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Prevalence and antibiotic susceptibility pattern of *Campylobacter* species isolated from broiler chicken meat samples in district Bannu, Pakistan

Inam Ullah[1,2], Hajra Khurshid[3], Nadeem Ullah[4,2*], Israr Aziz[3], Muhammad Junaid Khan[5], Basit Ali Khan[6], Junaid Ahmad[7], Zahid Ullah[7]

[1] *Department of Microbiology and Immunology, Hebei Medical University, Shijiajhuang, China.*
[2] *Department of Microbiology, Kohat University of Science and Technology, Kohat, Pakistan.*
[3] *Department of Biosciences, COMSATS University, Islamabad, Pakistan.*
[4] *Republic Department of China Pathogen Biology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.*
[5] *Department of Microbiology, Abdul Wali Khan University, Mardan, Pakistan.*
[6] *Department of Microbiology, Government College University, Lahore, Pakistan.*
[7] *Departemt of Microbiology ,Hazara University, Mansehra, Pakistan.*

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ABSTRACT

The *campylobacter* genus of bacteria is important in public health as it comprises many species causing diarrhea in humans. Poultry and their products are recognized as vital causes of campylobacteriosis in humans. For bacterial food-borne diseases, *Campylobacter* is considered as the leading cause. Higher prevalence has been reported in developed countries. Our study was a cross-sectional study directed to determine the prevalence of *Campylobacter* species in retail broiler meat in the Bannu district of Khyber Pakhtunkhwa, Pakistan, from January to June 2018. A total of 200 poultry meat samples were collected from four different areas of district Bannu Khyber Pakhtunkhwa province of Pakistan that includes Lakki gate, Tanchi bazar, Bannu Township and Mangal milla. Mueller-Hinton medium was used for disc diffusion method to determine antibiotic resistance of *Campylobacter* species. Amongst 200 broiler meat samples, 60 (30%) samples were found positive for *Campylobacter* species. The highest prevalence was observed in samples from Bannu Township (50%) while lowest prevalence (12%) was observed in samples from Mangal milla broiler meat samples. Amongst different types of meat samples, highest prevalence was found in thigh meat (46%), while lowest prevalence was observed in cloacal swab (20%). Highest resistance was observed against Amoxicillin (AMX) 80% while the resistance observed against other antibiotics were Ampicillin (AMP) 70%, Tetracycline (TET) 65%, Sulphamethoxazole + Trimethoprim (SXT) 60%, Chloramphenicol (CHL) 56.66%, Clarithromycin (CLR) 50%, Streptomycin (STR) 40%, Gentamycin (GEN) 36.66%, Ofloxacin (OFX) 20%, Ciprofloxacin (CIP) 15%, Levofloxacin (LEV) 15% and Azithromycin (AZM) 10%. The lowest resistance was observed against Ceftriaxone (CRO) 5%. Our Study concludes that *Campylobacter* species is prevalent highly in district Bannu, and it might be a hazard to public health.

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1. Introduction

Campylobacter species are spiral rod-shaped gram-negative bacteria. They are motile, non-spore-forming bacteria and grow in micro-aerobic conditions. In the globe, it is considered as one of the main public health problem (1).

*Corresponding author. Tel.: +923109724148
E-mail address: nadeem@hust.edu.cn

Campylobacter jejuni is the major disease-causing strain of thermo-tolerant *Campylobacter*. This strain is commonly associated with the campylobacteriosis (2). Campylobacteriosis is a zoonotic problem transmitted from animal or their product to human. While it causes self-limiting infection in human but some of them may cause complication such as bacteremia, abortion, reactive arthritis, and Guillain-Barre syndrome (3).

Eating contaminated raw or not properly cooked chicken products have been identified by many studies as the key vector for causing campylobacteriosis (4). *Campylobacter* species are pathogens mainly responsible for zoonosis, and they are commonly isolated from numerous species of animals, including poultry, pigs, sheep, cattle, wild birds, etc (5). The major route for infection in humans is considered due to the consumption and management of contaminated poultry meat (6). It has been considered a significant cause of human enteritis since 1970 (7). In comparison to Salmonella species, the major cause of gastroenteritis is considered as *campylobacter* (8).

