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Prevalence and Antimicrobial Resistance In Campylobacters of Kumaun of Uttarakhand

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ABSTRACT

A total of 730 samples comprising poultry caeca (210), chicken meat (111) and poultry droppings (180) along with faecal contents from goat (26), sheep (23), pigs (78), calves (20) and human (82) were collected from different places of Kumaun. Of these 39 (5.34%) were found positive for *Campylobacter*. The highest prevalence was in poultry caeca (7.62%) followed by poultry droppings (7.22%), calves faeces (5%), humans stools (3.66%), chicken meat (3.60%) and pigs faeces (2.56%). Any *Campylobacter* could not be recovered in samples collected from goat and sheep. Out of 39 pure cultures isolated, 19 were identified as *C. jejuni* and 17 as *C. coli*, whereas, 3 *C. jejuni* cultures negative for hippurate hydrolysis test. All the *C. jejuni* isolates (100%) were found to be sensitive to Amoxiclav, while 93.75% showed resistance towards Penicillin G. Among 7 *Campylobacter coli* isolates, 5 (71.43%) were sensitivity to Amoxyclav and Ampicillin. Only Penicillin G was found to be resistant in 5 (71.43%) of the *C. coli* isolates.

Keywords: Campylobacter, Prevalence, Antibiotic resistance.

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INTRODUCTION

Campylobacter cause serious complications related to acute bacterial enteric diseases in humans and animals of entire globe. The most significant Campylobacter species associated with human as well as food animals are *campylobacter jejuni* and *Campylobacter coli* (Wesley *et al.,* 2000). Worries regarding the prevalence of campylobacteriosis have increased many folds because of the frequent isolation of antimicrobial-resistant strains from humans and their foods. High prevalence and increasing resistance to antimicrobial drugs has been documented in human and animal strains of Campylobacter (Padungton and Kaneene, 2003). This is particularly annoying with fluoroquinolone and macrolide because these molecules are being used for treatment of human campylobacteriosis (Skirrow and Blaser, 2000). Therefore, present investigation was intended to know the latest disease and antimicrobial resistance status of bacteria.

MATERIALS AND METHOD

From various parts of Kumaun region of Uttarakhand 730 samples comprising poultry caeca (210), chicken meat (111) and poultry droppings (180) along with faecal contents from goat (26), sheep (23), pigs (78), calves (20) and human (82) were collected for the isolation of Campylobacter spp. The isolation and identification of Campylobacter spp. was carried out as per the procedures outlined by OIE terrestrial manual (2008) with necessary modification. Morphological, biochemical, serological and molecular characterization of the Campylobacter genus was done (Upadhyay *et al*., 2016). All the samples were screened for the presence of different species of thermophilic *Campylobacter.* Out of isolated, 23 isolates could be revived to study the antibiotic sensitivity as described by Upadhyay *et al.* (2016)).

RESULTS AND DISCUSSION

In the present study, the overall prevalence of *Campylobacter* was recorded as 5.34% with highest in poultry caeca (7.62%) followed by poultry droppings (7.22%), calves faeces (5%), humans stools (3.66%), chicken meat (3.60%) and pigs faeces (2.56%) **(Table 1)** disagreeing Rajkumar *et al*., (2010) observation of 18% from unorganized and 12% from organized observation farms in Uttar Pradesh. However, *Campylobacter* could not be recovered in any of the sample collected from goat and sheep in accordance with Wieczorek *et al*., (2012).

