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Prevalence and Drug Sensitivity Pattern of *Campylobacter jejuni* Isolated from Cattle and Poultry in and Around Gondar Town, Ethiopia

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Abstract: A cross sectional study was conducted from October 2013 to April 2014 to estimate the prevalence and drug sensitivity pattern of *Campylobacter jejuni* from fecal sample of cattle and chickens in and around Gondar town, Ethiopia. A total of 360 fecal samples were collected from cross and local breeds of cattle (N=270) and poultry (N=90) following random sampling method. The overall prevalence of *Campylobacter jejuni* in cattle and poultry were 21.5% (N=58) and 28.9% (N=26) respectively. Though the difference is not statistically significant, slightly higher prevalence (26.9%, N=28) was recorded in male cattle than in females (18.1%, N=30); in young (22.2%, N=8) than adults (21.4%, N=50) and in cross breeds (22.9%, N=35) than local breeds (19.7%, N=23). All isolates of *Campylobacter jejuni* were challenged with eight commonly used and newly introduced antimicrobials to see the resistance pattern. Isolates from cattle were resistant to Penicillin, Bacitracin, Erythromycin and Cephalothin at the rate of 96.6%, 93.1% 56.9% and 43.1% respectively but susceptible to Nalidixic acid (100 %), Ampicilin (87.9%), Streptomycin (77.6%) and Tetracycline (69.0%). On the other hand isolates from poultry were resistant to Penicillin (100%), to Bacitracin (80.8%), to Erythromycin (53.9%) and to Tetracycline (50%) though majority were susceptible to Nalidixic acid (100%), Ampicilin (80.8%) and Streptomycin (53.9%). High percentages of resistance to most of the antimicrobials tested may indicate the misuse of these agents in food animal treatment. Therefore, it is necessary to impose strictly the rules and regulations of drug administration and delivery system in the area so as to prevent the community from hazards of drug resistant, zoonotically important diseases.

Key words: *Campylobacter jejuni* · Cattle · Drug Sensitivity · Fecal · Poultry

INTRODUCTION

Campylobacter spp. particularly *Campylobacter jejuni* (*C. jejun*) is a leading causative agent of bacterial gastroenteritis with an estimated 400 million human cases worldwide [1]. Poultry is generally considered to be the most important reservoir for *Campylobacter* spp. Many types of food animals other than poultry including cattle, sheep and pigs also harbor this pathogen. In human *C. jejuni* infections occur mainly from contaminated poultry or other animal meat, meat products, raw milk, milk products and surface water [2]. Animal food products most commonly contaminated by this pathogen during slaughter and carcass dressing [3] and infections due to *C. jejuni* are generally self-limiting; with symptoms resolving in about three to five days though antibiotic therapy is required in immuno-compromised patients, in cases of bacteremia and in severe and long-lasting *Campylobacter* infections [4].

The spread of new generation of infections resistant to antibiotic treatments has serious consequences for public health. Antibiotic-resistant bacteria can keep people sick for long time and sometimes people are unable to recover at all. Children, the elderly and those with weakened immune systems (including cancer, HIV/AIDS and transplant patients) are particularly vulnerable because their immune systems are not as vigorous as those of healthy adults, therefore antimicrobial susceptibility testing has become more important than ever in routine clinical practice [5]. Taking this into

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consideration the objective of this study was to estimate the prevalence, assess associated potential risk factors and evaluate antimicrobial sensitivity pattern of *Campylobacter jejuni* isolated from fecal samples of cattle and poultry in and around Gondar town, Ethiopia.

MATERIALS AND METHODS

Study Area: The study was conducted from October, 2013 to April, 2014 in Gondar town, Ethiopia which is located 12°36'N and 33°28'E at altitude of 2300 m. above sea level. The average temperature and annual rainfall of the area is 20°C and 1800 mm respectively. The study area harbor a total 308,257 population, 8,202 cattle, 22,590 goats, 2,695 sheep, 1,065 horses, 9,001 donkeys [6] and 45,304 poultry based on the district agricultural office report.

Study Animals and Design: The study population comprises cross and local indigenous breeds of cattle and chicken selected randomly from intensive and semi intensive farms of Gondar town and its surroundings. The study was cross-sectional study which was aimed to determine the isolation rate of C*ampylobacter jejuni* and see their drug sensitivity patterns.

Sample Size Determination: Sample size were determined using 95% confidence interval and 5% desired level of precision with expected prevalence of 15.4% following the report of Lengerh *et al*. [7] and the formula given by Thrusfield as indicated below [8].

$$n = \frac{1.96^2 P_{exp}(1-P_{exp})}{d^2}$$

Where:
n = required sample size,
P_{exp} = expected prevalence,
d = desired absolute precision.

Based on the given formula, the total sample size was calculated to be 200 but to increase the level of precession and due to sample collection convenience, the sample size were increased to 360.

Sample Collection and Processing: Fecal samples were collected aseptically using sterile swabs from the rectum of cattle (N=270) and cloacae of poultry (N=90) and submitted to the laboratory in a test tube containing sterile alkaline peptone water that have 4% concentration activated charcoal to reduce the toxicity of oxygen, following ISO, 2006 protocol [9] and processed in the laboratory on the date of sample submission.