Amongst the 17 accurately named species in the *Campylobacter* genus, *C.jejuni ssp. jejuni*, *Campylobacter coli*, *Campylobacter fetus ssp*. fetus, *Campylobacter upsaliensis, Campylobacter lari* and *campylobacter hyointestinalis ssp. hyointestinalis* are the acknowledged cause of human intestinal infections (9). The most commonly *campylobacter* species reported is *C. jejuni* (80-90%) while *C. coli* is reported (5-10%) (10).

Poultry and their products are recognized as vital causes of Campylobacteriosis in human. For bacterial foodborne diseases, *Campylobacter* is considered as the leading cause. Higher prevalence has been reported in developed countries (11). Diseases caused by *C. jejuni* and *C. coli* in humans are common and generally transmitted through meat, milk and water (12). The most frequent source of infection is retail meat products, predominantly meat from poultry and for campylobacteriosis it is measured as main risk factor in human (13). By expressing characteristics of antibiotic resistance and diverse virulence determinants, the pathogenicity and survivability of *Campylobacter* strains is improved (14).

The main cause of *Campylobacter* infection in humans is considered as poultry meat, and their acquisition is via the fecal-oral route. Food Standards Agency shows 62.5% prevalence of *Campylobacter* species in poultry meat in the United Kingdom (15). Correspondingly, in poultry and chickens, *C. jejuni* have been reported in many earlier studies (16). Previous study done by Berrang *et al*. reported that the surface of poultry carcasses contamination by *Campylobacter* occur during handling of intestinal track (17). There might be a chance of cross-contamination from raw meat that can happen during cooking at home. The potential risk factors related with human *Campylobacter* and *Salmonella* infection is cross-contamination to other products and contaminated raw meats handling (18). Sporadic human incidence might occur due to eating not properly cooked poultry meat while epidemics are mostly associated with the raw milk (19). In the United States approximately half of the total sporadic *Campylobacter* problems are associated with foods prepared in restaurants (20). Antibiotic resistance has been developed in many microbes including *E. coli*, *Salmonella*, *Enterococcus* and *Campylobacter* due to use of antibiotic in animals envisioned for food. Through food chain this resistance can be transferred to human (21). Due to the extensive usage of the drug in animals and poultry production, an increase in drug resistance to fluoroquinolones in isolates of *Campylobacter* has been observed in the United States (22). In foodborne pathogens multidrug resistance is often noticed. In a number of studies multidrug resistance in *Campylobacter* have been observed. A previous study done by Zoran et al. in Serbia shows that 10% of *C. jejuni* and 16.3% of *Campylobacter coli* were observed to be resistant to three or at least one in three classes of antibiotics (23). Other earlier studies reported that in *C. coli* multidrug resistance was common than *C. jejuni* (24,25). Therefore, this study was conducted to found the prevalence of *Campylobacter* species in raw broiler meat and to determine the antibiotic resistance in *Campylobacter* species isolated from broiler meat samples.

2. Materials and Methods

2.1.Sampling

A total of 200 poultry meat samples were collected from four different areas of district Bannu Khyber Pakhtunkhwa province of Pakistan that includes Lakki gate, Tanchi bazar, Bannu Township, and Mangal milla. From each area, 50 broiler meat samples were collected from January to June 2018. In an icebox, all the collected samples were transported to the laboratory where they were handled on the same day for the isolation and identification of *Campylobacter* species.