Table 1: Prevalence of *Campylobacter* in different species

Sl. No.	Sample source	Total no. of samples	Positive samples
1	Poultry caeca	210	16 (7.62%)
2	Chicken meat	111	4 (3.60%)
3	Poultry droppings	180	13 (7.22%)
4	Pigs faeces	78	2 (2.56%)
5	Sheep and goats	49	0 (0%)
6	Calves faeces	20	1 (5%)
7	Human stools	82	3 (3.66%)
	Total	**730**	**39 (5.34%)**

* Figures in parenthesis are respective percentage prevalence

Species wise prevalence of *Campylobacter* isolates

Two species were identified namely *Campylobacter jejuni* and *Campylobacter coli*. The occurrence of *Campylobacter jejuni* was recorded higher than that of *Campylobacter coli* (Joshi, 2014). Out of 39 *Campylobacter* recovered from various sources, the poultry caeca had the highest number of recoveries followed by poultry droppings, and chicken meat **(Table 2).**

Table 2: Species distribution of *Campylobacter* in different host species

Sl. No.	Sample source	No. of positive samples	*C. jejuni*	*C. coli*
1	Poultry caeca	16 (7.62%)	7(43.75%)	9(56.25%)
2	Chicken meat	4 (3.60%)	3 (75%)	1(25%)
3	Poultry droppings	13 (7.22%)	7(53.85%)	6(46.13%)
4	Pigs faeces	2 (2.56%)	2 (100%)	0 (0%)
5	Sheep and goats	0 (0%)	0 (0%)	0 (0%)
6	Calves faeces	1 (5%)	0 (0%)	1 (100%)
7	Human stools	3 (3.66%)	2(66.67%)	1(33.33%)
Total		**39 (5.34%)**	**21(53.85%)**	**18(46.15%)**

Detection and distribution of virulent genes

Virulence gene PCRs confirmed presence of *wlaN* (672bp), *iam* (518bp), *ciaB* (986bp) and *dnaJ* (720bp) genes in 7 (17.95%), 18 (46.15%), 21 (53.84%) and 29 (74.36%) isolates, respectively. Isolates from poultry caeca and droppings showed the presence of all four genes, while isolates of chicken meat harboured *iam* and *cia*B genes. Moreover, *dna*J gene which was most prominent in isolates of poultry caeca and droppings was absent in the isolates recovered from chicken meat. Virulent gene *wla*N was detected in poultry caeca, poultry droppings and human stool. All isolates from pig feces revealed only *dna*J gene. The *iam* gene was detected in only one isolate of calf faeces. Out of 3 isolates from humans, *wla*N and *dna*J could be observed only in two samples. The third isolate did not reveal any virulent gene. Virulent genes *iam* and *cia*B were absent in all isolates (Table 3, Figure 1 to 4).

Table 3: Distribution of virulent genes among the *Campylobacter* isolates

Source	Species	Total No. of isolates	Virulent genes detected in *Campylobacter* species *wlaN*	*iam*	*ciaB*	*dnaJ*
Poultry caeca	*C. jejuni*	7	3	2	5	7
	C. coli	9	1	9	6	8
Chicken meat	*C. jejuni*	3	-	1	2	-
	C. coli	1	-	1	-	-
Poultry droppings	*C. jejuni*	7	2	1	5	5
	C. coli	6	-	3	3	6
Pig faeces	*C. jejuni*	2	-	-	-	2
Calves faeces	*C. coli*	1	-	1	-	-
Human stools	*C. jejuni*	2	1	-	-	1
	C. coli	1	-	-	-	-

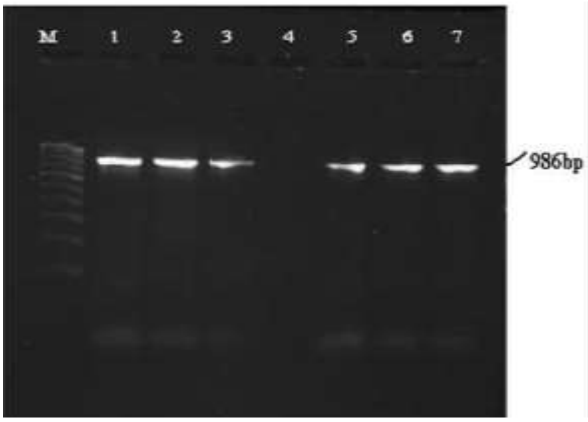

Figure 1: PCR for the detection of *cia*B gene (986bp) in *Campylobacter* isolates.

