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ANTIBIOGRAM OF *Campylobacter jejuni* AND *Campylobacter coli* ISOLATED FROM ANIMALS, FOODS OF ANIMAL ORIGIN AND HUMANS IN AREAS SURROUNDING GANNAVARAM, ANDHRA PRADESH

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Abstract: Food-borne *Campylobacter* spp. mainly *Campylobacter jejuni* and *Campylobacter coli* serve as major threat to developed and developing countries and ranks first among bacterial causes of gastroenteritis. A total of 25 *Campylobacter* isolates (6 *C. jejuni* and 19 *C. coli*) isolated from diverse sources were subjected to antibiotic susceptibility testing against 18 select antibiotics using disc diffusion method. A major fraction of the *Campylobacter* isolates showed sensitivity to colistin and co-trimoxazole (64%), chloramphenicol and azithromycin (56%) and ceftazidime (52%). All the *Campylobacter* isolates were resistant to at least one of the antibiotics tested. Higher resistance was observed for vancomycin and tetracycline (76%), cephalothin and ciprofloxacin (68%), erythromycin (56%) and doxycycline (52%). Notable percentages of isolates were intermediately resistant against nalidixic acid and nitrofurantoin (44%), gentamicin and streptomycin (32%) and clindamycin (28%). Resistance towards three or more classes were considered as Multi-Drug Resistant organisms. All 6 *C. jejuni* isolates and 17 out of 19 *C. coli* isolates were found to be Multi Drug Resistant. Multiple Antibiotic Resistance (MAR) index was calculated for all 25 isolates. Overall 84% (21/25) of the isolates had MAR index values above 0.2 which indicates that those strains were exposed to environments with high antibiotic usage. Hence the present finding indicates high levels of antibiotic resistance among *Campylobacter* isolates which bears public health significance.

Keywords: *Campylobacter jejuni, Campylobacter coli,* Multidrug Resistance, Multiple Antibiotic.

INTRODUCTION

Antimicrobial resistance in bacteria originating from foods of animal origin has become a major public health concern in both developed and developing countries. Food-borne disease transmission was estimated to contribute for 58% of global disease burden. The emergence of anti-microbial resistance is said to be hastened by globalisation of food system

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and increased trans-boundary transit of humans, animals, plants and associated products (Vemula *et al.,* 2012). *Salmonella* and *Campylobacter* cause an estimated 4,10,000 antibiotic-resistant infections in the United States every year (CDC, 2013). The reports of multiple drug resistant (MDR) *Campylobacter* infections have been increasing over the last few years and recently an extreme drug resistant strain was reported in Japan (Shin *et al*., 2015). Campylobacteriosis usually manifests as a self-limiting acute illness in humans but antimicrobial therapy is required in cases of severe and prolonged infection (Ge *et al.*, 2003). *Campylobacter* organisms are naturally susceptible to several classes such as macrolides, tetracyclines, aminoglycosides, ntirofurans and clindamycin (Walder, 1979). Therefore, erythromycins and fluoroquinolones were the drug of choice to treat acute illness (Engberg *et al.*, 2001). Eventually their indiscriminate use in poultry sector lead to emergence of resistant strains all over the world. (Sanad *et al.*, 2011). Over the years, increasing numbers of *Campylobacter* isolates have developed resistance to fluoroquinolones, macrolides, aminoglycosides and betalactams (Wieczorek and Osek, 2013).

MATERIALS AND METHODS

Reference strains

The American Type Culture Collection (ATCC) strains of *Campylobacter jejuni* (ATCC 29428) and *Campylobacter coli* (ATCC 33559) were used as reference strains for this study.

Bacterial isolates

A total of 25 *Campylobacter* isolates were recovered from samples of various sources including faecal swabs of animals (8), intestinal contents of slaughtered animals (12), foods of animal origin (4) and diarrhoeic human samples (1). The phenotypic characterization of the isolates were done by macroscopic, microscopic and biochemical methods. Macroscopically colonies were identified based on their colony morphology i.e. grey/white or creamy grey non-pigmented, moist colonies on modified Charcoal Cefoperazone Deoxycholate (mCCDA) agar and non-hemolytic, glistening, translucent and round colonies with irregular edge on 5% Blood agar. Microscopically, staining properties were used for identification. All the presumptive *Campylobacter* isolates were Gram-negative, curved slender rod shaped organisms, motile with corkscrew like movement. Biochemical characterization was done using catalase, oxidase and hippurate hydrolysis (Vandamme *et al.,* 2005). All the isolates were catalase and oxidase positive. 6 isolates were hippurate hydrolysis positive and 19 were negative. Further genotypic identification was done using a

multiplex PCR (m-PCR) which targeted 16S rRNA, *map* gene and *CeuE* gene specific for *Campylobacter* genus, *C. jejuni* and *C. coli*, respectively (Denis *et al.,* 2001).

