Poultry faeces
41	AMP CX	*C. coli*	2	2	*bla*OXA-61	Bindukhatta	Poultry faeces
42	AMP CX	*C. coli*	2	2	-	Haldwani	Poultry faeces