2.2.Isolation

Conventional methods were used for isolation and identification of *Campylobacter* species. In 1:10 buffered peptone water, the samples were homogenized in a stomacher for 2 min. Into 45 ml of Preston broth (Oxoid cat no SR0129, England) supplemented with antibiotics such as Polymyxin B, Rifampicin, Trimethoprim, and Cycloheximide (Oxoid), 5 ml of the homogenized sample was added for enrichment. The first media was incubated at 37ºC for 4 h, then under microaerophilic condition it was incubated at 44ºC for 44 h. Microaerophilic condition was created by using campy gas sachet (Gaspak EZ company container BBL 260680) in an anaerobic jar. After 48 h combined incubation, on surface of *Campylobacter* blood-free selective agar plate

(Oxoid cat no CM0739, england) comprising growth supplements (Oxoid cat no SR0155E england), 100 microliter of the broth culture was spread evenly. Under microaerophilic conditions, for 48 h the plates were incubated. Positive growth of *Campylobacter* isolates was further subjected to gram staining, motility test, and standard biochemical tests consisting of oxidase, nitrate reduction, catalase, indoxyl acetate.

2.3.Antibiotic susceptibility testing

The disc diffusion method described by CLSI, (2006) (26) was used for antibiotic susceptibility testing against all isolates. Mueller-Hinton broth supplemented with sodium pyruvate, sodium metabisulphite, and ferrous sulphate was used for the enrichment of each isolate. The inoculum was allowed to reach 0.5 McFarland standard turbidity level. After attaining the desired turbidity, then on the surface of Mueller- Hinton agar plate, supplemented with 5% lysed horse blood, 0.1 ml aliquot was spread. For drying, the plates were allowed, and then antibiotic disks were placed over the plates. The plates were then incubated for 48 h at 42ºC. By using a digital caliper, the zones of inhibition were measured. The following antibiotics including AZM (15 µg), AMP (10 µg), SXT (25 µg), CIP (5 µg), OFX (5 µg), LEV (5 µg), CLR (15 µg), CHL (30 µg), TET (30 µg), STR (10 µg), GEN (10 µg), AMX (20 µg) and CRO (30 µg) (Oxoid) were used for the susceptibility study.

Table 1. Distribution of samples

Sample type	Number of samples
Breast piece	50
Leg piece	50
Skin	50
Cloacal swab	50
Total	200

2.4. Statistical Analysis

SPSS version 22 for windows was used for the statistical analysis of data.

1. Results

A total of 200 poultry meat samples were collected from four different areas, microscopically the bacteria observed were gram negative rods (Fig. 1). Greyish small flat colonies were observed on selective medium (Fig. 2). The results of the identification tests are given in (Table 2). Among 200 broiler meat samples 60 (30%) samples were found positive for *Campylobacter* species (Table 3). Highest prevalence was observed in samples from Bannu Township (50%), followed by Lakki gate (30%) and Tanchi bazar (28%) while lowest prevalence (12%) was observed in samples from Mangal milla meat samples (Fig. 3). Among different type meat samples highest prevalence was found in thigh meat (46%), followed by breast piece (30%) and skin (24%) while lowest prevalence was observed in cloacal swab (20%) (Fig. 4). Highest resistance was observed against AMX (80%) while the resistance observed against other antibiotics were AMP (70%), TET (65%), SXT (60), CHL (56.66%), CLR (50%), STR (40%), GEN (36.66%), OFX (20%), CIP (15%), LEV (15%) and AZM (10%). Lowest resistance was observed against CRO (5%) (Table 4).

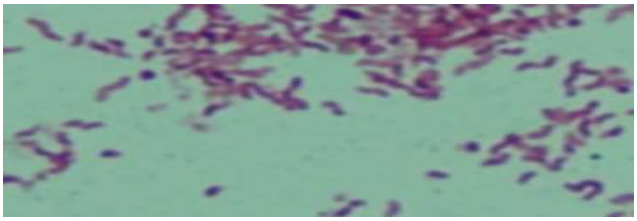

Figure 1. Gram staining of *Campylobacter* species

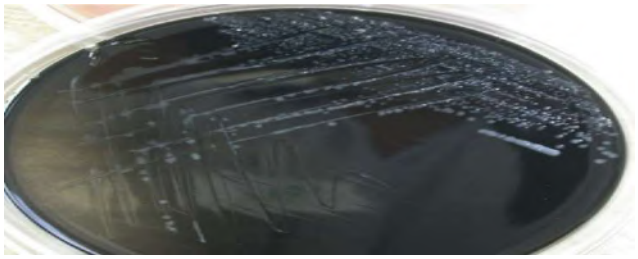

Figure 2. Growth of *Campylobacter* species on selective media

Table 2. Results of identification testing for *Campylobacter* species

Serial number	Test	Result
1	Catalase	Positive
2	Oxidase	Positive
3	Nitrate reduction	Positive
4	Indoxyl acetate	Positive
5	Motility	Positive