Antibiotic susceptibility testing

Kirby Bauer disc diffusion method (Bauer *et al.,* 1966) was followed to test the susceptibility of all the 25 isolates towards 18 antibiotics belonging to different classes of antibiotics. The antibiotics used were Ampicillin (10µg), Azithromycin (30µg), Ceftazidime (30µg), Cephalothin (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Clindamycin (2µg), Colistin (10µg), Co-trimoxazole (1.25/23.75µg), Doxycycline (30µg), Erythromycin (15µg), Gentamicin (10µg), Nalidixic acid (30µg), Nitrofurantoin (300µg), Norfloxacin (5µg), Vancomycin (30µg), Streptomycin (10µg) and Tetracycline (30µg). Initially *Campylobacter* isolates were sub-cultured on blood agar plates and incubated for 48 h under micro-aerophilic conditions at 42°C. Direct colony suspension of each isolate was made in PBS (pH 7.4) and the concentration was adjusted using 0.5 McFarland standard so as to have an approximate cell density of 1.5 x 10~8 CFU/ml which yields having absorbance of 0.132 at wavelength of 600 nm. About 200 µl of each inoculum was seeded on the Mueller Hinton (MH) agar supplemented with 5% defibrinized sheep blood using sterile cotton-tipped swab. The plates were incubated at 42°C for 24-48 h under micro-aerophilic conditions and the diameter of the inhibition zones was measured to determine antibiotic susceptibility patterns. Since break points of antimicrobial susceptibility are limited only to few antibiotics for the species of *Campylobacter* (El-baky *et al.*, 2014), the zone diameter interpretative breakpoints for *Enterobacteriaceae* as given in Clinical and Laboratory Standards Institute guidelines (CLSI, 2018) were taken as standards except for few antibiotics for which EUCAST breakeven points exclusive for *Campylobacter* spp. are available (EUCAST, 2012). Organisms showing resistance to three or more antimicrobial classes were considered as MDR. Multiple Antibiotic Resistance (MAR) index values were also calculated for all the isolates as suggested by Kruperman (1983).

RESULTS AND DISCUSSION

Antimicrobial resistance is one of emerging health issues globally and requires a "One Health" approach (Kahn, 2017). In this context, in vitro antibiotic sensitivity test was performed for a total of 25 *Campylobacter* strains isolated from diverse sources in and areas of Gannavaram, Andhra Pradesh. It was found that all the *Campylobacter* isolates were resistant to at least one of the eighteen antibiotics tested. A major fraction of the *Campylobacter* isolates showed sensitivity to colistin and co-trimoxazole (64%),

chloramphenicol and azithromycin (56%) and ceftazidime (52%). All the *Campylobacter* isolates were resistant to at least one of the antibiotics tested. Higher resistance was observed for vancomycin and tetracycline (76%), cephalothin and ciprofloxacin (68%), erythromycin (56%) and doxycycline (52%). Notable percentages of isolates were intermediately resistant against nalidixic acid and nitrofurantoin (44%), gentamicin and streptomycin (32%) and clindamycin (28%) as shown in Table 1. The species-wise and source-wise details of antibiotic resistance patterns were presented in Tables 2 and 3.