Lane M: 100bp DNA ladder;Lane 1-3 and 5-7: *ciaB* positive isolates; Lane 4: negative control.

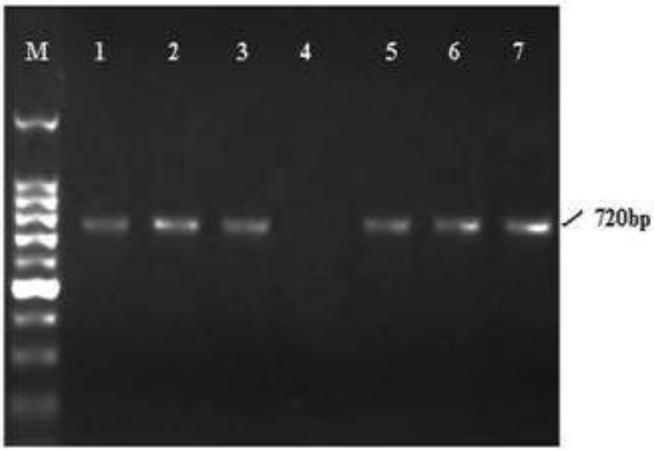

Figure 2: PCR for the detection of *dnaJ* (720bp) gene in *Campylobacter* isolates.

Lane M: 100 bp DNA ladder; Lane 1-3 and 5-7: *dnaJ* positive isolates; Lane 4: negative control.

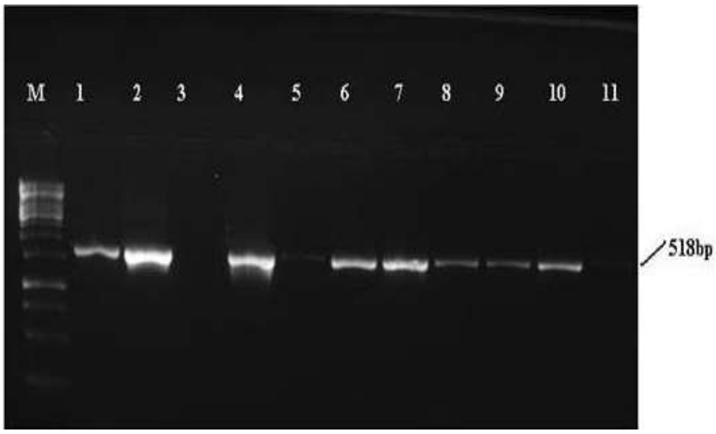

Figure 3: PCR for the detection of *iam* (518bp) gene in *Campylobacter* isolates.

Lane M: 100 bp DNA ladder; Lane 1-2 and 4-11: *iam* positive isolates; Lane 3: negative control.

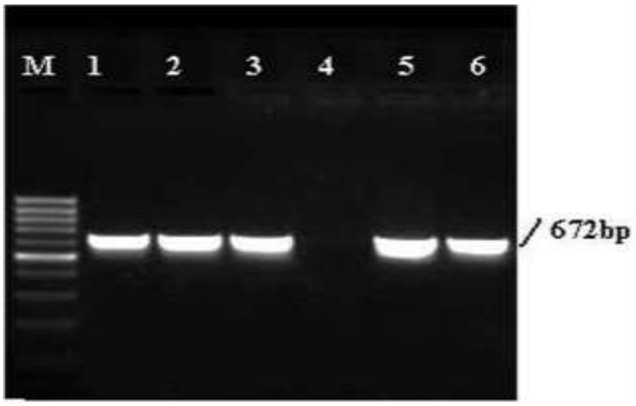

Figure 4: PCR for the detection of *wlaN* (672bp) gene in *Campylobacter* isolates