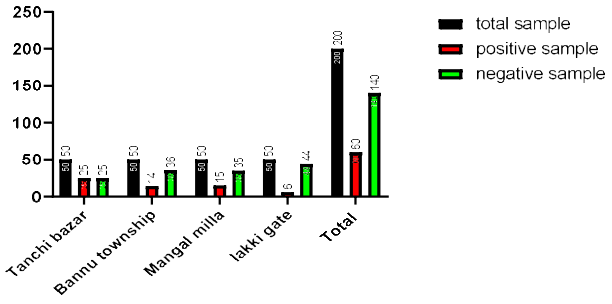

Figure 3. Area wise prevalence of *Campylobacter* species in broiler meat sample in district Bannu

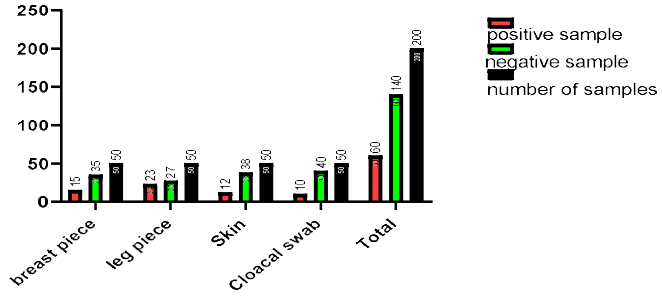

Figure 4. Sample wise prevalence of *Campylobacter* species in broiler meat sample in district Bannu

2. Discussion

Campylobacter species are spiral rod shaped gram negative bacteria. They are motile, non-spore-forming bacteria and grow in micro-aerobic condition. In the globe it is considered as one of the main public health problem (1). *C. jejuni* is the major disease causing strain of thermo-tolerant *Campylobacter*. This strain is commonly associated with the campylobacteriosis (2).

Campylobacteriosis is a zoonotic problem transmitted from animal or their product to human. While it cause self-limiting infection in human but some, it cause complication such as bacteremia, abortion, and reactive arthritis and Guillain-Barre syndrome (3). Eating of contaminated raw or not properly cooked chicken products have been identified by many studies as the key vector for causing campylobacteriosis (4).

Table 3. Over all prevalence of *Campylobacter* species in broiler meat sample in district Bannu

Sample	Positive n (%)	Negative n (%)
Broiler meat	60 (30)	140 (70)

Microscopically the bacteria observed were gram-negative rods. Greyish small flat colonies were observed on selective medium. The results of the identification tests are given in (Table 2). Among 200-broiler meat samples, 60 (30%) samples were found positive for *Campylobacter* species. Highest prevalence was observed in samples from Bannu Township (50%), followed by Lakki gate (30%) and Tanchi bazar (28%) while lowest prevalence (12%) was observed in samples from Mangal milla meat samples. Among different type meat samples highest prevalence was found in thigh meat (46%), followed by breast piece (30%) and skin (24%) while lowest prevalence was observed in cloacal swab (20%). Highest resistance was observed against AMX (80%) while the resistance observed against other antibiotics were AMP (70%), TET (65%), SXT (60), CHL (56.66%), CLR (50%), STR (40%), GEN (36.66%), OFX (20%), CIP (15%), LEV (15%) and AZM (10%). Lowest resistance was observed against CRO(5%).