Table-1: Antibiotic sensitivity/resistance patterns of *Campylobacter* isolates

Antimicrobial agent (dose)	Pattern of antibiogram					
	Sensitive		Intermediate		Resistant	
	No	%	No	%	No	%
Colistin (10µg)	16/25	64%	7/25	28%	2/25	8%
Co-trimoxazole (25µg)	16/25	64%	5/25	20%	4/25	16%
Clindamycin (2µg)	12/25	48%	7/25	28%	6/25	24%
Chloramphenicol (30µg)	14/25	56%	4/25	16%	7/25	28%
Ceftazidime (30µg)	13/25	52%	5/25	20%	7/25	28%
Ampicillin (10 µg)	11/25	44%	5/25	20%	9/25	36%
Nitrofurantoin (300µg)	5/25	20%	11/25	44%	9/25	36%
Azithromycin (30µg)	14/25	56%	-	-	11/25	44%
Nalidixic acid (30µg)	2/25	8%	11/25	44%	12/25	48%
Doxycycline (30µg)	8/25	32%	4/25	16%	13/25	52%
Gentamicin (10µg)	4/25	16%	8/25	32%	13/25	52%
Streptomycin (10µg)	4/25	16%	8/25	32%	13/25	52%
Erythromycin (15µg)	11/25	44%	-	-	14/25	56%
Norfloxacin (5µg)	7/25	28%	3/25	12%	15/25	60%
Ciprofloxacin (5µg)	8/25	32%	-	-	17/25	68%
Cephalothin (30µg)	8/25	32%	-	-	17/25	68%
Tetracycline (30µg)	6/25	24%	-	-	19/25	76%
Vancomycin (30µg)	5/25	20%	1/25	4%	19/25	76%

Table-2: Antibiotic resistance among *Campylobacter* isolates (species-wise)

Source		Resistant strains / No. of strains examined																
		CL	**COT**	**CD**	**C**	**CAZ**	**AMP**	**NIT**	**AZM**	**NA**	**DO**	**GEN**	**S**	**E**	**NX**	**CIP**	**CEP**	**TE**	**VA**
1	***C. jejuni***																		
	PC (2)	0/2	0/2	1/2	0/2	0/2	1/2	1/2	1/2	2/2	1/2	2/2	1/2	1/2	1/2	2/2	1/2	1/2	1/2
	PI (2)	0/2	0/2	1/2	0/2	1/2	0/2	0/2	2/2	2/2	1/2	1/2	0/2	1/2	1/2	2/2	1/2	2/2	2/2
	CM (1)	0/1	1/1	0/1	1/1	1/1	1/1	0/1	1/1	0/1	0/1	0/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
	HD (1)	0/1	0/1	0/1	1/1	0/1	0/1	1/1	1/1	0/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1
	TOTAL (6)	**0/6** (0%)	**1/6** (16.7%)	**2/6** (33.4%)	**2/6** (33.4%)	**2/6** (33.4%)	**2/6** (33.4%)	**2/6** (33.4%)	**4/6** (66.7%)	**4/6** (66.7%)	**3/6** (50%)	**4/6** (66.7%)	**3/6** (50%)	**3/6** (50%)	**3/6** (50%)	**6/6** (100%)	**4/6** (66.7%)	**5/6** (83.3%)	**5/6** (83.3%)
2	***C. coli***																		
	PC (1)	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1	1/1	1/1	1/1	0/1	1/1	1/1	0/1
	SF (5)	1/5	0/5	2/5	2/5	1/5	1/5	1/5	2/5	2/5	1/5	2/5	1/5	2/5	2/5	2/5	3/5	5/5	4/5
	PI (6)	0/6	1/6	0/6	2/6	1/6	3/6	3/6	1/6	2/6	4/6	3/6	3/6	4/6	5/6	3/6	3/6	4/6	5/6
	SI (4)	1/4	0/4	1/4	1/4	1/4	1/4	2/4	1/4	3/4	2/4	2/4	3/4	3/4	2/4	3/4	3/4	3/4	3/4
	PK (3)	0/3	2/3	1/3	0/3	2/3	2/3	1/3	2/3	0/3	3/3	2/3	2/3	1/3	2/3	3/3	3/3	1/3	2/3
	TOTAL (19)	**2/19** (10.5%)	**3/19** (15.8%)	**4/19** (21.1%)	**5/19** (26.3%)	**5/19** (26.3%)	**7/19** (36.8%)	**7/19** (36.8%)	**6/19** (31.6%)	**8/19** (42.1%)	**10/19** (52.6%)	**9/19** (47.4%)	**10/19** (52.6%)	**11/19** (57.9%)	**12/19** (63.1%)	**11/19** (57.9%)	**13/19** (68.4%)	**14/19** (73.7%)	**14/19** (73.7%)
	GRAND TOTAL (25)	**2/25** (8%)	**4/25** (16%)	**6/25** (24%)	**7/25** (28%)	**7/25** (28%)	**9/25** (36%)	**9/25** (36%)	**10/25** (40%)	**12/25** (48%)	**12/25** (48%)	**13/25** (52%)	**13/25** (52%)	**14/25** (56%)	**15/25** (60%)	**17/25** (68%)	**17/25** (68%)	**19/25** (76%)	**19/25** (76%)