Lane M: 100 bp DNA ladder; Lane 1-3 and 5-6: *wlaN* positive isolates; Lane 4: Negative control

Antibiotic sensitivity pattern of *Campylobacter* isolates

Out of 39 thermophilic *Campylobacter* species, only 23 isolates could be revived to study the antibiotic sensitivity pattern. All the *C. jejuni* isolates (100%) were found to be sensitive to Amoxiclav, while 93.75% of them showed resistance towards Penicillin G agreeing with our findings of Rajagunalan (2010). Most of the *C. jejuni* isolates (62-87%) were intermediately sensitive against Ciprofloxacin, Chloramphenicol, Amoxacillin and Cephalexin. Among the 7 *Campylobacter coli* isolates, 5 (71.43%) of them showed sensitivity towards Amoxyclav and Ampicillin in alliance with the finding by Rahimi *et al*., (2011). Most of the isolates were intermediately sensitive to all the antibiotics. Only Penicillin G was found to be resistant in 5 (71.43%) of the *C. coli* isolates **(Table 4)**. In the present study *C. coli* were found to be more resistant than *C. jejuni* to most of the drugs in conformity with the findings of Adzitey *et al.*, (2012).

Table 4: Antibiotic sensitivity pattern of *Campylobacter* isolates

S. No	Antibiotics	*Campylobacter*spp.	No. of Isolates		
			Resistant	**Intermediate**	**Sensitive**
1.	Chloramphenicol	*C. jejuni*	0	11 (68.75%)	5 (31.25%)
		C. coli	2 (28.57%)	5 (71.43%)	0
2.	Ciprofloxacin	*C. jejuni*	2 (12.5%)	10 (62.5%)	4 (25%)
		C. coli	3 (42.86%)	4 (57.14%)	0
3.	Gentamicin	*C. jejuni*	0	2 (12.5%)	14 (87.5%)
		C. coli	0	5 (71.43%)	2 (28.57%)
4.	Amoxacillin	*C. jejuni*	0	14 (87.5%)	2 (12.5%)
		C. coli	0	4 (57.14%)	3 (42.86%)
5.	Cephalexin	*C. jejuni*	1 (6.25%)	12 (75%)	3 (18.75%)
		C. coli	2 (28.57%)	5 (71.43%)	0
6.	Levofloxacin	*C. jejuni*	1 (6.25%)	2 (12.5%)	13 (81.25%)
		C. coli	0	3 (42.86%)	4 (57.14%)
7.	Kanamycin	*C. jejuni*	1 (6.25%)	1 (6.25%)	14 (87.5%)
		C. coli	0	3 (42.86%)	4 (57.14%)
8.	Penicillin G	*C. jejuni*	15 (93.75%)	1 (6.25%)	0
		C. coli	5 (71.43%)	2 (28.57%)	0
9.	Amoxyclav	*C. jejuni*	0	0	16 (100%)
		C. coli	0	2 (28.57%)	5 (71.43%)
10.	Ampicillin	*C. jejuni*	0	5 (31.25%)	11 (68.75%)
		C. coli	0	2 (28.57%)	5 (71.43%)

CONCLUSION

Out of 730 samples collected, overall prevalence of *Campylobacter* estimeted to be 5.34% with highest in poultry caeca (7.62%) trailing poultry droppings (7.22%), calves faeces (5%), humans stools (3.66%), chicken meat (3.60%) and pigs faeces (2.56%). Samples collected from goat and sheep exhibited absence of bacteria. Out of 39 isolated Campylobacters, 19 were identified as *C. jejuni* and 17 as *C. coli*, while, 3 *C. jejuni* cultures could not respond to hippurate hydrolysis test. *C. jejuni* isolates were found to be 100% sensitive to Amoxiclav, while 93.75% of them showed resistance towards Penicillin G and of 7 *Campylobacter coli* isolates, 5 (71.43%) showed sensitivity towards Amoxyclav and Ampicillin.

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