In poultry meat, different prevalence of *Campylobacter* have been reported in different countries such as in Northern Ireland 85% (27), in Poland 87% (28), in Estonia 20.8% (29) and in Italy 73-81% (30,31). These results are not in accordance with our reported prevalence and this might be due to different climatic conditions, techniques of slaughtering etc. Sampling type may also be the reason for this. In the world after infections, antibiotic resistance is one of the greatest hazard to the public health. Different countries reported different pattern of antimicrobial resistance in *Campylobacter* species. In Switzerland, Ledergerber et al. observed 28.7% resistance to Ciprofloxacin followed by 12.6% to Tetracycline, 11.8% to Sulphonamide and observed lowest (10.3%) resistance to Ampicillin (32).

Table 4: Antibiotic resistance profile of *Campylobacter* species isolates

Serial No	Antimicrobial drugs	Concentration	Code	Drug susceptibility	*Campylobacter species* (n)%
1	Tetracycline	30µg	TET	*Susceptible*	21 (35)
				Resistant	39 (65)
2	Azithromycin	15µg	AZM	*Susceptible*	54 (90)
				Resistant	6 (10)
3	Chloramphenicol	30µg	CHL	*Susceptible*	26 (43.33)
				Resistant	34 (56.66)
4	Ciprofloxacin	5µg	CIP	*Susceptible*	51 (85)
				Resistant	9 (15)
5	Levofloxacin	5 µg	LEV	*Susceptible*	51 (85)
				Resistant	9 (15)
6	Ampicillin	10µg	AMP	*Susceptible*	18 (30)
				Resistant	42 (70)
7	Sulphamethoxazole + Trimethoprim	25 µg	SXT	*Susceptible*	24 (40)
				Resistant	36 (60)
8	Ofloxacin	5 µg	OFX	*Susceptible*	48 (80)
				Resistant	12 (20)
9	Clarithromycin	15 µg	CLR	*Susceptible*	30 (50)
				Resistant	30 (50)
10	Streptomycin	10 µg	STR	*Susceptible*	36 (60)
				Resistant	24 (40)
11	Gentamycin	10 µg	GEN	*Susceptible*	38 (63.33)
				Resistant	22 (36.66)
12	Amoxicillin	20 µg	AMX	*Susceptible*	12 (20)
				Resistant	48 (80)
13	Ceftriaxone	30 µg	CRO	*Susceptible*	57 (95)
				Resistant	3 (5)

AMP (47.4%), CIP (42.1%), Erythromycin (12.1%), GEN (25.6%), Nalidixic acid (46.4%) and TET (45.3%) resistance was observed by Mattheus et al. in a study done in Belgium (33). For Tetracycline 18.4% resistance and for Ampicillin 17.6% resistance was reported by Miflin *et al*. in a study done on *C. jejuni* (34). In *Campylobacter* species from broiler meat in South Africa Bester *et al*. observed highest resistance for Tetracycline and Ceftriaxone as 98.2% and 96.4% respectively (35). Extensive resistance of *Campylobacter* to various antibiotics including Lincomycin (51-100%), Ampicillin (33.3-60.2%) and Tetracycline (5.6-40.7%) have been observed in a study done by Obeng *et al*. (36). In a recent study done by Wieczorek *et al*. in poultry observed 92.5% resistance to Ciprofloxacin followed by 88.9% in Nalidixic acid and 68.4% in Tetracycline in Poland (37). In Poland another study done by Wysok *et al*. observed 52.7% resistance to Ciprofloxacin, 56% to Nalidixic acid and 61.3% to Doxycycline (38). All these previous studies along with current study give a clue of antimicrobial resistance in *Campylobacter* species in the world. The result difference might be due to variability in geographical location, change in climatic condition and use of different antibiotics in feeds of poultry and other animals.

3. Conclusion

Our Study conclude that *Campylobacter* species is highly prevalent in district Bannu. Potential source of *Campylobacter* might be raw meat from broiler and this can be a possible source of infection to people by consuming raw or not properly cooked meat. To most of the available antimicrobial agents, high percentages of resistance was observed and this might be due to use of antimicrobial agents for growth promotion or treatment in poultry. Hence it require quick attention of the concerned authorities for veterinary and public health as the diseases is zoonotic that could be hazardous to public health.

Conflict of interests

The authors declare no competing interests.

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