PC-poultry faeces, **PI**-poultry intestines, **CM**- chicken, **HD**-human diarrhoeic samples, **SF**-pig faeces, **SI**-pig intestine, **PK**-pork; **CL-**colistin, **COT**-co-trimoxazole, **CD**-clindamycin, **C**-chloramphenicol, **CAZ**-ceftazidime, **AMP**-ampicillin, **NIT**-nitrofurantoin, **AZM**-azithromycin, **NA**-nalidixic acid, **DO**-doxycycline, **GEN**-gentamicin, **S**-streptomycin, **E-**erythromycin, **NX**-norfloxacin, **CIP**-ciprofloxacin, **CEP**-cephalothin, **TE**-tetracycline, **VA**-vancomycin

Table-3: Antibiotic resistance among *Campylobacter* isolates (source-wise)

Source		Resistant strains / No. of strains examined																
		CL	**COT**	**CD**	**C**	**CAZ**	**AMP**	**NIT**	**AZM**	**NA**	**DO**	**GEN**	**S**	**E**	**NX**	**CIP**	**CEP**	**TE**	**VA**
1	**FAECAL SWABS**																		
	PC (3)	0/3	0/3	1/3	0/3	0/3	1/3	1/3	1/3	3/3	1/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	1/3
	SF (5)	1/5	0/5	2/5	2/5	1/5	1/5	1/5	2/5	2/5	1/5	2/5	1/5	2/5	2/5	3/5	3/5	5/5	4/5
	TOTAL (8)	**1/8** (12.5%)	**0/8** (0%)	**3/8** (37.5%)	**2/8** (25.0%)	**1/8** (12.5%)	**2/8** (25.0%)	**2/8** (25.0%)	**3/8** (37.5%)	**5/8** (62.5%)	**2/8** (25.0%)	**4/8** (50.0%)	**3/8** (37.5%)	**4/8** (50%)	**4/8** (50%)	**5/8** (62.5%)	**5/8** (62.5%)	**7/8** (87.5%)	**5/8** (62.5%)
2	**INTESTINAL CONTENTS**																		
	PI (8)	0/8	1/8	1/8	2/8	2/8	3/8	3/8	2/8	4/8	5/8	4/8	3/8	5/8	6/8	4/8	4/8	6/8	7/8
	SI (4)	1/4	0/4	1/4	1/4	1/4	1/4	2/4	1/4	3/4	2/4	2/4	3/4	3/4	2/4	3/4	3/4	3/4	3/4
	TOTAL (12)	**1/12** (8.3%)	**1/12** (8.3%)	**2/12** (16.7%)	**3/12** (25.0%)	**3/12** (25.0%)	**4/12** (33.3%)	**5/12** (41.7%)	**3/12** (25.0%)	**7/12** (58.3%)	**7/12** (58.3%)	**6/12** (50.0%)	**6/12** (50.0%)	**8/12** (66.7%)	**8/12** (66.7%)	**7/12** (58.3%)	**7/12** (58.3%)	**9/12** (75.0%)	**10/12** (83.3%)
3	**FOODS OF ANIMAL ORIGIN**																		
	CM (1)	0/1	1/1	0/1	1/1	1/1	1/1	0/1	1/1	0/1	0/1	0/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1
	PK (3)	0/3	2/3	1/3	0/3	2/3	2/3	1/3	2/3	0/3	3/3	2/3	2/3	1/3	2/3	3/3	3/3	1/3	2/3
	TOTAL (4)	**0/4** (0%)	**3/4** (75.0%)	**1/4** (25.0%)	**1/4** (25.0%)	**3/4** (75.0%)	**3/4** (75.0%)	**1/4** (25.0%)	**3/4** (75.0%)	**0/4** (0%)	**3/4** (75.0%)	**2/4** (50.0%)	**3/4** (75.0%)	**2/4** (50.0%)	**2/4** (50.0%)	**4/4** (100%)	**4/4** (100%)	**2/4** (50.0%)	**3/4** (75.0%)
4	**HUMAN DIARRHOEIC SAMPLES**																		
	HD (1)	0/1	0/1	0/1	1/1	0/1	0/1	1/1	1/1	0/1	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	1/1
	TOTAL (1)	**0/1** (0%)	**0/1** (0%)	**0/1** (0%)	**1/1** (100%)	**0/1** (0%)	**0/1** (0%)	**1/1** (100%)	**1/1** (100%)	**0/1** (0%)	**1/1** (100%)	**1/1** (100%)	**1/1** (100%)	**0/1** (0%)	**1/1** (100%)	**1/1** (100%)	**1/1** (100%)	**1/1** (100%)	**1/1** (100%)
	GRAND TOTAL (25)	**2/25** (8%)	**4/25** (16%)	**6/25** (24%)	**7/25** (28%)	**7/25** (28%)	**9/25** (36%)	**9/25** (36%)	**10/25** (40%)	**13/25** (48%)	**13/25** (52%)	**13/25** (52%)	**13/25** (52%)	**14/25** (56%)	**15/25** (60%)	**17/25** (68%)	**17/25** (68%)	**19/25** (76%)	**19/25** (76%)