Isolation and Identification of *C. jejuni* Strain: Modified Preston's *Campylobacter* selective agar (Oxoid, UK) with antibiotic supplements (Polymixin-B 2,500 IU, Rifampicine 5.00 mg, trimethoprume 5.00mg and Cyclohexamide 50 mg concentration per litter volume) was used for the isolation and identification of *C. jejuni.* After 48 hrs of incubation at 42°C in a microaerophilic atmosphere using CampyGen® gas generating kits (5% O_2 and 10% CO_2) (Oxoid, UK) in anaerobic jars, characteristic colonies showing flat, grayish, finely granular and translucent with an irregular edge and a tendency to spread along the direction of streak; or colonies that have 1-2 mm diameter, round, raised, convex, smooth, shiny with an entire translucent edge and a darker, opaque center with or without a metallic sheen, were selected as suspected *C. jejuni.* The type strains *C. jejuni* (NCTC 11351) were used as positive control.

Suspected isolates which gave Gram's negative stain with spiral or vibrioid morphology, catalase and oxidase positive and showing cork screw or darting motility were taken as tentative *C. jejuni* and streaked on blood free charcoal based modified Preston's *Campylobacter* agar without antibiotic supplements for further biochemical tests including Hippurate test.

Antimicrobial Susceptibility Test: Antimicrobial susceptibility test following disk diffusion method mentioned by National Committee for Clinical Laboratory Standards (NCCLS) [10] was applied to determine the susceptibility of *C. jejuni* to commonly used antimicrobial agents including Tetracycline (TE-30), Streptomycin (S-10), Bacitracin (B-10), Cephalothin (KF-30), Nalidilix acid (NA-30), Penicillin (P-10), Erythromycin (E-15) and Ampicilin (AM-10). Briefly by taking fresh 48 hour old pure isolated bacterial colonies were inoculated in 0.85% NaCl suspension to the level of turbidity equivalent to 0.5 McFarland standards (which forms a suspension approximately equivalent to 1 x 10^8 to 4 x 10^8 CFU/ml) and sided with sterile cotton swab onto a Mueller-Hinton agar (Oxoid). Antibiotic discs were applied after drying the plates for 3–5 minute and the plates were incubated in microaerophilic atmosphere at 42°C for 48 hours. The zone of inhibition around the disc were measured to the nearest millimeter using a digital caliper, the size of the inhibition zone was compared with the disc manufacturer's standard and classified as sensitive (S), intermediate (I) or resistant (R) to the given drug.

RESULTS

The prevalence of *C. jejuni* in fecal samples of cattle (N=270) and poultry (N=90) were 21.5% (N=58) and 28.9% (N=26) respectively and statistically significant association were not observed between the occurrence of *Campylobacter jejuni* in cattle and breed, sex, age group and origin of the animal (Table 1).

For antimicrobial susceptibility tests a total of 84 *C. jejuni* isolates from cattle (N=58) and poultry (N=26) were challenged with eight commonly used as well as newly introduced antimicrobials as indicated in Table 2 and all isolates of *Campylobacter jejuni* were resistant at least to one of the antimicrobials used in the test. *C. jejuni* isolates from cattle were found highly resistant to Penicillin, Bacitracin and Erythromycin at the rate of 96.6%, 93.1% and 56.9% respectively while 46.6% of them were intermediate resistant to Cephalothin. On the other hand all were highly susceptible to Nalidixic acid, 87.9% to Ampicilin, 77.6% to Streptomycin and 69.0% to Tetracycline (Table 2).

Likewise *C. jejuni* isolates from poultry was resistant to Penicillin (100%), Bacitracin (80.8%) and Erythromycin (53.9%) though they are susceptible to Nalidixic acid, Ampicilin and Streptomycin at the rate of 100%, 80.8% and 53.9% respectively and intermediate resistant to Cephalothin (46.2%) and Erythromycin (34.6%) (Table 2).

DISCUSSION

Campylobacter jejuni is considered to be a commensal organism of chicken gut and the leading causative agent of gastroenteritis in humans worldwide which even sometimes surpassing other infections due to *Salmonella*, *Shigella* and *Escherichia coli.* It is also considered as a major triggering agent of Guillain-Barre syndrome (GBS) which is a molecular mimicry between sialylated lipo-oligosaccharide structures on the cell envelope of these bacteria and ganglioside epitopes on the human nerves that generates cross-reactive immune response and results in autoimmune-driven nerve damage [11].