PC-poultry faeces, **PI**-poultry intestines, **CM**- chicken, **HD**-human diarrhoeic samples, **SF**-pig faeces, **SI**-pig intestine, **PK**-pork; **CL-**colistin, **COT**-co-trimoxazole, **CD**-clindamycin, **C**-chloramphenicol, **CAZ**-ceftazidime, **AMP**-ampicillin, **NIT**-nitrofurantoin, **AZM**-azithromycin, **NA**-nalidixic acid, **DO**-doxycycline, **GEN**-gentamicin, **S**-streptomycin, **E-**erythromycin, **NX**-norfloxacin, **CIP**-ciprofloxacin, **CEP**-cephalothin, **TE**-tetracycline, **VA**-vancomycin

C. jejuni isolates showed highest resistance to ciprofloxacin (6/6, 100%) followed by vancomycin and tetracycline (5/6, 83.3%), cephalothin, gentamicin, nalidixic acid and azithromycin (4/6, 66.7%), norfloxacin, erythromycin, streptomycin and doxycycline (3/6, 50%), nitrofurantoin, ampicillin, ceftazidime, chloramphenicol and clindamycin (2/6, 33.4%) and co-trimoxazole (1/6, 16.7%). No resistance was observed towards colistin.

C. coli isolates showed highest resistance to vancomycin and tetracycline (14/19, 73.7%) followed by cephalothin (13/19, 68.4%), norfloxacin (12/19, 63.1%), ciprofloxacin and erythromycin (11/19, 57.9%), streptomycin and doxycycline (10/19, 52.6%), gentamicin (9/19, 47.4%), nalidixic acid (8/19, 42.1%), nitrofurantoin and ampicillin (7/19, 36.8%), azithromycin (6/19, 31.6%), ceftazidime and chloramphenicol (5/19, 26.3%), clindamycin (4/19, 21.1%), co-trimoxazole (3/19, 15.8%) and colistin (2/19, 10.5%).

The presence of resistance to erythromycin (56%) and azithromycin (44%) is matter of concern, as they are first line of treatment for *Campylobacter* infection in humans (Houf *et al*., 2004). In the present study, 56% of isolates exhibited resistance towards erythromycin which is considered as a classic drug of choice for *Campylobacter* infections in humans. This goes in line with a range of resistance rates reported by various researchers right from 0.3% (Wieczorek *et al*., 2012) through 37.6% (Ibrahim *et al*., 2018) to 99.75% (Karikari *et al*., 2017). The resistance towards azithromycin (44%) observed in the present study was higher compared to the reports of Mason *et al*. (2017) with 2 % resistance towards azithromycin but lower compared to the findings of Chen *et al*. (2010) where they reported 62.35% resistance level.

The emergence of *Campylobacter* spp. strains resistant to fluoroquinolones and tetracyclines have been attributed to the use of sub-therapeutic levels of antibiotics at intensive animal rearing units. The fluoroquinolone antibiotics used in this study were ciprofloxacin and norfloxacin which showed resistance rates of 68% and 60%. The resistance rate for ciprofloxacin in the present study was almost in tune with the findings of Perez-boto *et al*. (2013) and Wieczorek *et al*. (2012) who reported a resistance rates of 73.9% and 88.1% towards ciprofloxacin, respectively. However lower rates have been demonstrated by Ibrahim *et al*. (2018) and Karikari *et al*. (2017) who reported a rates of 28.1% and 38.95% respectively. Resistance towards norfloxacin of the present study (60%) was almost comparable with the findings of Vlieghe *et al*. (2008) who reported a rate of 78.8%. However, Karikari *et al*. (2017) reported only 14.2% which is lower than the present finding. Karikari *et al*. (2017) in their study in Ghana, reported a tetracycline resistance rate of 79.8%

which is in line with the present findings (76%). Lower levels of resistance to tetracycline i.e. 57.6% and 43.8% have been reported by Wieczorek *et al*. (2012) and Ibrahim *et al*. (2018), respectively. Zhou *et al*. (2015), in their study in Japan reported high resistance levels of *Campylobacter* towards doxycycline (80.8%) which is almost comparable with that of the present findings (52%). The present study depicts 48% percent of *Campylobacter* isolates to be resistant against nalidixic acid, which is in tune with the findings of Ibrahim *et al*. (2018) who reported the resistance to be 50%. However higher levels of resistance towards nalidixic acid (86.8% and 86.7%) have been reported by Wieczorek *et al*. (2012) and Zhou *et al*. (2016). Among aminoglycosides, the present study depicts a resistance rate of 52% for both streptomycin and gentamicin and similar values have been reported by Wieczorek *et al*. (2012) and Ibrahim *et al*. (2018).

The present study depicted a resistance level of 28% and 16% towards chloramphenicol and co-trimoxazole whereas, a lower resistance level of 12.3% for chloramphenicol was reported by Zhou *et al*. (2016) and higher resistance level of 42.5% for co-trimoxazole reported by Ibrahim *et al*. (2018) respectively. Kuana *et al*. (2008) reported a 80% susceptibility of *Campylobacter* strains to co-trimoxazole and colistin, respectively which is comparable with the present finding of 64%. Uzunovic-Kamberovic (2003) reported a resistance level of 10% to nitrofurantoin, which is lower than the findings of the present study (36%). Tang *et al*. (2017) reported a vancomycin-resistance level of 86% which is similar to the resistance level of 76% reported in the present study. Cha *et al*. (2017) reported a resistance level of 2% towards clindamycin which is lower when compared to findings in the present study (24%).

Out of 6 *C. jejuni* isolates highest resistance was found towards ciprofloxacin (6/6, 100%) followed by vancomycin and tetracycline (5/6, 83.3%), cephalothin, gentamicin, nalidixic acid and azithromycin (4/6, 66.7%), norfloxacin, erythromycin, streptomycin and doxycycline (3/6, 50%), nitrofurantoin, ampicillin, ceftazidime, chloramphenicol and clindamycin (2/6, 33.4%) and co-trimoxazole (1/6, 16.7%). No resistance was observed towards colistin. The resistance level towards ciprofloxacin (100%) in the present study was in agreement with the findings of Wieczorek *et al*. (2012) who reported 91% among *C. jejuni* isolates. The resistance levels observed towards ampicillin (33.4%), gentamicin (66.7%), norfloxacin (50%), tetracycline (83.3%) and erythromycin (50%) were in agreement with the findings of Abamecha *et al*. (2015) who reported resistance levels of 46.6%, 50%, 64.9%, 82.2% and 60.3%, respectively for *C. jejuni* strains in a study in Ethiopia.

Out of 19 *C. coli* isolates, highest resistance was found towards vancomycin and tetracycline (14/19, 73.7%) followed by cephalothin (13/19, 68.4%), norfloxacin (12/19, 63.1%), ciprofloxacin and erythromycin (11/19, 57.9%), streptomycin and doxycycline (10/19, 52.6%), gentamicin (9/19, 47.4%), nalidixic acid (8/19, 42.1%), nitrofurantoin and ampicillin (7/19, 36.8%), azithromycin (6/19, 31.6%), ceftazidime and chloramphenicol (5/19, 36.8%), clindamycin (4/19, 21.1%), co-trimoxazole (3/19, 15.8%) and colistin (2/19, 10.5%). The present findings depict resistance levels of 15.8% for co-trimoxazole and 36.8% for ampicillin, which were in agreement with the findings of Ibrahim *et al*. (2018) who reported resistance levels of 14.3% and 19%, respectively for *C. coli* strains. Wieczorek *et al*. (2012) reported resistance levels of 63.3% and 31.1% towards tetracycline and gentamicin which is similar to the findings in the present study i.e. 73.7% and 47.4%, respectively for *C. coli* strains.