Table 1: Relative prevalence of *Campylobacter jejuni* from fecal samples of cattle in and around Gondar town, Ethiopia

Characteristics	Classification	Total examined	Positives	Prevalence (%)	OR*	*p-value*
Breed	Cross	153	35	22.88	0.825	$p_{(1)}=0.524$
	Local	117	23	19.66		
Age	Adult	234	50	21.37	1.051	$p_{(1)}=0.907$
	Young	36	8	22.22		
Sex	Female	166	30	18.07	1.670	$p_{(1)}= 0.085$
	Male	104	28	26.92		
Origin	Intensive farm	199	39	19.60	-	$p_{(2)}= 0.435$
	Semi intensive farm	10	3	30.00		
	Vet Clinics	61	16	26.23		

** OR= odds ratio*

Table 2: Drug sensitivity pattern of *Campylobacter jejuni* isolated from fecal samples of bovine and poultry in and around Gondar town, Ethiopia

		Tested drugs and frequency (percentage) of sensitivity pattern							
Spp.	Sensitivity	KF-30	B-10	TE-30	NA-30	S-10	E-15	P-10	AM-10
Bovine	R	25(43.1%)	54(93.1%)	17(29.3%)	0(0.0%)	6(10.3%)	33(56.9%)	56(96.6%)	3(5.2%)
	I	27(46.6%)	1(1.7%)	1(1.7%)	0(0.0%)	7(12.1%)	17(29.3%)	2(3.4%)	4(6.9%)
	S	6(10.3%)	3(5.2%)	40(69%)	58(100%)	45(77.6%)	8(13.8%)	0(0%)	51(87.9%)
Poultry	R	9(34.6%)	21(80.8%)	13(50%)	0(0.0%)	5(19.2%)	14(53.9%)	26(100%)	4(15.4%)
	I	12(46.2%)	4(15.4%)	4(15.4%)	0(0.0%)	7(26.9%)	9(34.6%)	0(0%)	1(3.8%)
	S	5(19.2%)	1(3.8%)	9(34.6%)	26(100%)	14(53.9%)	3(11.5%)	0(%)	21(80.8%)

Remark:-Cephalothin (KF-30), Bacitracin (B-10), Tetracycline (TE-30), Nalidixic acid (NA-30), Streptomycin (S-10), Erythromycin (E-15), Penicillin (P-10), Ampicilin (AM-10); R= Resistant; I= Intermediate; S= Susceptible.

Based on the Community Zoonoses Reports of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) campylobacteriosis is one of the most commonly reported zoonosis in the EU and by the year 2008 it was the principal cause of zoonotic disease in humans with 190,566 reported confirmed cases [12,13]. Most *C. jejuni* infections are acquired due to consumption of raw or undercooked poultry products, unpasteurized milk and contaminated water. In the present study an overall prevalence of 23.3% of *C. jejuni* is recorded, which is relatively less than the report of Kassa *et al.* in Jimma town, Ethiopia (27.84%) [14] hence positive flock is generally more frequent among organic and free-range animals than among intensively reared animals due to increased environmental exposure [15]. Likewise the study shows higher relative prevalence of *C. jejuni* in poultry (28.9%) than in cattle (21.5%) and it was also higher than the study reports from developed countries' such as Sweden (1.93%) and Germany (9.2%) [16, 17]. Variation of prevalence in different country could be associated mainly with variations in animal management system. A relatively higher prevalence of *C. jejuni* in males (26.9%) than females (18.1%) may be also associated with better management trends for milking animal than male animals in the study area and the result showing greater relative prevalence in young (22.2%) than in adults (21.4%) could be associated to stronger immune status of adults than younger age groups. This result was coherent to the study report of Baserisalehi *et al.* [18] though the variation was statistically not significant.

The drug sensitivity pattern of the isolates in this study is alarming. Many of the isolates are resistant to multiple antimicrobial agents tested (Table-2), which coincides with the study result of Ebrahim [19] in Iran and Nawal in Egypt [20]. The antimicrobial resistance record of *C. jejuni* to Penicillin and Bacitracin is similar to the study done in Bulgaria by Boyanova *et al*. from clinical patients [21] where antibiotic usage is common and the probability of the agent to adapt and become resistant is higher. Likewise the result of this study showed relatively higher resistance pattern of *C. jejuni* on Streptomycin (19.2%) and Erythromycin (53.9%) than poultry farm isolates in the Czech Republic (9% and 6% respectively) [17]. In principle, in countries where the usage of antibiotics in animal production is uncommon, the prevalence of resistant strains is very low [22]. But in this study, higher antibiotic resistance pattern identified as compared to studies done in developed countries. This may be a good indicator of improper usage and insufficient regulation of medicaments in the study area, specifically in animal production and treatments which intern could be the source of zoonotically important drug resistant pathogenic microorganisms to the public.

CONCLUSION AND RECOMMENDATIONS

Historically it is true that unregulated use of antimicrobial agents in food animal production has led to the emergence and spread of antibiotic resistant *Campylobacter* spp. A good example of this is increment of fluoroquinolone resistant *Campylobacter* spp. isolated from animals and human patients in Europe and US following the approval and use of fluoroquinolones in poultry production [23, 24].

Higher prevalence of multi drug resistant *C. jejuni* occurrence among food animals (poultry and cattle) in this study is a clear indicator of higher risk to the public in acquiring zoonotically important drug resistant pathogens. Therefore, reinforcing hygienic practices at each link in the food chain from farm-to-fork is critical together with strict implementation of antibiotic utility rules and regulation for animals so as to prevent public health hazard and avoid economic losses in industries that process foods of animal origin.

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