Among livestock faecal isolates, higher resistance levels were observed towards tetracycline (7/8, 87.5%), followed by ciprofloxacin, cephalothin, nalidixic acid and vancomycin (5/8, 62.5%), gentamicin, erythromycin, norfloxacin and ciprofloxacin (4/8, 50%), azithromycin, streptomycin and clindamycin (3/8, 37.5%), chloramphenicol, nitrofurantoin, ampicillin and doxycycline (2/8, 25%), colistin and ceftazidime (1/8, 12.5%). All strains were susceptible to co-trimoxazole. Among livestock intestinal isolates, high resistance level was observed towards vancomycin (10/12, 83.3%), tetracycline (9/12, 75%), norfloxacin and erythromycin (8/12, 66.7%), nalidixic acid, doxycycline, ciprofloxacin and cephalothin (7/12, 58.3%), gentamicin and streptomycin (6/12, 50%), nitrofurantoin (5/12, 41.7%), ampicillin (4/12, 33.4%), chloramphenicol, ceftazidime and azithromycin (3/12, 25%), clindamycin (2/12, 16.7%), co-trimoxazole and colistin (1/12, 8.3%). Higher levels of resistance were observed among 4 isolates recovered from foods of animal origin,, higher resistance were observed for ciprofloxacin (4/4, 100%) and cephalothin (4/4, 100%) followed by vancomycin, streptomycin, doxycycline, azithromycin, ampicillin, ceftazidime and co-trimoxazole where 75% resistance was observed. 50 % resistance level was exhibited towards tetracycline, norfloxacin, erythromycin and gentamicin. Resistance level of 25% was exhibited towards nitrofurantoin, chloramphenicol and clindamycin. All 4 isolates were susceptible to colistin and nalidixic acid. Human diarrhoeic isolate was found to be resistant to chloramphenicol, nitrofurantoin, azithromycin, doxycyline, gentamicin, streptomycin, norfloxacin, ciprofloxacin, cephalothin, tetracycline and vancomycin.

According to Ziech *et al*. (2016) organisms were considered as Multi-drug resistant (MDR) when they do not show susceptibility to at least three classes of antibiotics (at least one drug from each class). In the present study, out of 25 *Campylobacter* isolates, 80% of isolates were showing multi-drug resistance, MDR was found in 6 (100%, 6/6) *C. jejuni* isolates and 17 (89.4%) *C. coli* isolates. These findings were in agreement with the reports of Abamecha *et al*. (2015) and Ibrahim *et al*. (2018) who demonstrated MDR values of 46.6-80.5% and 62.5% for *Campylobacter* isolates, respectively. Before concluding any *Campylobacter* isolate as MDR, we must take into consideration the breakpoint values recommended by CLSI (2018) which does not provide an uniform platform for all classes of antibiotics as values for many antibiotics are not available.

Screening of 25 isolates for MAR index revealed 5 different values for *C. jejuni* and 10 different values for *C. coli* and their frequencies were calculated as in Table-4. Overall 84% (21/25) of the isolates had MAR index values above 0.2 which indicates that those strains were exposed to environments with high antibiotic usage. These findings were in agreement with the findings of Ghimire *et al*. (2014) who reported that 77.8% of the *Campylobacter* isolates in their study had MAR index values above 0.2.

Table-4: Multiple Antibiotic Resistance (MAR) indices of *C. jejuni* and *C. coli*

MAR index	Percentage frequency of MAR index (%)	
	C. jejuni	***C. coli***
0	0	0
0.1	16.67	15.7
0.2	0	5.26
0.3	0	15.7
0.4	16.67	21.12
0.5	16.67	15.7
0.6	33.32	15.7
0.7	16.67	5.26
0.8	0	5.26
0.9	0	0
1	0	0

CONCLUSION

These findings could be considered as an eye-opener for the future threats to come in terms of antibiotic resistance prevalent in food-borne pathogens and also necessitated the importance of continuous surveillance over the levels of antibiotic resistance especially in terms of MDR. MDR in foods of animal origin can be attributed to the use of non-therapeutic antibiotics at the farm level. These inept practices cannot be condoned as they come back to haunt the human healthcare system as a whole as they tend to make them ineffective. In *Campylobacter* spp. the main mechanism by which resistance is brought about towards the different classes of antibiotics is by the presence of CmeABC efflux pump mechanism which plays a major role in intrinsic and acquired resistance and studies can be converged on this idea for understanding the resistance mechanisms of *Campylobacter* spp.

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